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ANA BEATRIZ CAROLLO ROCHA LIMA

INFLUÊNCIA DA PREDAÇÃO E DE ASPECTOS GEOCLIMÁTICOS SOBRE A COLORAÇÃO DAS ESPÉCIES DE BRACHYCEPHALIDAE NA MATA ATLÂNTICA

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“"Influência da predação e de aspectos geoclimáticos sobre a coloração das espécies de Brachycephalidae na Mata Atlântica"”

Este exemplar corresponde à redação final da Dissertação defendida pela candidata
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RESUMO

A família Brachycephalidae compreende dois gêneros e 52 espécies. O gênero *Brachycephalus* apresenta, em sua maioria, espécies tóxicas e de coloração conspícua, enquanto o gênero *Ischnocnema* possui muitas espécies polimórficas e crípticas quanto a sua coloração dorsal. No primeiro capítulo foi realizado um experimento com modelos de plasticina para testar se os padrões coloridos e brilhantes do gênero *Brachycephalus* estão relacionados com aposematismo. Além disso, foi testada a hipótese alóctone/autóctone, que prediz que os predadores vão responder mais fortemente a um padrão autóctone do que a um alóctone, dado que eles supostamente são ingênuos ao padrão introduzido e/ou experientes quanto ao padrão autóctone. O experimento foi realizado em duas localidades de Mata Atlântica no estado de São Paulo, Brasil, onde ocorrem tanto espécies camufladas quanto conspícuas de Brachycephalidae. Foram encontradas diferenças na intensidade de ataque entre os modelos crípticos e vistosos, suportando a hipótese de que a coloração conspícua de *Brachycephalus* é sinal de sua toxicidade. No segundo capítulo foi investigado como fatores ambientais e espaciais afetam os padrões de coloração de espécies do complexo de *Ischnocnema guentheri-henselii* e *I. parva*, comparando populações em diferentes latitudes depositadas no acervo de quatro coleções científicas. Os diferentes padrões foram plotados em mapas e, através de análises de agrupamento, foram detectadas diferenças na proporção da frequência dos padrões nas populações situadas no limite meridional da ocorrência das espécies. A segregação de populações abaixo da latitude -24º é coincidente com variações morfológicas observadas em outras espécies, ou mesmo com limites de distribuição de espécies irmãs de anuros. Assim, é evidente que esta região caracteriza-se por forte promotora de diversificação e o clima pode ser uma das explicações para nossos resultados. Desta forma os resultados desta dissertação sugerem que a predação pode estar exercendo pressão no gênero *Brachycephalus* e que fatores climáticos e geológicos podem estar influenciando a coloração no gênero *Ischnocnema*.

Palavras-chave: Anuro, Predação (Biologia), Polimorfismo.

ABSTRACT

Brachycephalidae family comprises two genera and 52 species. *Brachycephalus* genus presents mostly toxic species with conspicuous coloration, while *Ischnocnema* genus has many polymorphic and cryptic species as their dorsal coloration. In the first chapter we conducted an experimental essay, with plasticine frog simulacra to test if the bright colorful patterns of *Brachycephalus* species are related to aposematism. Furthermore, we tested the allochthonous/autochthonous hypothesis, which predicts that predators will respond strongly to an autochthonous pattern than to an allochthonous one, as they supposedly are naïve to this introduced pattern and/or experienced to the autochthonous pattern. The experiment was made in two Atlantic forest sites in the state of São Paulo, Brazil, where both cryptic and conspicuous Brachycephalidae species occur. We found differences in predation attack between cryptic and conspicuous simulacra, supporting that the conspicuous coloration of *Brachycephalus* is a sign of their toxicity. In the second chapter we investigated how environmental and spatial factors affect color patterns of species complex *Ischnocnema guentheri-henselii* and *I. parva* comparing populations at different latitudes deposited in four scientific collections. Different patterns were plotted on maps and, through cluster analysis, differences were detected in the ratio of the frequency of patterns in populations located in the southern limit of species occurrences. The segregation of populations below -24 ° latitude coincides with morphological variations observed in other species, or even distribution limits sister species of frogs. Thus, it is clear that this region is characterized by a strong promoter of diversification and the weather can be one of the explanations for our study. Thus, the results of this dissertation suggest that predation may be exerting pressure on the *Brachycephalus* genus and geological and climatic factors may be influencing the coloration in the *Ischnocnema* genus.

