Polymorphism in a neotropical toad species: An ontogenetic, population and geographic approach to chromatic variation in *Proceratophrys cristiceps* (Wied-Neuwied, 1824) - Amphibia, Anura, Odontophrynidae

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Knowing the extent of variability is important in understanding how evolution operates in polymorphic species such as those of the genus *Proceratophrys* Miranda-Ribeiro, 1920. This genus is comprised of South American toads which are amply distributed across this continent. The subject of this study was *P. cristiceps*, whose distribution in Brazil is limited to the Caatinga biome. Our goal was to examine and describe its chromatic variation from a populational perspective. We looked for different phenetic polymorphism levels and its probable chromotypic association by applying statistical and GIS tools in a way which would facilitate future taxonomic research regarding this and other species. We characterized *P. cristiceps* colour patterns and re-evaluated its geographic variation, and have also highlighted the potential consequences to the taxonomy of the genus. The results revealed six principle chromotypes, and although their frequencies varied between sex and ontogenetic classes, the phenotypic expression appeared to respect defined proportions and show selective value for the species. We conclude that individual variation jointly with typological traditionalism may overestimate the polymorphic magnitude at the population level and be the cause of taxonomic inflation. Our data support the usefulness of *P. cristiceps* as a model for microevolutionary studies.

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Abstract

Knowing the extent of variability is important in understanding how evolution operates in polymorphic species such as those of the genus *Proceratophrys* Miranda-Ribeiro, 1920. This genus is comprised of South American toads which are amply distributed across this continent. The subject of this study was *P. cristiceps*, whose distribution in Brazil is limited to the Caatinga biome. Our goal was to examine and describe its chromatic variation from a populational perspective. We looked for different phenetic polymorphism levels and its probable chromotypic association by applying statistical and GIS tools in a way which would facilitate future taxonomic research regarding this and other species. We characterized *P. cristiceps* colour patterns and re-evaluated its geographic variation, and have also highlighted the potential consequences to the taxonomy of the genus. The results revealed six principle chromotypes, and although their frequencies varied between sex and ontogenetic classes, the phenotypic expression appeared to respect defined proportions and show selective value for the species. We conclude that individual variation jointly with typological traditionalism may overestimate the polymorphic magnitude at the population level and be the cause of taxonomic inflation. Our data support the usefulness of *P. cristiceps* as a model for microevolutionary studies.

Keywords: Amphibia; Chromatism; Polymorphism; Populations; Variation.

INTRODUCTION

Morphological variation plays a fundamental role in the evolution of species. Although not all characteristics of organisms are heritable, at least those which are transmissible to new generations natural selection can, potentially, act without major problems (Ridley 2004). Understanding how evolutionary mechanisms operate on populations through morphological variability in individuals has been the main objective of the most different studies since the times of Darwin (Futuyma 1987; Huxley 1940).

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

The genus *Proceratophrys* Miranda-Ribeiro, 1920 belongs to a group of South American amphibians popularly known as small ox-toads, or horned minor frogs. They have wide distribution across Brazil and also occur in Argentina and Paraguay (Frost 2021; Napoli et al. 2011). It was traditionally a taxon which is difficult to classify, as the species that comprise this taxon were almost always confused with those of the genus *Ceratophrys* Wied-Neuwied, 1824 or even compared to them, often with the aim of congeneric positioning (Boulenger 1882; Braun 1973; Gravenhorst 1829; Günther 1873; Miranda-Ribeiro 1920; 1923; Müller 1884).

The genus *Proceratophrys* was originally described by Miranda-Ribeiro (1920) when indicating the presence of a “dilated post-typanic bone, spiculated eyelid and the absence of a keratoid appendix” as diagnostic characteristics. The species included in the description were *P. appendiculata* (Günter, 1873); *P. boiei* (Wied-Neuwied, 1824); *P. cristiceps* (Müller, 1883) and *P. renalis* (Miranda-Ribeiro, 1920). Miranda-Ribeiro highlighted a portion of morphological traits, with a few being morphometric and other 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). They are distributed in different biomes and morphoclimactic dominions, such as the Amazon, Caatinga, Cerrado, Chaco, Atlantic Forest and Pampas (Barrio & Barrio 1993; Giaretta et al. 2000; Izecksohn et al. 1998; Martins & Giaretta 2011). Although much has been discussed about *Proceratophrys cristiceps* in the last decade, not only the proposed taxonomy would raise doubts (Cruz et al. 2012), but the distribution suggested by Junior et al. (2012) and Mângia et al. (2020) show somewhat dubious and questionable records. This is mainly because some are syntopic with those of species in the *goyana* group, or because that they are located in an unusual biome for the species.

Similar to some other anurans, *P. cristiceps* displays chromatic and morphometric polymorphism (Vieira & Vieira 2012). At least two chromotypes were described in this species (Vieira et al. 2008), whose polymorphism may be come from environmental fluctuations and/or genetic events on populations (Dias & Gonçalves da Cruz 1993; Smith & Skúlason 1996). This information has gone unnoticed in recent studies, but if it is extended to other species, it may partly explain the taxonomic inflation (Aleixo 2009; Alroy 2003; Isaac et al. 2004; Padial & De la Riva 2006) observed in the genus in recent decades, and specially, if we consider the high number of species described in a short period of time when in the absence of more accurate taxonomic revisions (Junior et al. 2012).

Chromatic variability is common in anurans (Hoffman & Blouin 2000; Kakazu et al. 2010), as this facilitates the survival of species in areas where there are many predators (Bourke & Bakker 2011). In cases such as these, chromatic polymorphism provides these species with a varying amplitude which enables them to occupy, adapt and reproduce for generations in determined environments (Hoffman & Blouin 2000). Furthermore, the clear description of external characteristics, such as intra and interspecific colouration patterns, has the potential for reduction or even the solution for serious taxonomic problems (Grismer et al. 2002).

The adaptive importance of polymorphism for organisms, including *P. cristiceps*, lies in the improvement of the reproductive and survival capacities of individuals in response to stress caused by the environment or predators (Ridley 2004). Natural populations are constantly exposed to many variable conditions. Regardless of the degree of changes, a limiting factor for survival is seen in the ability for morphophysiological modification, whether intraspecific or

interspecific (Ricklefs 2008). In this case, the survival and adaptation of organisms depends on the maintenance of a number of individuals in these populations in such a way that the evolutionary mechanisms can act on them, and thus give rise to what we call biodiversity (Badii et al. 2007; O'Neill et al. 2012).

Knowing the importance of populational polymorphism in taxonomic and evolutionary research, we have based our study precisely on examining of the chromatic variation in our model organism *P. cristiceps*. Thus, we seek to determine the extent of chromatic variability in this species at both the ontogenetic and population levels by looking for explanatory patterns along defined geographic gradients in order to corroborate or to question some taxonomic proposals. In turn, producing information that facilitates identifying the species and its congeners, and thus favouring future work in ecology, biogeography and systematics of the genus, as well as for other species.

MATERIALS AND METHODS

Origin of the examined material

A total of 634 *Proceratophrys cristiceps* specimens from 37 localities were analysed (Appendix). All the individuals were obtained from the Animal Ecophysiology Laboratory (*UFPB*) and the Herpetological Collection in the Universidade Federal da Paraíba (*CHUFPB*). It was possible to locate excellent samples from different areas in northeastern Brazil in these collections, and their geographic information proved to be important in producing of habitat suitability maps and spatial similarity for the study species.

Sexual, ontogenetic identification and specimens maturity

The sex of the preserved animals was identified through an incision in the posterior ventrolateral portion with the aim of verifying the corresponding sexual structures: ovaries or testicles (Heyer 2005). The ontogenetic classification adopted herein for the metamorphosed

animals followed Izecksohn & Peixoto (1980; 1981) and Mercadal de Barrio & Barrio (1993). The individuals considered as juveniles were those with cloacal-rostrum lengths less than or equal to 25 mm; subadult lengths were from 26 to 35 mm, whereas adults had lengths greater than or equal to 36 mm. The compatibility of these classes with the maturity of individuals (animals potentially capable of competing for sexual partners) was tested through their correlation with gonad 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) and was performed using Observed minus Expected Frequencies Pearson's Chi-square.

The aforementioned classification enabled us to identify operational ontogenetic units (OOUs) consistent with each post-larval developmental phase suggested (Vieira & Vieira 2012), whose chromatic patterns were statistically functional with respect to the analysed frequencies. In the case of the studied local populations (*sensu* Mayr, 1977), the ontogenetic categorization used herein expanded our understanding of the variation in *P. cristiceps*, both at chromatic and morphometric levels.

Chromatic characterization

The chromatic characterization in both living and preserved *Proceratophrys cristiceps* specimens was performed through the standardisation suggested by the colour catalogue for field herpetologists (Köhler 2012). This was done with the aim of decreasing or avoiding ambiguity issues in relation to the terminology and description of the observed hues. The study of live animal colours was achieved through *in loco* observations. Preserved specimens were immersed in water with the intention of enhancing the contrasts of the spots, stripes and colouration in both natural and artificial light. This enabled improved pattern identification, as well as for the description and classification of possible chromotypes.

The colours and dorsal spot patterns in *P. cristiceps* was registered in digital photographs (DSC-H10 Sony, 8.1 Megapixels). They were all taken at the same distance (25 cm) with the camera lens in a horizontal position using flash and a white background to highlight the contrast. We considered the number and size of the dark spots on the dorsal surface of the specimen's

158 body (Rabbani et al. 2015). The dark spots were defined according to their contrast with the
159 dorsal surrounding colour (Fig. S1 B). The chromatic area of these spots was calculated in
160 ImageJ vol. 1.8.0 (Rasband 2018). The images (.tiff) were processed, then converted to 8-bit
161 (grey value), and quantified after. The measurement interval was 0.1-infinity, which enabled
162 calculating (in pixels/mm^2) even the smallest of the particles (by gradient), with the total body
163 area of the each specimen having been isolated (Fig. S1 C). The reference scale used herein was
164 20 mm.

165

166 ***Analysis of interpopulation chromatic and morphometric variation***
167

168 As a continuous or discrete property, morphological variation, can lead to mistakes when
169 certain categories and explanatory variables are disregarded in comparative tests. Thus, it is
170 necessary to first verify the magnitude of the likely variation in recognized variables and factors
171 in order to avoid fragile comparisons and mistaken conclusions as to their simultaneous effects
172 (Zar 2014).

173 For example, morphometric variation in animal research can be identified as either sexual
174 dimorphism or originated from ontogeny (allometry), often failing to be noticed when
175 comparable categories are separately (or simultaneously) tested in recognized populations. It was
176 with this in mind that we tried to identify different forms of variability in our samples, in order to
177 test them within and among the chromatic categories observed herein.