Keywords: Anura, Predation (Biology), Polymorphism.

SUMÁRIO

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“In the past, the approach to studying selection has been to begin with the assumption that interpopulation differences result from selection, and then studying the mechanisms by which selection maintains those differences. However, the results of this study clearly indicate that the assumption of a selective maintenance should be revisited.”

HOFFMAN, E. A.; SCHUELER, F. W.; JONES, A. G. & BLOUIN, M. S. 2006

In: *An analysis of selection on a colour polymorphism in the northern leopard frog*.

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INTRODUÇÃO GERAL

Coloração em animais tem três funções básicas: comunicação, termorregulação e evasão à predação (ENDLER, 1978). Coloração em animais e suas funções tem sido compilados por alguns autores como Cott (1940), Wickler (1968), Edmunds (1974), Ruxton *et al.* (2004) e Rudh & Qvarnström (2013).

Muitas espécies de anuros exibem cores surpreendentes ou diferentes morfotipos cromáticos, incluindo espécies crípticas e polimórficas ou com padrões de coloração vistosa e contrastante com o fundo (HOFFMAN & BLOUIN, 2000; SCHAEFER *et al.*, 2002; TOLEDO & HADDAD, 2009). A coloração dos anuros resulta da seleção natural atuando simultaneamente em diferentes aspectos da história natural, em especial em suas estratégias defensivas (e.g., COTT, 1940; WICKLER, 1968; EDMUNDS, 1974; TOLEDO & HADDAD, 2009) ou reprodutivas (e.g., ENDLER, 1978; BELL & ZAMUDIO, 2012), termorregulação (e.g., TATTERSALL *et al.*, 2006), comunicação intraespecífica e estratégias defensivas (ENDLER, 1978) ou em processos relacionados com a proteção contra a radiação (e.g., KAUL & SHOEMAKER, 1989), osmorregulação e metabolismo do nitrogênio (e.g., SCHMUCK & LINSENMAIR, 1988). O uso da coloração como estratégia defensiva em anfíbios é um grande campo de conhecimento e bastante inexplorado até o momento (TOLEDO & HADDAD, 2009), em especial a natureza da aprendizagem do predador para evitar presas conspícuas (SERVEDIO, 2000).

Cores e padrões em anuros são controlados basicamente por melanóforos, que contêm melanina e por cromatóforos, que contêm pigmentos coloridos. Estes pigmentos são influenciados por fatores como temperatura, luz e umidade: condições quentes e secas contraem os grânulos de pigmento tornando o anuro mais pálido, enquanto condições frias e úmidas dispersam o pigmento tornando o anuro mais escuro. Um anuro de cor escura perderá calor mais rapidamente quando a temperatura é mais elevada do que a entorno, ou absorverá mais calor quando exposto ao sol do que um anuro de cor clara; alguns anuros fazem termorregulação através da mudança de cor (PASSMORE & CARRUTHERS, 1979; FROST-MASON *et al.*, 1994; HOFFMAN & BLOUIN, 2000; TATTERSALL *et al.*, 2006). Apesar de todos os anuros poderem clarear ou escurecer o tom de pele existente, alteração de cor real é incomum e mudanças de cor observadas na natureza geralmente são relacionadas à idade e em uma direção (HOFFMAN & BLOUIN, 2000).

Aposematismo é um fenômeno defensivo que inclui a presença de sinais visíveis e contrastantes, geralmente relacionados à presença de toxinas ou indicando que o animal pode morder ou mostrar outro comportamento agressivo. Padrões de coloração vistosa aliados a propriedades desagradáveis da presa são uma estratégia de defesa contra predadores visualmente orientados, visto que estes associam a conspicuidade com a impalatabilidade ou outros meios de defesa (COTT, 1940; EDMUNDS, 1974; GITTLEMAN *et al.*, 1980; SERVEDIO, 2000; ENDLER & MAPPES, 2004; RUXTON *et al.*, 2004; TOLEDO & HADDAD, 2009). Apesar de o aposematismo estar presente em ampla variedade de táxons (ENDLER & MAPPES, 2004; RUXTON *et al.*, 2004), apenas alguns grupos de anuros podem ser caracterizados como genuinamente aposemáticos (SCHAEFER *et al.*, 2002). Alguns autores rejeitam total ou parcialmente a ideia de coloração de advertência, com o argumento de que indivíduos conspícuos de uma população são mais visíveis do que os seus coespecíficos e estão, portanto, expostos a maiores riscos de predação e também que novos mutantes, por serem raros, podem facilmente tornar-se extintos por ataques de predadores que não aprenderam a evita-los (MALLET & SINGER, 1987; GUILFORD, 1988; SPEED, 2001; RUXTON *et al.*, 2004).

Já o polimorfismo cromático pode ser definido como a ocorrência simultânea de dois ou mais fenótipos cromáticos fixos, de base genética em populações, em que a frequência do tipo mais raro é maior do que pode ser mantida por mutação recorrente (FORD, 1975; TOLEDO & HADDAD, 2009). Alguns anuros apresentam mais de um morfotipo (KAKAZU *et al*., 2010), podendo ocorrer variação na coloração do corpo ou no padrão dorsal, comumente com presença ou ausência de listras escuras ou manchas no dorso; é comum o polimorfismo dorsal para o vermelho, verde, marrom ou cinza, e combinações destes padrões (HOFFMAN & BLOUIN, 2000). O polimorfismo cromático é amplamente distribuído em anfíbios, presente em pelo menos 225 espécies; a maioria das espécies dos gêneros com padrões de coloração crípticos *Craugastor*, *Eleutherodactylus, Pristimantis* e *Ischnocnema* são polimórficas cromáticas (HOFFMAN & BLOUIN, 2000; TOLEDO & HADDAD, 2009).

A seleção por parentesco é a explicação mais provável para a evolução da coloração aposemática em espécies impalatáveis (HARVEY & GREENWOOD, 1978; SILLEN-TULLBERG & BRYANT, 1983; RUDH & QVARNSTRÖM, 2013). Já a origem e manutenção de polimorfismos cromáticos podem ter sido originadas pela seleção sexual e seleção apostática (diferencial): a especialização perceptiva dos predadores (formação de imagem de busca) pode levar à manutenção de polimorfismos, uma vez que presas com fenótipos raros seriam pouco afetadas por predadores que buscam o tipo mais comum (TINBERGEN, 1960; OWEN, 1963; VASCONCELLOS-NETO & GONZAGA, 2000).

A família Brachycephalidae abriga dois gêneros e 52 espécies, sendo que o gênero *Brachycephalus* apresenta espécies miniaturizadas de coloração conspícua e o gênero *Ischnocnema* possui muitas espécies crípticas e polimórficas quanto a sua coloração dorsal. O gênero *Brachycephalus* é restrito à Mata Atlântica costeira do leste para o sul do Brasil

(POMBAL-JR *et al.*, 1994; FROST, 2013) e conta com 20 espécies descritas (FROST, 2013). Boa parte das espécies conhecidas foi descrita nos últimos anos (e.g. *Brachycephalus ferruginus* e *B. pombali* ALVES, RIBEIRO, HADDAD & REIS, 2006; *B. pitanga* ALVES, SAWAYA, REIS & HADDAD, 2009; *B. toby* HADDAD, ALVES, CLEMENTE-CARVALHO & REIS, 2010; *B. pulex* NAPOLI, CARAMASCHI, CRUZ & DIAS, 2011; *B. guarani* CLEMENTE-CARVALHO, GIARETTA, CONDEZ, HADDAD & REIS, 2012; e *B. tridactylus* GAREY, LIMA, HARTMANN & HADDAD, 2012) e provavelmente novas espécies ainda serão descritas para o gênero (POMBAL-JR *et al.*, 1994; ALVES *et al.*, 2009). As espécies conhecidas de *Brachycephalus* têm uma distribuição insular, ocorrendo em sua maioria isoladamente das espécies congêneres em montanhas das regiões sul e sudeste do Brasil em altitudes entre 600 e 1800 m (ALVES *et al.*, 2009).