178 The morphometric (Fig. S2; Vieira and Vieira, 2012) and chromatic differences in
179 *Proceratophrys cristiceps* were tested using multiway ANOVA with unequal replications and the
180 Kruskal-Wallis test. The latter is indicated for samples with unknown distributions. The
181 comparison between frequency proportions was achieved through cross tabulation, which were
182 carried out using Pearson's Chi-square tests. This representation was found to be very
183 informative, enabling to examine the data in a simplified manner (line plots).

184

185 ***Population analysis***
186

187 The collection locality was accepted herein as a true population for strictly operational
188 reasons. This was done with the intention of producing sub-samples, presumably considered as
189 distinct populations (following the traditional definition that they need to be contiguous, but
190 situated in different territories), and separated by geographical gaps of relative lengths
191 (Dobzhansky 1970; Mayr 1977). As such, the premise was that the separation of samples by
192 location would generate exclusive and independent populational sets (not intercross).

193 Thus, we decided to find presumably intercrossing sets aiming to mitigate
194 methodological eventualities, or the "*demes*" so to speak (Gilmour & Gregor 1939; Winsor
195 2000). According to our terminological redefinition (with strictly operational application), *deme*
196 would be any cluster of local populations closely related by sharing at least one exclusive
197 characteristic (*phenon*), without necessarily supporting a possible taxonomic distinction at the
198 species level, but which confers a particular identity (since it is more frequent and statistically
199 significant).

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

212 The graphical representation of the distances between the *demes* had a multiple
213 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) by 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 x 86.58 km along the equator line). The geographical similarity was calculated to compose a map based on the coefficients of variation of eight phenotypic traits (Hijmans et al. 2012; Scheldeman & van Zonneveld 2010): chromatic (spots size; area occupied by spots) and morphometric (CRL; HW; HL; Thl; TL and ThL. See Fig. S2).

The principal components analysis was an option regarding population variation in our model species. With this we aimed to determine a factor that could simply explain the probable variability found based on the possible linear combination of our variables.

We checked the normality of the residuals (probability-probability plot) and the symmetry of the multivariate population distributions prior to the analyses (Figs. S3 and S4). 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 (Foundation 2018) and Past v.3.1.5 (Hammer 2016) software programmes.

In addition to the metric data and with the purpose of interpreting the probable variations between the *demes*, we collected information from some explanatory variables such as vegetation cover; climate in accordance with the Köppen-Geiger classification (Peel et al. 2016); altitude; rainfall and temperature (min and max) of all the locations where the specimens were collected. This information was obtained from the National Meteorological Institute (INMET 2020) and from freemeteo (2019). Both provide regular climatological data (monthly and annual means) which comprise a historical series from 1960 until the present day, with a minimum radius of 2 km distance for each coordinate on UTM (Universal Transverse Mercator).

Environmental niche modelling

The potential distribution maps were generated with the intention of interpreting the distribution of *P. cristiceps* according to determined and defined predictor variables. We used two software programmes with the goal of mitigating the possible effects caused by $P_M(g)$ and $P_B(g)$ in the BAM diagram (biotic, abiotic, and movements) of probabilities (errors of omission and commission) for species with restricted vagility (Soberón 2007 ; Soberón 2009): the DIVA-GIS (Hijmans et al. 2005) and the MaxEnt (Philips et al. 2017; Phillips et al. 2006). We then estimated the proportional quantity of probable presence based on the real records of the sample through MaxEnt (Soberón 2009), whereas we balanced 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 to dispersing individuals) by including them in a relatively larger prediction compared to Maxent (Qiao et al. 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 2020). All the maps presented herein are in the resolution from 30 arc seconds (~ $1km^2$) in GCS WGS 1984 projections.

Checking the taxonomic functionality of phenetic characteristics

We analysed the ambiguity and frequency of the diagnostic characteristics commonly used at the taxonomic level inside the genus *Proceratophrys*. We tested the functionality of information from the authors (see below) by comparing with each other and with the phenotypic traits of our sample *P. cristiceps* individuals. Our objective was to verify if identical diagnostic features could be found among different species (refutability principle). Then we built 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 (we considered the description of the head form herein); 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 which could explain 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 et al. (2000); Gonçalves da Cruz et al. (2005); Ávila et al. (2011); Napoli et al. (2011); Martins & Giaretta (2011); Cruz et al. (2012); Junior et al. (2012); Ávila et al. (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 of the controversial *cristiceps* group (Dias et al. 2014; Giaretta et al. 2000).

RESULTS

Chromatic analysis

Our observations indicated the existence of at least six main chromatic variations in the *Proceratophrys cristiceps* (Fig. 1), which were:

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, impedes recognizing a characteristic dorsal geometric figure – “arrowhead” (Miranda-Ribeiro 1937). Conspicuous suborbital bands. Animals moderately melanised and with two or more 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 the brown colouration and suborbital or interorbital bands (two, with one of them being in the shape of a "Y"), however, there was a well-defined dorsal geometric figure which was laterally limited by dark bands (maroon – C38) in the orbit-cloaca direction. There were also lighter nuances on the flanks (salmon – C57 to C59) and on the limbs, stomach and snout (cyan white – C155). Usually occur in leaf litter (97.02%) or gravel (2.98%);

Chromotype 3 (n=39, 6%): very clear brown-grey colouration and slightly variegated (C256 to C259). Evident dorsal figure and yellow-brown colouration (C84), distributed in the orbit-cloaca direction; limited by two bands (in opposite toothed arches) and lines of semi-parallel glandular nodules. Single interocular stripe and two well-defined suborbitals. May present 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%): there is evident trichromatic colouration, whose rusty red hue (C35 and C253) covers a large part of the body. Clear dorsal 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 and gravel (93.75%);

Chromotype 5 (n=51, 8%): general colouration monochromatic in comparison 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 colouration is brown-grey (C19) and in diverse nuances, with evident yellow-brown spots (or a lighter hues C12 and C111) distributed in characteristic areas: snout and suprascapula. The dorsal figure is laterally outlined by spots in a toothed arch shape, but unclear. Generally inhabiting earthy or sandy soils (81.26%) and even in leaf litter (18.74%).

Figure 1. **Chromatic variation in *Proceratophrys cristiceps* individuals. The diversity found is characterised according to the general colour pattern, saturation and distribution of dorsal spots.**

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. 2). 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).

Figure 2. Chromotypes of *Proceratophrys cristiceps* with a distribution of their frequencies varying in terms of sex, maturity and ontogenetic development.

The frequencies of Chrom5 were found to be higher in juveniles compared to sub-adults and adults when analysing these same samples for ontogenetic class. We also verified the ontogenetic class frequencies for each sex, which demonstrated a pattern with little difference to that observed for the species as a whole. Unlike females, the chromotypic variation in the males was significant between Chrom3, Chrom4 and Chrom5, therefore moving away from the general species' pattern (Fig. 3).

Figure 3. Chromotypes of *Proceratophrys cristiceps* with the distribution of their frequencies varying between sexes according to maturity and ontogenetic development (post-larval). The significant differences for the males suggest a curious and discreet effect of the factors acting on the sex variable.

The chromotypes also showed different frequencies in terms of maturity, with slightly lower frequencies in Chrom4 and higher in Chrom5, mainly varying between mature individuals (Fig. 1). The variation revealed a smaller number of Chrom4 individuals compared to Chrom3 and Chrom5 adult individuals. This was maintained for both males and females when analysing the samples separately.

Another peculiarity of the studied specimens was the integumentary saturation (proportional quantity of dark in relation to light background). The Chrom5 individuals found herein were less saturated than the others (Fig. 4), with a lower average size of dorsal patches,

and the area occupied by these same patches (as well as their distribution) was reduced. Such variation, which characterised the form and extension of the dorsal designs, was significant and independent of sex, ontogenetic class and maturity, either in the species as a whole or internally in the samples (Figs. S5 to S8).

Figure 4. Saturation of chromotypes of *Proceratophrys cristiceps*. The dorsal design 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.

Although the distribution of dorsal spots did not vary significantly between males and females, the average size (in mm^2) of the referred spots was greater in females, which were also more saturated than males (Figs. S5 to S8). The results also indicated that the Chrom6 juvenile female (but not Chrom6 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 DRN), but this variation was absent in juveniles and even in sub-adults (Figs. S9 to S12). Males and females did not differ morphometrically in the permutations performed in terms of chromotypes. However, only adult males (Chrom6 and Chrom3) or mature males (Chrom3, Chrom1 and Chrom2) differed from each other when internally analysing the samples, with differences found in the cephalic region and 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. S13). The others were almost uniformly distributed in the cartesian space, without any variable (active or supplementary, morphometric or chromatic) supporting the composition of the *demes*, and these were not easily explained by the environmental predictors. However, geographically supported and consistent groups were produced 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 where phenons were shared (Fig. 5), constituting a strong indicator of the occurrence of genetic flow between populations.

Figure 5. 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.

The phenetic trait diversity mapping indicated the existence of at least five *demes* in the *P. cristiceps* species (Fig. 6 B), being exclusively distributed in the Caatinga and transition phyto physiognomies according to their area of habitat suitability. The referred species is most likely found in predominantly arboreal-shrubby vegetation, under direct influence of precipitation and annual minimum temperatures (Figs. 6 and S14).

Figure 6. 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).

DISCUSSION

The probable meaning of variation in P. cristiceps

Species are a multidimensional phenomenon (Wheeler & Meier 2002; Zachos 2016), therefore studying variation in organisms provides essential information in the field of experimental taxonomy (Sneath & Sokal 1973; Sokal & Rohlf 1995), and consequently to the fields of systematics, biogeography and ecology. As such, taxonomic characteristics (defining or diagnostic) must be thoroughly discriminated and understood, mainly with respect to probable intraspecific varieties.

When dealing with these probable varieties, it is necessary to identify categories which can be in themselves equivalent in an experimentally comparable way. Thus, it is not difficult to perceive that the variation is expressed by altering phenotypes, and within its own limits determines the different morphological forms that we usually identify when dealing with individual and population variations (Nicoglou 2015). With this in mind, the results in our particular case indicated two clear variation levels in *P. cristiceps*: morphometric and chromatic, and both with apparent adaptive value.

The variations observed in *Proceratophrys cristiceps* at the chromatic level indicated selective value in the studied species. This fact is reflected in the differential frequencies of the chromotypes that seem to signal some advantage of individual survival. The distribution of animals in the soil found herein suggests having a predominant role in the bias of chromotypic frequencies throughout post-larval development, i.e. it indicates a frequency-dependent selection (Bond 2007). In this case, the number of less saturated animals decrease as maturity or adulthood is reached, suggesting that certain phenotypes may be reinforced by local edaphic conditions (Figs. 7 and S15), i.e. crypsis may play a importante role (Bonte & Maelfait 2004; Endler 1981; Moreno-Rueda 2020; Rabbani et al. 2015). The contrasts of colours and spots comprise disruptive patterns which should function as a highly effective strategy against predators when combined with general colouration and saturation (Cuthill et al. 2005). Together, the two mechanisms (disruptive colour and crypsis) can thereby partly explain the observed variation in the frequencies, especially in juveniles, but they cannot explain the relative sample proportionality.

Figure 7. Juveniles of *Proceratophrys cristiceps* observed in the Pedra de Boca State Park. (A) Chrom3; (B) Chrom6; (C) Chrom4 and (D) Chrom5. The contrast in colouration in relation to the soil suggests reinforced adaptability in individual survival ability (crypsis and disruptive colouration). Photo credit: Kleber S. Vieira.

As the chromatic expressivity (observed percentage that a given phenotype presents itself) found in *Proceratophrys cristiceps* was not exclusive to specific samples, but was maintained even within and between categories (Table S1), the phenotypic divergence by local effects (polyphenism) can be easily discarded as an alternative explanation for the identified patterns. Therefore, we deduce that it may be a chromatic polymorphism with a strong genetic basis (White & Kemp 2016), due to the differential abundance and almost invariability of the

chromotypes, whose poly or dichromatism has already been identified (Mângia et al. 2020; Nunes et al. 2015; Vieira et al. 2008).

However, another important factor in relation to the biogeographic aspect of our results was the existence of *demes* (understood herein as conglomerate populations) which were morphometrically smaller on average in the north-western portions (hotter and drier) of the Caatinga. The most likely explanation may involve temperature associated effects in determining this phenomenon (Fig. 8). This appears to be plausible when we draw our attention to the determinants of potential distributions (Fig. S14), where the mean annual minimum temperature and the precipitation of the last quarter of the year significantly contributed to the model of habitat suitability.

Figure 8. Morphometric gradient (cline and isophenes) observed in the distribution of the analysed populations of *Proceratophrys cristiceps*. 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 the temperatures are higher. Maps of South America showing the average annual temperature cover (A) and maximum temperature of the hottest month (B) for the years 1970-2000. The outlined space corresponds to the area occupied by the Caatinga biome. Climate data source: WorldClim (2020).

This cline effect appears to point to Bergmann's rule (Bergmann 1848; Blackburn et al. 1999; Salewski & Watt 2017). However, because they are anuran amphibians, a phenotypic plasticity controlled by genes may be involved (Ashton 2002; Berven 1982a; Berven 1982b), which would therefore result in adaptation strategies to avoid thermoregulatory imbalance and the hydric stress – 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, this morphometric variation observed in *Proceratophrys cristiceps* is partly a consequence of sexual dimorphism and of ontogenetic effects (Vieira & Vieira 2012), as well as probable random events involving skull conformation to food resources (Atencia et al. 2020; Brito et al. 2012; Emerson 1985). However, the metric variation is negligible when comparing the chromotypes to each other, either between sexes or developmental categories (maturity and ontogenesis as described herein). Thus, although the chromotypic variation is evident and quite informative in *P. cristiceps*, it can sometimes prove to be deceptive and lead to serious taxonomic problems if misinterpreted and examined in isolation. Thus, the evolutionary implication of variation (if chromatic or morphometric) is difficult to

484 approach experimentally and often taxonomic practices look at operational morphological units
485 (OMUs) as different species or even sub-species. Furthermore, there are underlying factual (and
486 experimental) requirements to explain the morphologic divergence and the alleged taxonomic
487 diversity (Van Holstein & Foley 2020).

488

489 ***Taxonomic implications of variation in P. cristiceps***

490

491 We have seen a large increase in the number of descriptions of species of the genus
492 *Proceratophrys* in the past 20 years. The taxonomic inflation rate between the years 2011 and
493 2021 was 44% (Fig. 9 A), While only those of the *cristiceps* group (with reduced eyelid
494 appendages), which inhabit open and dry environments of Cerrado and Caatinga (Dias et al.
495 2014) reached 12% (Fig. 8 B). Although the taxonomy of *Proceratophrys cristiceps* (and other
496 species of the genus) has been debated for decades (Barrio & Barrio 1993; Cruz et al. 2012;
497 Lynch 1971; Mângia et al. 2020), it is difficult to say if this increase in group diversity really
498 reflects the true species diversity, or if it is just a typification of intraspecific variability already
499 observed (Junior et al. 2012).

500

501 **Figure 9. Number of species described among three diverse genera of anuran amphibians (A) and among**
502 **those of *Proceratophrys* (B). The lines represent the least squares regressions, while the numbers over the dots**
503 **represent the periodic rate (%) of descriptions (A). We found that *Leptodactylus* and *Rhinella* genera**
504 **increased at similar rates over the decades, being later surpassed by *Proceratophrys* due to faster rates of**
505 **annual descriptions in the latter (A). When compared between congeneric groups (B), the highest description**
506 **rates are observed in the *cristiceps* group. The *bigibbosa* group is reasonably stable, but the rate in the *boiei***
507 **group declines in relation to the total. Data obtained from Frost, D. R. (2021). Amphibian Species of the**
508 **World: an Online Reference. Version 6.1.**

509 When we revisit the original descriptions, we can see that not only the coloration, but
510 especially the size and appearance of nodules and tubercles are among the most common
511 diagnostic (or defining) characteristics pointed out for all species in the *cristiceps* group (and
512 also in the others) (Fig. 10). This suggests that the species were defined based on traits with
513 significant phenotypic plasticity.

514 **Figure 10. Grouping of authors formed the identifying regions with high density of common values (Two-**
515 **Way Joining). The highlighted blocks in warm colours reflect the greater set 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. a – Gravenhorst (1829); b – Miranda-Ribeiro (1937); c – Lynch (1971); d – Jim & Caramaschi (1980); e – Eterovick & Sazima (1998); f – Ávila et al (2011); g – Napoli et al (2011); h – Günter (1873); i – Müller (1884); j – Cruz et al (2012); k – Mângia et al (2020); l – Braun (1973); m – Izeckshohn & Peixoto (1981); n – Mângia et al (2018); o – Barrio & Barrio (1993); p – Caramaschi (1996); q – Giaretta et al (2000); r – Junior et al (2012); s – Brandão et al (2013); t – Martins & Giaretta (2013); u – Cruz et al (2005); v – Godinho et al (2013); w – Martins & Giaretta (2011); x – Ávila et al (2012).

For example, our observations indicated that the nodules (including warts and tubercles) were extremely variable in terms of number, shape and distribution, either isolated or regionally, in the same individual or between specimens. Some animals present large and round nodules; distributed regularly or irregularly; with glandular appearances and standing out; or smaller and more conical, or even flat. This serves little purpose as a defining or diagnostic characteristic for chromotypes. In addition to the nodules, the shape of the snout was equally variable when viewed laterally or dorsally, and was equally variable, not only due to allometric factors (Vieira & Vieira 2012), but also in terms of the position of the specimens in the vision plane. The difficulty in using this information is also highlighted by other taxonomists (Brandão et al. 2013).

Another common characteristic in the description of these species are the rows of opposite oculum-dorsal nodules and their associated spots and stripes. It seems that such rows of dorsal glandular nodules are important in forming the arrowhead shape (Miranda-Ribeiro 1937). This shape dissolves when discontinued, resulting in a pattern of irregular spots and bands (very variable between individuals) which interconnect at various points, especially in the middle dorsal portion (Chrom1). The nodules in these discontinuities can spread in the suprascapular direction and hang to the flanks, 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 victimised by a singular typological traditionalism (see Fig. 10). This seems to interfere in the researchers' perception, making them choose those characteristics which are more traditionally used, but which less explain the proposed species, ignoring the evident plasticity of these same morphological traits or their ambiguity between the alleged taxa. The consequence of acting in such a way, i.e. disregarding probable variation, is

549 unfortunately that the description of species may not be sustainable in reality (Dobzhansky 1970;
550 Mayr 1996).

551 By reviewing the descriptions of the species of genus *Proceratophrys*, and then
552 comparing the information of the authors with each other and with the characteristics of the
553 individuals in our samples and testing the probable ambiguity of the proposed diagnostic traits,
554 we can verify that some species described in recent decades would not actually be that
555 morphologically different from *P. cristiceps* or *P. goyana*, or even among themselves – see *P.*
556 *carranca* (Godinho et al. 2013), *P. branti* (Brandão et al. 2013), *P. huntingtoni* (Ávila et al.
557 2012) and *P. dibernardoi* (Brandão et al. 2013). Furthermore, the same chromatic varieties
558 observed in *P. cristiceps* may be equally recognisable in their congeners (Ávila et al. 2011;
559 Brandão et al. 2013; Junior et al. 2012; Martins & Giaretta 2013). This leads us to the conclusion
560 that these patterns are, to a greater or lesser extent, common to the genus.

561 Species considered to be distinct in the last decades often presuppose the hypothesis of
562 sympatric speciation in the absence of an evident vicariant element (Godinho et al. 2013; Mângia
563 et al. 2018; Martins & Giaretta 2013). This is the case with taxa (cryptic) which share
564 similarities, and whose distinctions, mostly performed through colour, warts or tubercles, or even
565 sometimes by acoustic (not immune to variability as supposed) and genetic analysis, are
566 conceptually confused and ambiguous. Furthermore, they are not even tested under an
567 experimental model of diversification dynamics (Ajmal Ali et al. 2014; Annibale et al. 2020;
568 Schindel & Miller 2005; Van Holstein & Foley 2020). This is mainly the case for the species of
569 the *P. goyana* and *P. cristiceps* sets (Martins & Giaretta 2011); and why not for the *P. biggibosa*,
570 *P. boei* and *P. appendiculata* groups whose taxonomic history involves controversies and the use
571 of equally variable phenetic traits? Where evidence of pre- or post-zygotic barriers or their
572 biogeography continue to be elusive?

573 In light of these facts, we suggest that future studies with traditionally used characteristics
574 be preliminarily recognized as diagnostic characteristics based on significant sampling and
575 statistically tested. Furthermore, we cannot discount that the hypothesis of taxonomic inflation in
576 the genus *Proceratophrys*, especially for those of the *cristiceps* group, has been due to poorly
577 interpreted population peculiarities emerging from microevolutionary processes (Amaro et al.