Brachycephalus ephippium é uma espécie pequena (até 18 mm de comprimento rostro-cloacal – CRC), de coloração amarela a alaranjada, que vive na serapilheira em matas de 750 a 1200 m (SEBBEN *et al.*, 1986; POMBAL-JR *et al.*, 1994) no sudeste do Brasil (FROST, 2013). A espécie é diurna (POMBAL-JR *et al.*, 1994) e apresenta toxinas em sua pele (SEBBEN *et al.*, 1986; PIRES-JR *et al.*, 2002). *Brachycephalus pitanga* é conhecida apenas da vizinhança da localidade-tipo, habitando a serapilheira entre os municípios de Ubatuba e São Luís do Paraitinga, SP, sudeste do Brasil (ALVES *et al.*, 2009; FROST, 2013). Também é diurno e pequeno (até 16 mm CRC), de coloração alaranjada com manchas avermelhadas (ALVES *et al.*, 2009). A espécie foi descrita recentemente e pouco se publicou a respeito da história natural da mesma.

O gênero *Ischnocnema* inclui muitas espécies que são importantes membros das comunidades de anfíbios de serapilheira da Mata Atlântica do Brasil (MARTINS *et al.*, 2010).

Um estudo recente descobriu que várias populações tradicionalmente consideradas como *I. guentheri* formam um complexo de espécies, incluindo *I. henselii* (na maioria das populações) e outras que, provavelmente, são espécies não descritas (GEHARA *et al*., 2013). Portanto, neste trabalho, a espécie *Ischnocnema guentheri* foi denominada por "complexo de *Ischnocnema guentheri-henselii*".

O complexo de *I. guentheri-henselii* e *I. parva* apresentam um padrão dorsal extremamente variável, sendo que o padrão dorsal do complexo de *I. guentheri-henselii* varia entre uniformemente escuro ou claro a um padrão complexo incluindo manchas escuras na região escapular ou com ondulações escuras e linhas longitudinais vertebrais ou laterais claras (HEYER, 1984; HEYER *et al*., 1990). *Ischnocnema parva* também apresenta grande número de morfotipos, incluindo indivíduos uniformemente claros ou escuros, com ou sem linhas vertebrais ou dorsolaterais; com flanco mais claro ou mais escuro do que o dorso, ou rajados (obs. pessoal). Ambas as espécies habitam as áreas florestadas da Mata Atlântica e possuem as fêmeas maiores do que os machos. Apresentam desenvolvimento direto e atividade noturna e crepuscular (HEYER *et al,*, 1990; HADDAD *et al*. 2013; MARTINS *et al*., 2010).

O complexo de *I. guentheri-henselii* ocorre nas regiões sul e sudeste do Brasil, nos estados de Santa Catarina, São Paulo, Rio de Janeiro e Minas Gerais (HEYER *et al*., 1990; HADDAD *et al*. 2013; FROST, 2013). *Ischnocnema parva* ocorre no sudeste do Brasil (FROST, 2013). O complexo de *I. guentheri-henselii* possui hábito arborícola, costuma vocalizar a partir de folhas ou galhos sobre a vegetação baixa (20-60 cm), normalmente do final da tarde ao início da noite (HEYER, 1984; HEYER *et al*., 1990; HADDAD *et al*. 2013). Suas estratégias de defesa são tanatose e camuflagem visual eucríptica, i.e., assemelhando-se a uma parte aleatória do substrato (HADDAD *et al*. 2013; TOLEDO & HADDAD, 2009). Habitam matas preservadas, podendo ser utilizada como indicadora ambiental (PONTES *et al*., 2009). Evidências sugerem que possa viver mais de um ano (HEYER *et al.*, 1990). *Ischnocnema parva* é frequente no chão da mata, habitando a serapilheira. É camuflado e preda principalmente artrópodes, como formigas e tatuzinhos de jardim (HADDAD *et al*. 2013; MARTINS *et al*., 2010).

Tanto coloração críptica com seus polimorfismos quanto padrões de coloração vistosos ocorrem em anfíbios e seus significados adaptativos ainda são pouco estudados. Neste trabalho foram investigados dois casos abordando os dois tipos de coloração protetiva em anuros.