2012; Mângia et al. 2020) instead of a taxonomic quality, evoked due to the simple and unfortunate confusion of methods and concepts.

Finally, we conclude that individual variation jointly with typological traditionalism may overestimate the polymorphic magnitude 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.

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861

List of Supporting Information

Figure S1. (A) *Proceratophrys cristiceps* (WLSV1463) individual immersed in water with enhanced contrast of spots and stripes. (B) Chromatic pattern dorsal (characteristic) in 8-bit. (C) Total area of particles (red colour) calculated along the dorsal surface of the specimen. Set of measurements: area; minimum and maximum grey value and mean grey value. Bar: 56 mm. Photo credit: Kleber Vieira.

Figure S2. Measurements taken for the *Proceratophrys cristiceps* specimens (digital caliper/0.01 mm precision): Cloacal Rostrum Length (CRL); Eye Diameter (ED); Foot Length (FoL); Forearm Length (FL); Hand Length (HaL); Head Length (HL); Head Width (HW); Internal Metatarsal Callus Length (IMCL); Internarinal Distance (ID); Interocular Distance (InD); Nostril Eye Distance (NED); Thigh Length (ThL); Tibial Length (TL) and Rostrum Nostril Distance (RND). More details in Vieira and Vieira (2012) and Watters et al. (2016). Image credit: Kleber Vieira.

Figure S3. Normality of the residues and relative morphometric symmetry in the multivariate population distributions (probability-probability plot).

Figure S4. Normality of residues and relative morphometric symmetry in the multivariate population distributions (probability-probability plot).

Figure S5. Average size of the dorsal spots in female *Proceratophrys cristiceps* in terms of maturity and ontogenetic class (post-larval). Chrom5 individuals are significantly different (α=0.05) from the other chromotypes, demonstrating smaller spots. Curiously, females generally demonstrated a greater average spot size compared to males.

Figure S6. Area occupied by dorsal spots in female *Proceratophrys cristiceps* in terms of maturity and ontogenetic class (post-larval). Chrom5 individuals are significantly different (α=0.05) from the other chromotypes, demonstrating smaller spots and located further apart from one another.

Figure S7. Average size of dorsal spots in *Proceratophrys cristiceps* males in terms of maturity and ontogenetic class (post-larval). Chrom 5 individuals are significantly different (α=0.05) from the other chromotypes, demonstrating smaller spots. Some values not observed.

Figure S8. Area occupied by dorsal spots in *Proceratophrys cristiceps* males in terms of maturity and ontogenetic class (post-larval). Chrom5 individuals are significantly different (α=0.05) from the other chromotypes, demonstrating smaller spots and located further apart from one another. Males exhibit a smaller average distribution area compared to females. Some values not observed.

Figure S9. The multifactorial permutations of variance did not show significant morphometric differences (α=0.05) for the chromotypes of *Proceratophrys cristiceps*, indicating that males and females are equivalent when comparing them for ontogenetic class (post-larval). Wilks' lambda = 0.81; F(117, 4400, 6) = 1.05; p = 0.34. Vertical bars demote 0.95 confidence intervals (weighted marginal means, some means not observed).

Figure S10. The multifactorial permutations of variance did not show significant morphometric differences (α=0.05) in the chromotypes of *Proceratophrys cristiceps*, indicating that males and females

were equivalent when comparing ontogenetic class (post-larval). Wilks' lambda = 0.81; F(117, 4400, 6) = 1.05; p = 0.34. Vertical bars denote 0.95 confidence intervals (weighted marginal means, some means not observed).

Figure S11. The multifactorial permutations of variance did not show significant morphometric differences (α=0.05) in the chromotypes of *Proceratophrys cristiceps*, indicating that the males and females were equivalent when comparing maturity (Immature and Mature). Wilks' lambda = 0.80; F(52, 1218, 2) = 1.33; p = 0.063. Vertical bars denote 0.95 confidence intervals (weighted marginal means, some means not observed).

Figure S12. The multifactorial permutations of variance did not show significant morphometric differences (α=0.05) in the chromotypes of *Proceratophrys cristiceps*, indicating that the males and females were equivalent when comparing maturity (Immature and Mature). Wilks' lambda = 0.80; F(52, 1218, 2) = 1.33; p = 0.063. Vertical bars denote 0.95 confidence intervals (weighted marginal means, some means not observed).

Figure S13. Chromotypes (A) and *demes* (B) represented against the first two principal components scaled for morphometric and chromatic variables. PC1 is correlated to size dimensions, whereas PC2 is correlated with saturation. It is possible to verify that Chrom5 and Dem5 are more concentrated and distributed along the superior portion of the second component, suggesting the presence of low saturated specimens. The environmental predictors did not explain the chromatic variance observed, indicating the existence of underlying operating factors.

Figure S14. AUC curves and test Jackknife of the environmental variables of the climate model (default parameters) of *Proceratophrys cristiceps*. The data indicated that the species is typical of the Caatinga, being found in greater probability in the zones of tropical savanna and semi-arid climate of this biome according to the Köppen-Geiger classification.

Figure S15. *Proceratophrys cristiceps* adults observed in the Reserva Particular do Patrimônio Nacional Fazenda Almas. (A) Chrom1 and (B) Chrom2. The contrast of the animals' coloring in relation to the soil suggests adaptive reinforcement in the individual survival capacity (crypsis). Photo credit: Washington L. S. Vieira.

Table S1. Proportion of chromotypic expression in *Proceratophrys cristiceps*. Relative frequency little variable between the analyzed sample categories: ≈14:43:6:9:8:20. Significant variations not observed (α=0.05).

930 **Appendix**
931

932 Specimens Examined

933 BAHIA| Paulo Afonso (-9.401130556°S.; -38.20623333°W): UFPB12112, UFPB12114, UFPB12115,
934 UFPB12116, UFPB12118, UFPB12124. Santa Terezinha (-12.771725°S; -39.52416389°W): CHUFPB24169.
935 CEARÁ| Crato (-7.229958333°S; -39.41229722°W): CHUFPB19690, CHUFPB20690. Ipu (-4.321944444°S; -
936 40.71083333°W): UFPB6127. Jaguaribe (-5.901030556°S; -38.62215°W): CHUFPB19946, CHUFPB20656,
937 CHUFPB20657, CHUFPB20675, CHUFPB20940, CHUFPB21058, CHUFPB22183, CHUFPB22188,
938 CHUFPB22195, CHUFPB22233. Junco (-4.814325°S; -38.98613889°W): UFPB10033, UFPB10034,
939 UFPB10035, UFPB10036, UFPB10037. Quixadá (-4.972555556°S; -39.01541389°W): CHUFPB19935,
940 CHUFPB22177, CHUFPB22191. Santa Quitéria (-4.324272222°S; -40.14281111°W): UFPB10752,
941 UFPB10759, UFPB10760. Ubajara (-8.616025°S; -37.16555833°W): CHUFPB19726, CHUFPB19729,
942 CHUFPB19886, CHUFPB19925, CHUFPB19969, CHUFPB20654, CHUFPB20662, CHUFPB20671,
943 CHUFPB20680, CHUFPB20681, CHUFPB20683, CHUFPB20792, CHUFPB20818, CHUFPB20820,
944 CHUFPB20821, CHUFPB20822, CHUFPB20827, CHUFPB20830, CHUFPB20854, CHUFPB20876,
945 CHUFPB20894, CHUFPB20896, CHUFPB20921, CHUFPB20930, CHUFPB20933, CHUFPB20938,
946 CHUFPB20939, CHUFPB20943, CHUFPB20946, CHUFPB21056, CHUFPB21347, CHUFPB21349,
947 CHUFPB21351, CHUFPB21355, CHUFPB22178, CHUFPB22179, CHUFPB22187, CHUFPB22190,
948 CHUFPB22194, CHUFPB22201, CHUFPB22205, CHUFPB22217, CHUFPB22222, CHUFPB22225. PARAÍBA|
949 Boa Vista (-7.260538889°S; -36.24889444°W): UFPB1571, UFPB1572, UFPB1573, UFPB1574, UFPB1575,
950 UFPB1576, UFPB1577, UFPB1579, UFPB1580, UFPB1581. Cabaceiras (-7.469663889°S; -
951 36.30575833°W): UFPB11266, UFPB11267, UFPB11268, UFPB11269, UFPB11270, UFPB11271,
952 UFPB11272, UFPB11273, UFPB11274, UFPB11275, UFPB11276, UFPB6691, UFPB6692, UFPB6693,
953 UFPB6694. Desterro (-6.875280556°S; -37.53213333°W): UFPB1582, UFPB1583, UFPB1584, UFPB1585,
954 UFPB1586. Fazenda Almas (-7.470833333°S; -36.88083333°W): FA01, FA44, FA45, FA46, FA149, FA154,
955 FA158, FA159, UFPB4267, UFPB4270, WLSV1308, WLSV1346, WLSV1349, WLSV1463, WLSV1470,
956 WLSV1472, WLSV1474, WLSV1475, WLSV1476, WLSV1477, WLSV1485, WLSV1487, WLSV1488,
957 WLSV1497, WLSV1505, WLSV1566, WLSV1567, WLSV1572, WLSV2021, WLSV2026, WLSV2042,
958 WLSV2131, WLSV2170, WLSV2252, WLSV2259, WLSV2260, WLSV2339, WLSV2340, WLSV2341,
959 WLSV2388, WLSV2391, WLSV2935, WLSV3007, WLSV3016, WLSV3017A, WLSV3018, WLSV3019,
960 WLSV3031, WLSV3032, WLSV3303, WLSV3304, WLSV3305, WLSV3318, WLSV3319, WLSV3320,
961 WLSV3321, WLSV3990, WLSV4057, WLSV4063, WLSV4091, WLSV4093, WLSV4095, WLSV4207,
962 WLSV4208, WLSV4209, WLSV4237, WLSV4335, WLSV4365, WLSV4375, WLSV4388, WLSV4397,
963 WLSV4398, WLSV4399, WLSV4411, WLSV4492, WLSV4493, WLSV4494, WLSV4515, WLSV4529,
964 WLSV4530, WLSV4533, WLSV4604, WLSV4646, WLSV4647, WLSV4765, WLSV4766, WLSV4767,
965 WLSV4768, WLSV4769, WLSV4770, WLSV4771, WLSV4772, WLSV4773, WLSV4774, WLSV4775,
966 WLSV4776, WLSV4777, WLSV4778, WLSV4779, WLSV4780, WLSV4789, WLSV4791, WLSV813, WLSV814,
967 Y039. Patos (-6.986025°S; -37.31695278°W): KSV041, KSV053, KSV055, KSV079, KSV113, KSV196,
968 KSV232, KSV233, KSV237, KSV246, KSV247, KSV248, KSV251, KSV266, KSV278, KSV313, KSV319, KSV320,
969 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.

Figure 1

Chromatic variation in *Proceratophrys cristiceps* individuals.

The diversity found is characterised according to the general colour pattern, saturation and distribution of dorsal spots.

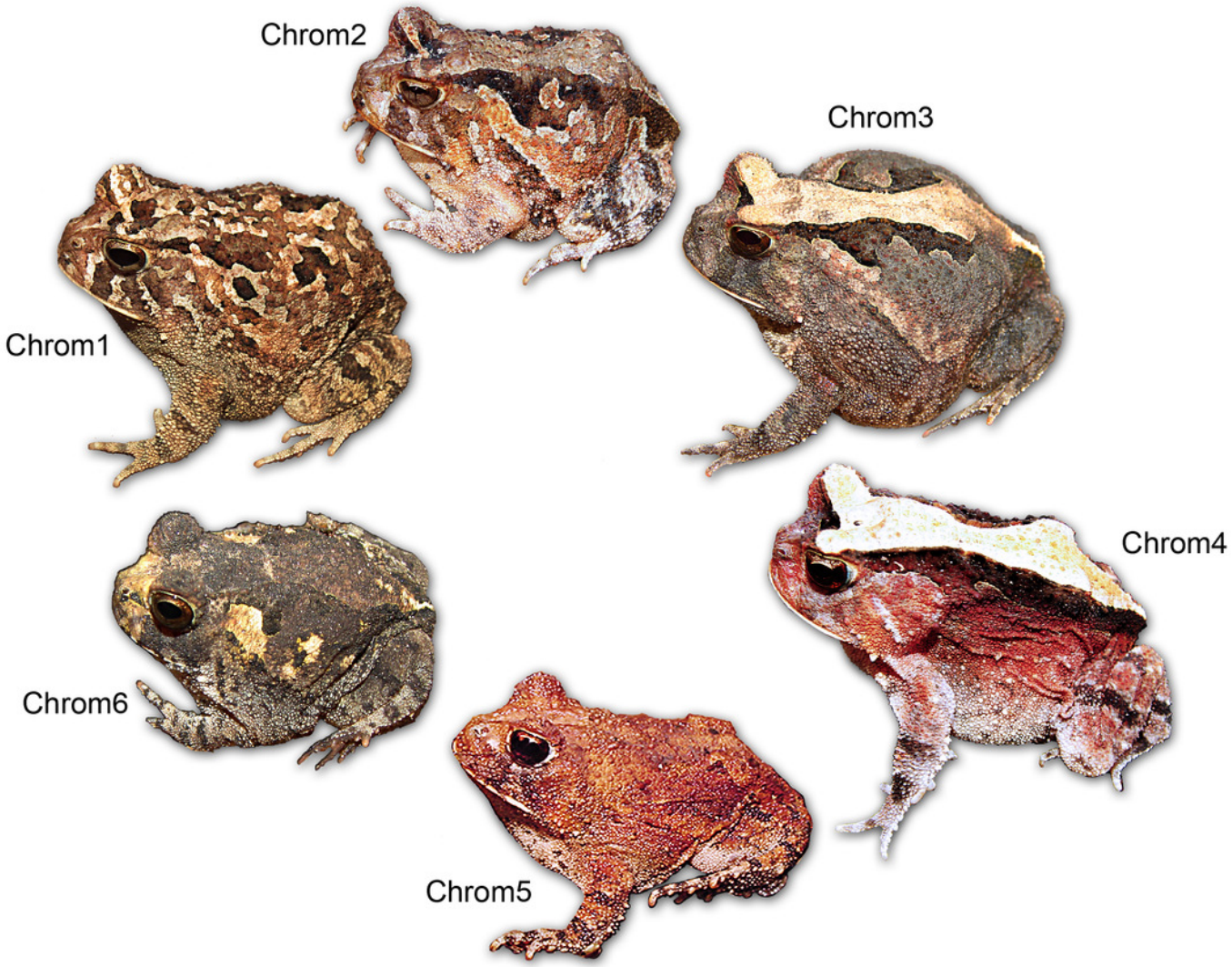


Figure 2

Chromotypes of *Proceratophrys cristiceps* with a distribution of their frequencies varying in terms of sex, maturity and ontogenetic development.

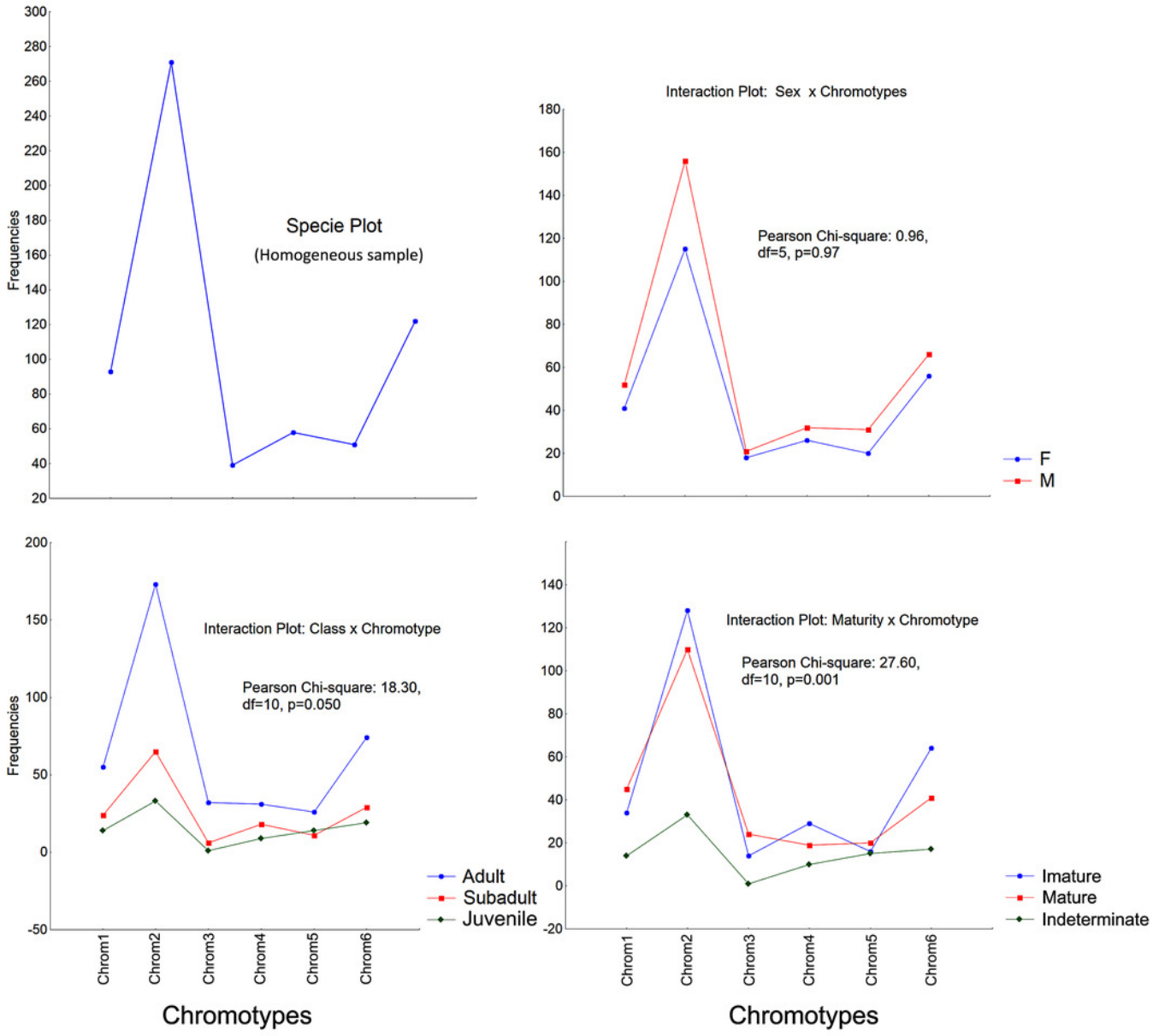


Figure 3

Chromotypes of *Proceratophrys cristiceps* with the distribution of their frequencies varying between sexes according to maturity and ontogenetic development (post-larval).

The significant differences for the males suggest a curious and discreet effect of the factors acting on the sex variable.