No primeiro capítulo do presente trabalho foi testada experimentalmente a hipótese de aposematismo associada às espécies de coloração conspícua *Brachycephalus ephippium* e *B. pitanga* através de um experimento com modelos de plasticina.

No segundo capítulo foi analisado de que maneira os fatores climáticos associados à latitude, como temperatura e umidade relativa, podem afetar a ocorrência dos diferentes padrões de coloração do complexo de *I. guentheri-henselii* e *I. parva* comparando a coloração de populações depositadas no acervo de coleções científicas em diferentes latitudes.

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CAPÍTULO 1

Is the conspicuous pattern of the Atlantic forest pumpkin toadlets aposematic?

Abstract

Aposematism includes the presence of conspicuous or contrasting signals, indicating that the animal may be unpalatable or harmful in anyway, such as presenting poison, hidden weapons, or aggressive behaviors. Many contrasting and colorful species are considered aposematic, however, few experiments were made to confirm this hypothesis. Therefore, we conducted an experimental essay with plasticine frog simulacra to test if the bright colorful patterns of *Brachycephalus* species are related to aposematism. Furthermore, we tested the allochthonous/autochthonous hypothesis, which predicts that predators will respond strongly to an autochthonous pattern than to an allochthonous one, as they supposedly are naïve to this introduced pattern. The experiment was made in two Atlantic forest sites in the state of São Paulo, Brazil, where both cryptic and conspicuous *Brachycephalus* species occur. We set trios of plasticine simulacra, one brown cryptic (representing species such as *B. bruneus* or *B. hermogenesi*), one yellow conspicuous (such as *B. ephippium*) and one red conspicuous (such as *B. pitanga*), directly on the leaf litter or on white cardboard sheets. We found differences in rates of attack between cryptic and conspicuous simulacra. Our results may provide evidences supporting that the conspicuous coloration of *Brachycephalus* can be considered aposematic.

Key Words: *Brachycephalus*, Aposematism, Conspicuousness, Coloration, Predation, Simulacra

Introduction

Anurans show great variety of colors patterns, including species with aposematic contrasting patterns with the background (HOFFMAN & BLOUIN, 2000; SCHAEFER *et al.*, 2002; TOLEDO & HADDAD, 2009). The coloration of frogs results from natural selection acting simultaneously on different aspects of their natural history (TOLEDO & HADDAD, 2009), such as sexual selection (e.g. ENDLER, 1978; BELL & ZAMUDIO, 2012). Coloration in amphibians is related to processes such as radiation protection, thermoregulation, osmoregulation, nitrogen metabolism, intra and interspecific communication and defense mechanisms against predation (COTT, 1940; EDMUNDS, 1974; TATTERSALL *et al.*, 2006; TOLEDO & HADDAD, 2009). Therefore, several selective pressures may be selecting for different color morphs within species. Among these possible selective pressures, predation seems to be a major one, however, still understudied (e.g., ALLEN & GREENWOOD, 1988; OWEN & WHITELEY, 1989; HOFFMAN & BLOUIN, 2000; VASCONCELLOS-NETO & GONZAGA, 2000; BOND & KAMIL, 2002; TOLEDO & HADDAD, 2009).

Aposematism is a defensive strategy that consists in the presence of conspicuous and contrasting signs, usually related to the presence of toxins in the skin of the individuals or indicating that the animal can bite or display other aggressive behaviors. Aposematic coloration may protect the frog from visual predators (COTT, 1940; GITTLEMAN *et al.*, 1980; SERVEDIO, 2000; ENDLER & MAPPES, 2004; RUXTON *et al.*, 2004; TOLEDO & HADDAD, 2009). Aposematism is present in a wide variety of taxa (ENDLER & MAPPES, 2004; RUXTON *et al.*, 2004), nevertheless, only a few groups of anurans can be characterized as genuinely aposematic (SCHAEFER *et al.*, 2002).