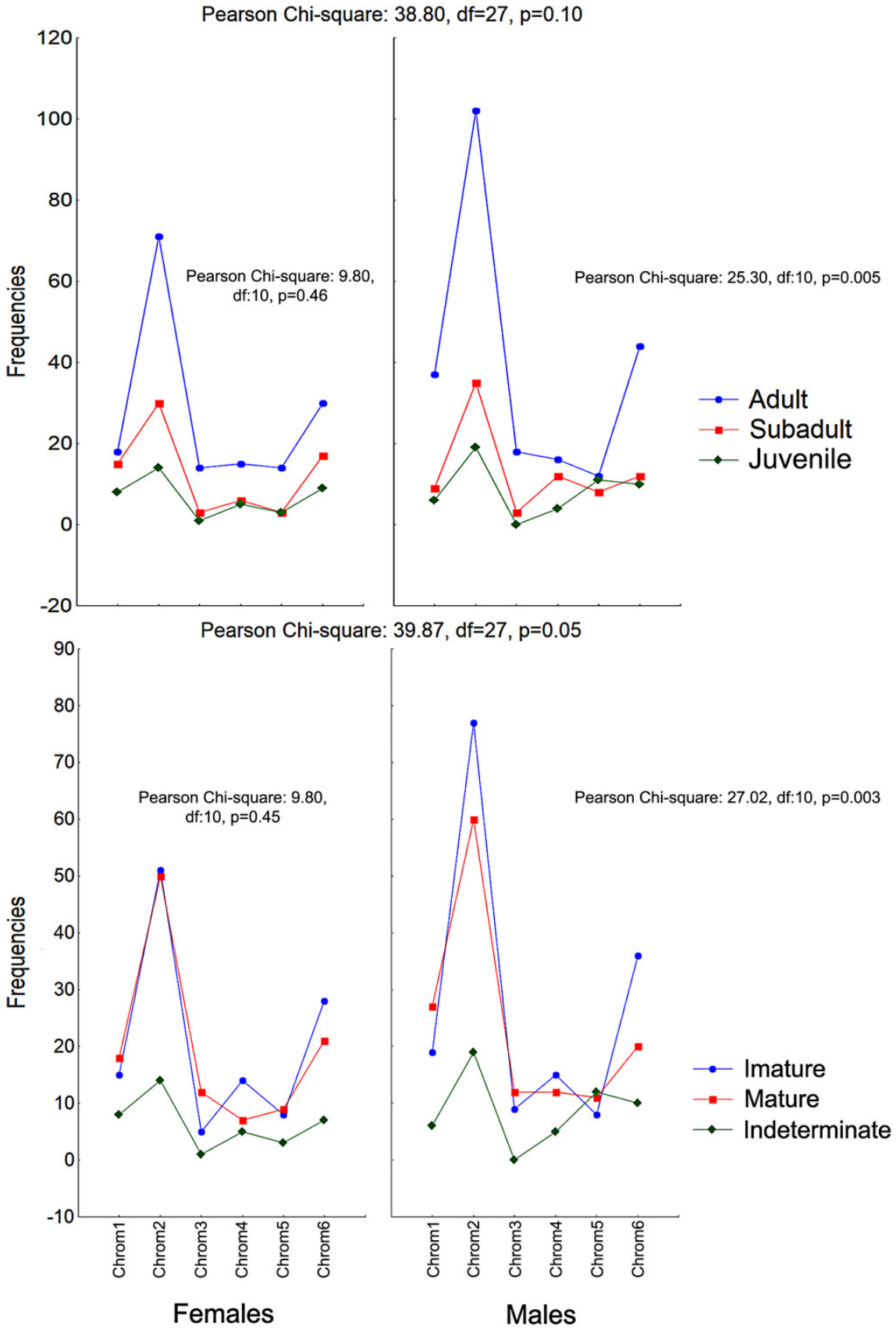

Pearson Chi-square: 38.80, df=27, p=0.10
Pearson Chi-square: 9.80, df:10, p=0.46
Pearson Chi-square: 25.30, df:10, p=0.005
Adult
Subadult
Juvenile
Frequencies
Pearson Chi-square: 39.87, df=27, p=0.05
Pearson Chi-square: 9.80, df:10, p=0.45
Pearson Chi-square: 27.02, df:10, p=0.003
Imature
Mature
Indeterminate
Chrom1
Chrom2
Chrom3
Chrom4
Chrom5
Chrom6
Females
Males

Figure 4

Saturation of chromotypes of *Proceratophrys cristiceps*.

The dorsal design 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.

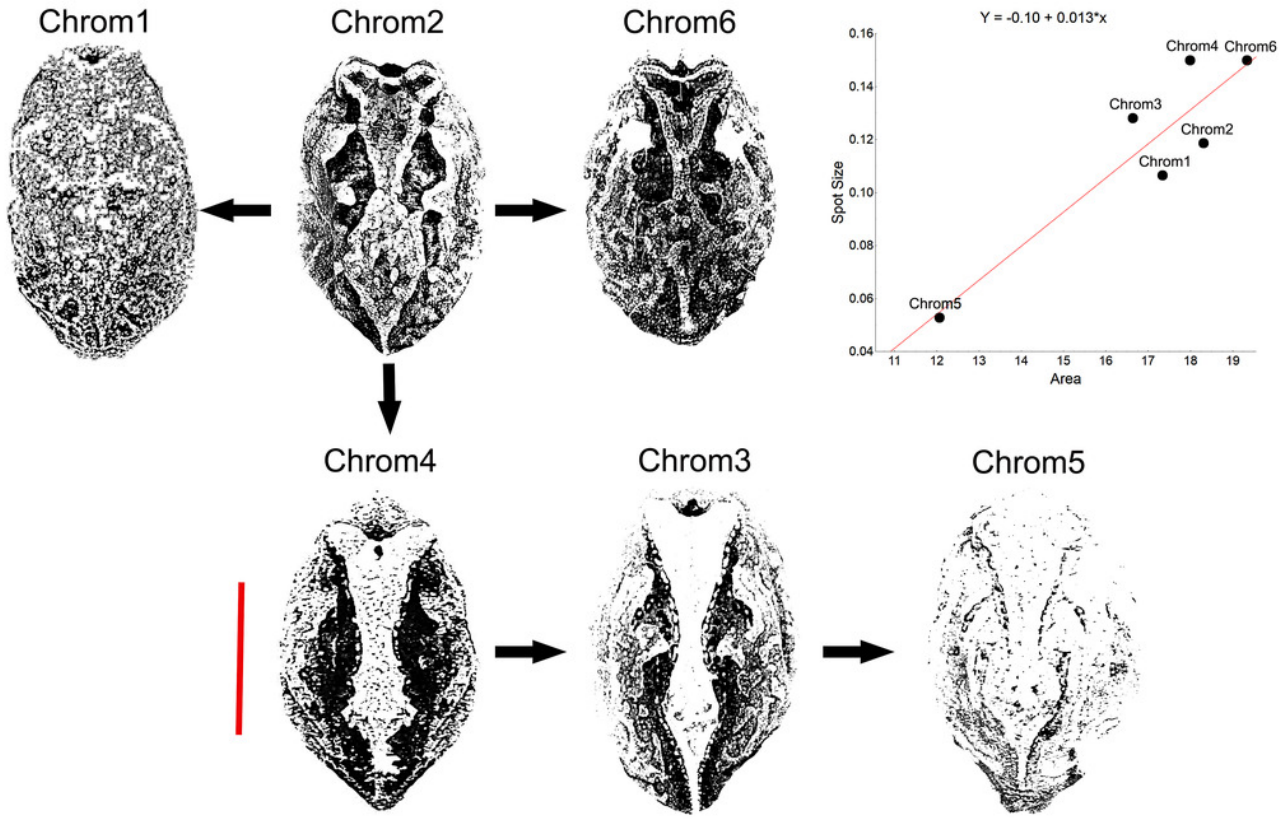


Figure 5

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.

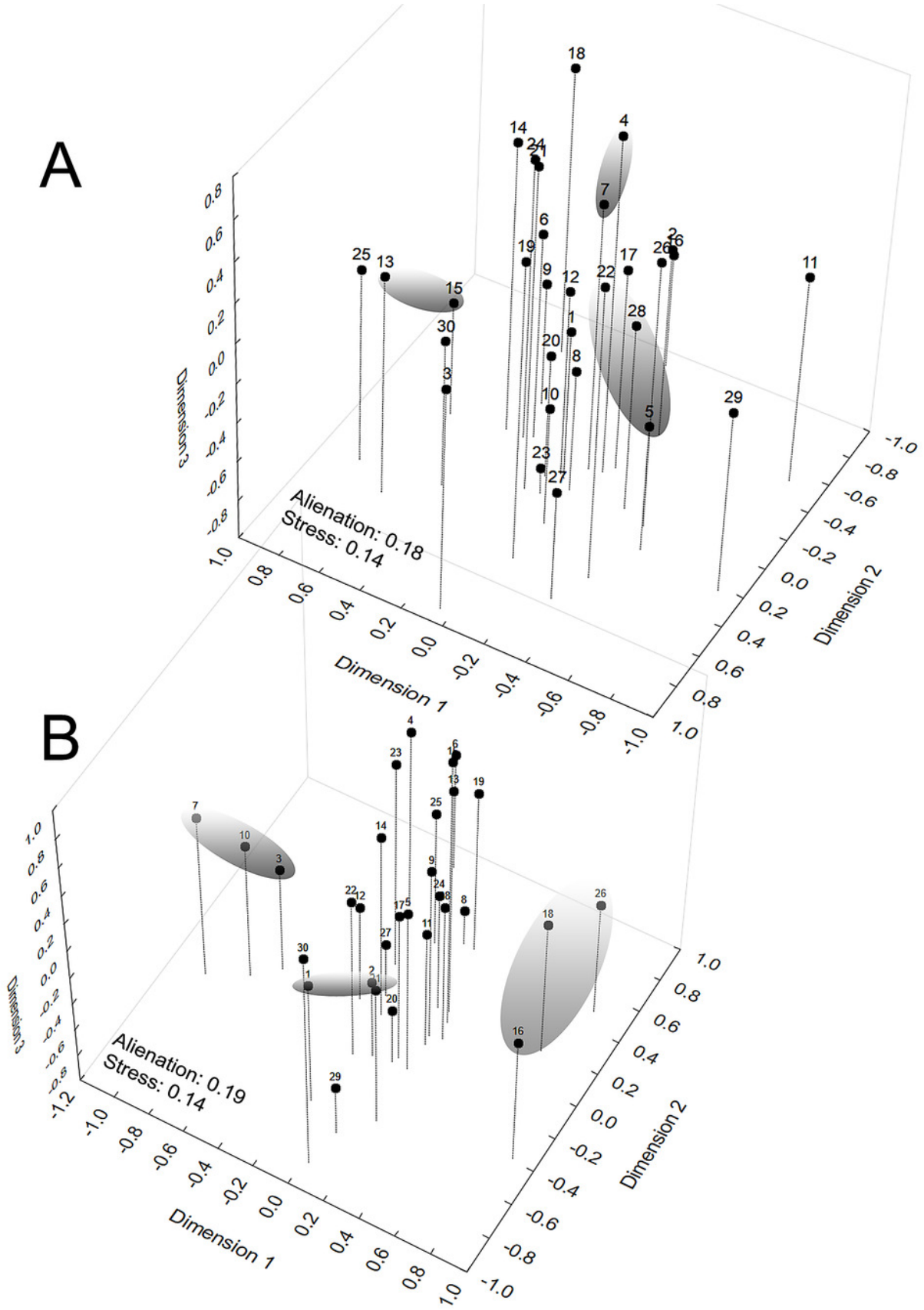

A
Alienation: 0.18
Stress: 0.14
Dimension 1
Dimension 2
Dimension 3
B
Alienation: 0.19
Stress: 0.14
Dimension 1
Dimension 2
Dimension 3

Figure 6

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).

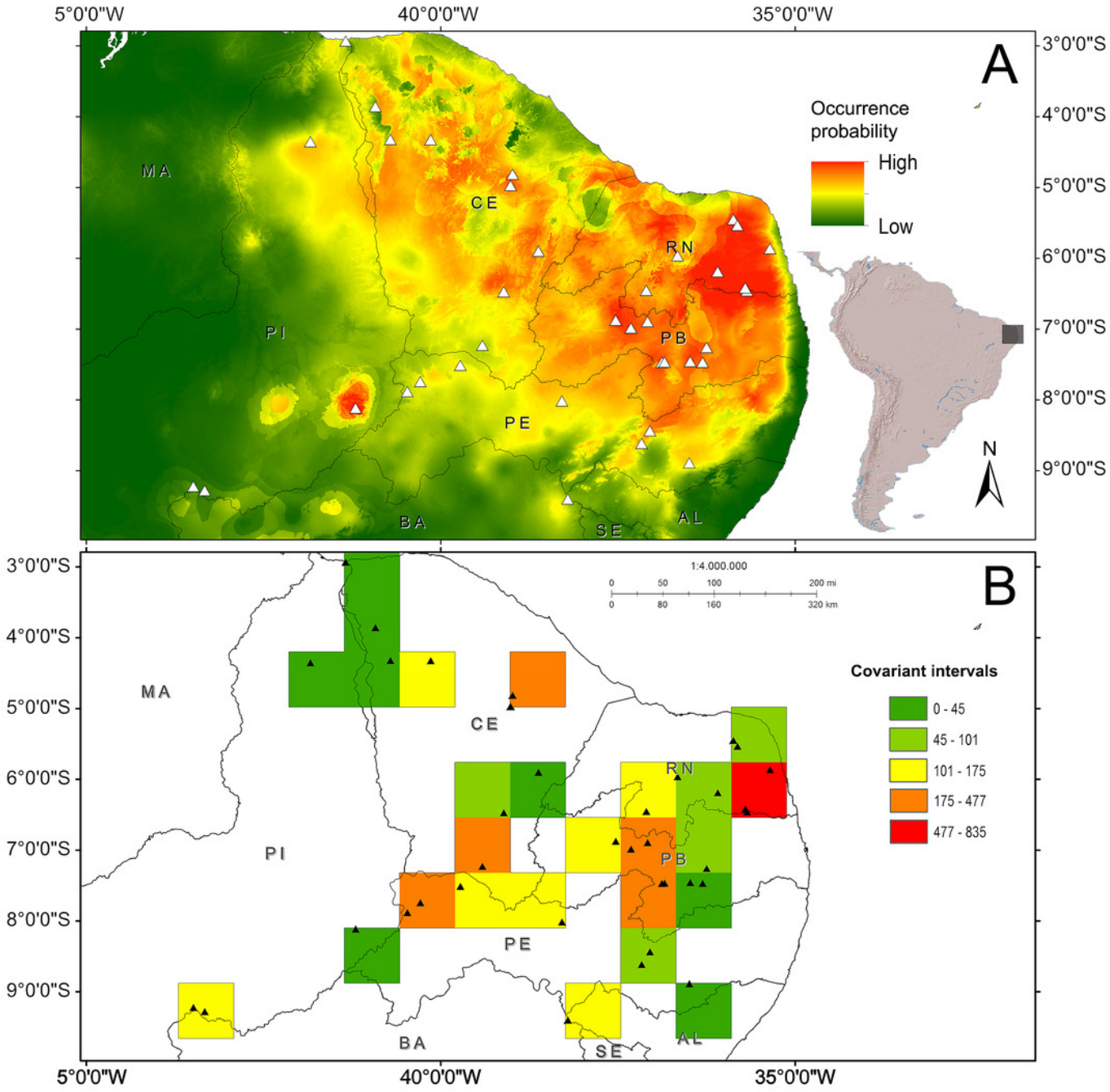

5°0'0"W
40°0'0"W
35°0'0"W
3°0'0"S
4°0'0"S
5°0'0"S
6°0'0"S
7°0'0"S
8°0'0"S
9°0'0"S
A
Occurrence probability
High
Low
MA
CE
RN
PI
PB
PE
BA
SE
AL
N
B
1:4.000.000
0 50 100 200 mi
0 80 160 320 km
Covariant intervals
0 - 45
45 - 101
101 - 175
175 - 477
477 - 835

Figure 7

Juveniles of *Proceratophrys cristiceps* observed in the Pedra de Boca State Park. (A) Chrom3; (B) Chrom6; (C) Chrom4 and (D) Chrom5.

The contrast in colouration in relation to the soil suggests reinforced adaptability in individual survival ability (crypsis and disruptive colouration). Photo credit: Kleber S. Vieira.

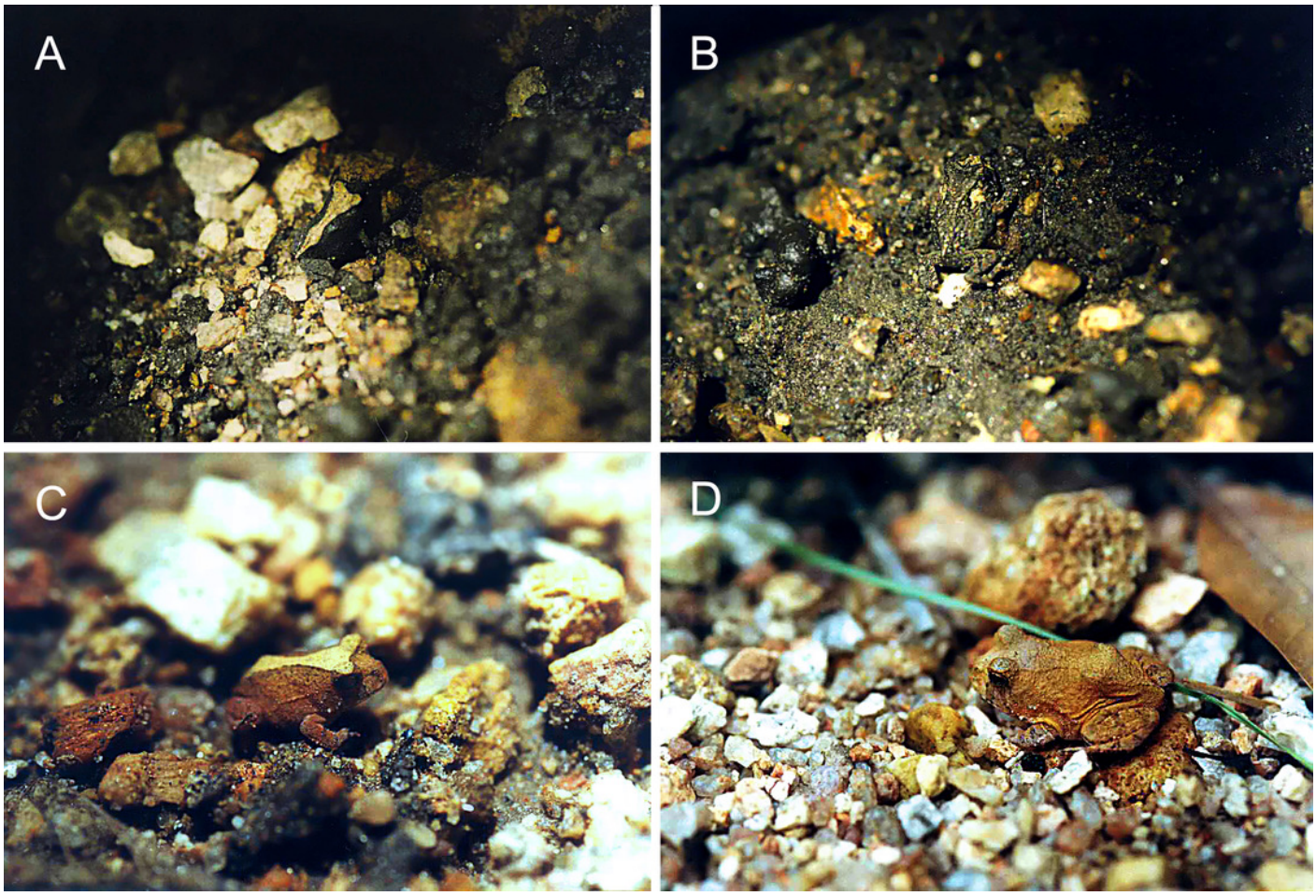


Figure 8

Morphometric gradient (cline and isophenes) observed in the distribution of the analysed populations of *Proceratophrys cristiceps*.

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 the temperatures are higher. Maps of South America showing the average annual temperature cover (A) and maximum temperature of the hottest month (B) for the years 1970-2000. The outlined space corresponds to the area occupied by the Caatinga biome. Climate data source: WorldClim (2020).

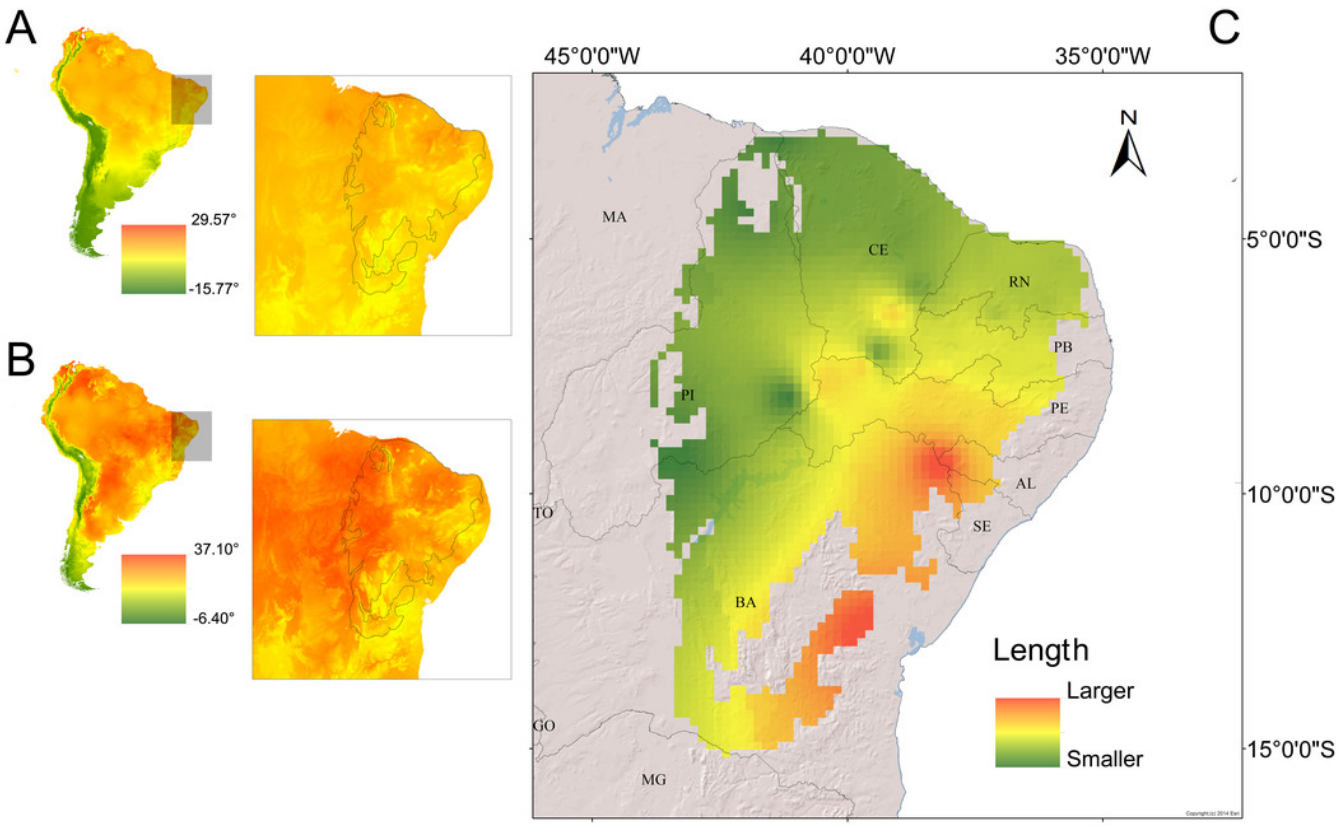


Figure 9

Number of species described among three diverse genera of anuran amphibians (A) and among those of *Proceratophrys* (B).