Few studies have specifically tested the hypothesis that the conspicuous coloration in frogs is considered an warning (aposematic) signal to potential predators (SAPORITO *et al*., 2007). Experiments with clay models were conducted to test effects of patterns considered aposematic in predators on snakes (BRODIE, 1993; WÜSTER *et al*. 2004; NISKANEN & MAPPES, 2005), birds (LINDSTRÖM *et al*., 1999) and other groups, but few studies have been conducted involving aposematic amphibians. Kuchta (2005) conducted an experiment with salamanders, comparing earthenware models conspicuous and cryptic, and the predation of the first was significantly lower; and Saporito *et al*. (2007), produced clay models from cryptic and conspicuous frogs like *Oophaga pumilio* and the predation of cryptic models was almost twice than the conspicuous models.

Most of the species of *Brachycephalus* are small (about 2 cm of SVL), diurnal, with coloration varying between yellow, orange, pink and red, and foraging and reproducing on the dark brown background composed by wet leaf litter inside the Brazilian Atlantic rainforest (SEBBEN *et al*., 1986; POMBAL-JR *et al*., 1994). It is also known that some of the surveyed species secrets highly toxic skin compounds, such as tetrodotoxin, ephippiotoxin and 11 other similar toxins (PIRES-JR *et al*., 2002). Therefore, the presence of bright contrasting coloration and skin toxins supports the aposematism hypothesis for this genus (POMBAL-JR *et al*., 1994). However this hypothesis was never tested. Furthermore, if the species is shown to be aposematic, there is no information if the predator assemblage would respond in the same manner to an autochthonous and an allochthonous color morph. It is suggest that predators would respond strongly to an autochthonous pattern than to an allochthonous one, as they supposedly are naïve to this introduced pattern (hereafter, the autochthonous recognition hypothesis).

Methods

The experiment was performed in September of 2012 in two sites: Serra do Japi, municipality of Jundiaí, state of São Paulo, Brazil, where *B. ephippium* occurs and PESM Núcleo Santa Virgínia, municipality of São Luís do Paraitinga, state of São Paulo, Brazil, where *B. pitanga* occurs. At Serra do Japi the experiment site chosen was a forested area near a swamp (23.24141 S, 046.93776 W, 1058 m asl) and at Núcleo Santa Virgínia, the experiment was performed on the "Pirapitinga trail", next to a stream (23.335553 S, 45.146235 W, 902 m asl). Secretaria do Meio Ambiente de Jundiaí and Instituto Florestal provided the working permits n° 012.192/2011.

We prepared models (simulacra) with plasticine representing cryptic frogs as those of brownish species of *Brachycephalus*, and simulacra representing conspicuous frogs, such as *B. ephippium* and *B. pitanga*. We used odorless modeling plasticine Acrilex®, manufactured in a plaster mold with a layer of enamel, made from a preserved specimen of *B. ephippium*, covered with nail varnish (Colorama®) to cover imperfections from layer.

With this method, our goal was to test two hypotheses: (1) the aposematism hypothesis, in which we assumed that predators have innate avoidance to conspicuous patterns and therefore avoid both conspicuous simulacra (yellow and red), and (2) the autochthonous recognition hypothesis, in which we assumed that predators who had previous experience with autochthonous pattern will avoid them and the predation of allochthonous conspicuous pattern simulacra would be higher.

To test this hypothesis, 1200 simulacra were arranged in trios, in a total of 200 trios by locality, so predators could choose between one or more simulacra. Half of the trios were placed upon a white cardboard (15 x 21 cm), ensuring the visualization of all simulacra (both exposed), and herein we call them "exposed group". The other half of the trios were distributed over the leaf litter, where the brown simulacra were cryptic, and herein we call them "cryptic group" (Figure 1a, 1b). Each trio consisted of a brown simulacra (resembling *B. hermogenesi*, figure 1c), a yellow simulacra (mimicking *B. ephippium*, figure 1d) and a red simulacra (like *B. pitanga*, figure 1e). Simulacra were distributed along four transects of approximately 100 m, with 50 trios each, in both localities (Santa Virgínia and Serra do Japi). Each trio was placed approximately 2 meters from each other.

We checked for predations every day, in the afternoon, so that simulacra were exposed for 24 hours for four consecutive days. When we found evidence of predation, the whole trio was removed. Predation events were considered when the simulacra disappeared, or when there were "marks" such as pecks, bites and stings. A photographic record was made of all the simulacra that showed marks of predation for recognition of the types of predators.