The lines represent the least squares regressions, while the numbers over the dots represent the periodic rate (%) of descriptions (A). We found that *Leptodactylus* and *Rhinella* genera increased at similar rates over the decades, being later surpassed by *Proceratophrys* due to faster rates of annual descriptions in the latter (A). When compared between congeneric groups (B), the highest description rates are observed in the *cristiceps* group. The *bigibbosa* group is reasonably stable, but the rate in the *boiei* group declines in relation to the total. Data obtained from Frost, D. R. (2021). Amphibian Species of the World: an Online Reference. Version 6.1.

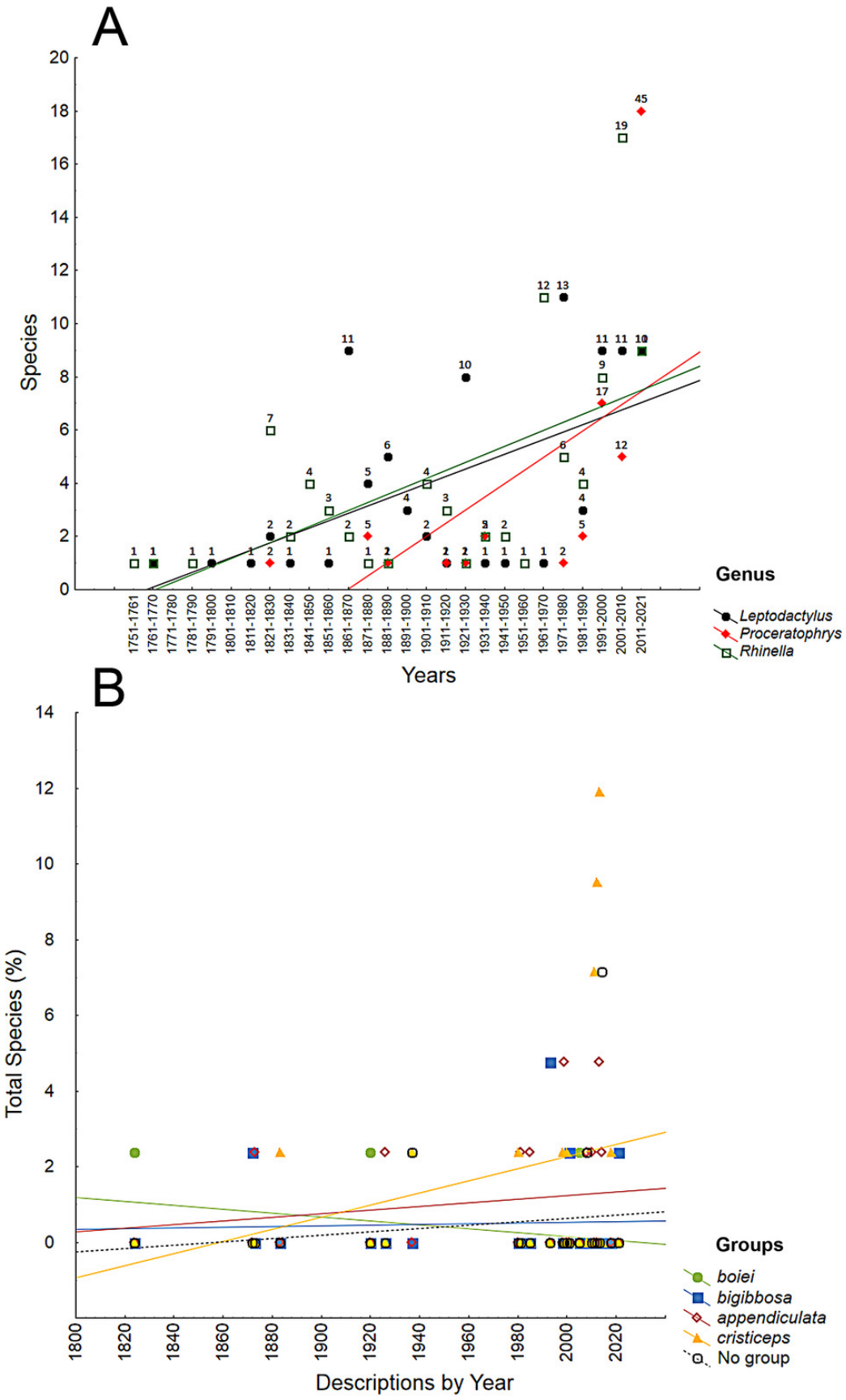
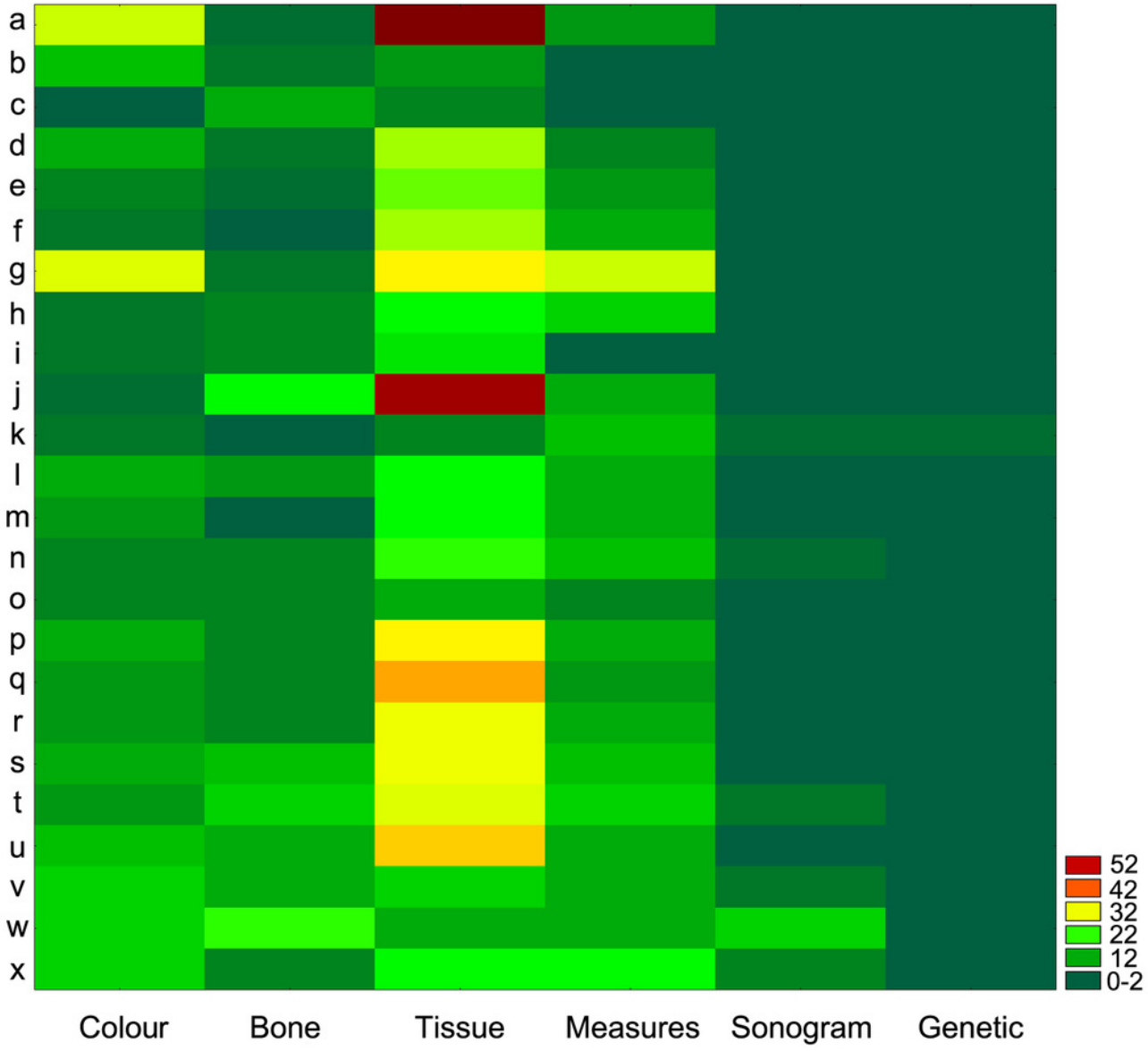

A
Species
Years
Genus
Leptodactylus
Proceratophrys
Rhinella
B
Total Species (%)
Descriptions by Year
Groups
boiei
bigibbosa
appendiculata
cristiceps
No group

Figure 10

Grouping of authors formed the identifying regions with high density of common values (Two-Way Joining).

The highlighted blocks in warm colours reflect the greater set 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. a – Gravenhorst (1829); b – Miranda-Ribeiro (1937); c – Lynch (1971); d – Jim & Caramaschi (1980); e – Eterovick & Sazima (1998); f – Ávila et al (2011); g – Napoli et al (2011); h – Günter (1873); i – Müller (1884); j – Cruz et al (2012); k – Mângia et al (2020); l – Braun (1973); m – Izeckshohn & Peixoto (1981); n – Mângia et al (2018); o – Barrio & Barrio (1993); p – Caramaschi (1996); q – Giaretta et al (2000); r – Junior et al (2012); s – Brandão et al (2013); t – Martins & Giaretta (2013); u – Cruz et al (2005); v – Godinho et al (2013); w – Martins & Giaretta (2011); x – Ávila et al (2012).


a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u
v
w
x
Colour
Bone
Tissue
Measures
Sonogram
Genetic
52
42
32
22
12
0-2