We performed chi-square test with three categorical variables to compare the frequency of predation observed and expected. The expected frequencies of the chi-square were calculated by the arithmetic mean of the rate of predation of the three simulacra in each group. The chi-square test was applied for the entire experiment and separately for the exposed and cryptic groups in both localities. We made the test between cryptic and both conspicuous simulacra and only between the conspicuous simulacra, from the exposed and cryptic groups and separately for both localities.

Results

We found simulacra with three different types of marks (Figure 1f, 1g, 1h): V-shaped marks were associated with birds (pecking); dotted or crumbly marks were associated with arthropod predation and U-shaped with tooth signs were associated with small mammals/lizards bites. Besides that, were also registered cases of disappeared simulacra.

Out of the 200 trios arranged directly on the leaf litter (cryptic group), 34 yellow conspicuous simulacra, 39 red conspicuous simulacra and 70 brown cryptic simulacra were attacked. Out of the 200 trios arranged in the cardboard (exposed group), 30 yellow conspicuous simulacra, 34 red conspicuous simulacra and 56 brown cryptic simulacra were attacked upon (figure 2a).

In Serra do Japi, out of the 100 trios of the exposed group, six yellow conspicuous simulacra, eight red conspicuous simulacra and 17 brown cryptic simulacra were predated. Out of the 100 trios of the cryptic group, three yellow conspicuous simulacra, eight red conspicuous simulacra and 11 brown cryptic simulacra were predated (figure 2b). At Núcleo Santa Virgínia, out of the 100 trios from exposed group, 28 yellow conspicuous simulacra, 31 red conspicuous simulacra and 53 brown cryptic simulacra were predated. Out of the 100 trios of the cryptic group, 27 yellow conspicuous simulacra, 26 red conspicuous simulacra and 45 brown cryptic simulacra were predated (figure 2c).

The results of the chi-square for the aposematic hypothesis (between the three simulacra) and autochthonous recognition hypothesis (only between the two conspicuous simulacra) are presented in the table 1. All the results of the chi-square for the aposematic hypothesis was statistically significant ($P < 0,05$), except for the control group of Serra do Japi. Although this result was not significant, there was a difference in the proportion of the preyed simulacra between the cryptic simulacra (11 predated) and the conspicuous simulacra (eight red and three yellow predated); the cryptic simulacra had the same predation than both conspicuous simulacra. The results of the chi-square for the autochthonous recognition hypothesis were not statistically significant in any case.

Discussion

Based on the aposematism hypothesis (COTT, 1940; EDMUNDS, 1974; ENDLER, 1978) it was expected that attacks upon cryptic simulacra would be less intense when the simulacra are over the leaf litter than when they are on contrasting backgrounds (e. g. KUCHTA, 2005; SAPORITO *et al*, 2007; FARALLO & FORSTNER, 2012). However, in our study, there was no difference in the attack on the cryptic simulacra in both contrasting and cryptic backgrounds. Thus, it is possible that our simulacra were not too cryptic, or that predators can distinguish them even in a cryptic scenario, choosing the cryptic instead of the conspicuous. Our study shows experimentally that predators prefer cryptic prey even if they are over similar backgrounds; this fact may indicate the avoidance of conspicuous *Brachycephalus* by the predator, and perhaps reinforces the aposematism hypothesis (POULTON, 1887; COTT, 1940; RETTENMEYER, 1970).

In regarding to the autochthonous recognition hypothesis were expected that the simulacra that represented the alien species (*B. pitanga* at Serra do Japi and *B. ephippium* at Santa Virgínia) would be those more preyed upon, as the predator could be naïve. However, our results indicate that predators may present an innate avoidance to conspicuous colorations (COPPINGER, 1970; SHETTLEWORTH, 1972; MARPLES *et al.*, 1998). An alternative explanation is that as the color patterns are similar (red and yellow), predators may avoid unusual patterns that are phenotypically similar to common ones (RODRÍGUEZ *et al.*, 2012). Besides that, it is possible that the same predator species can be (or could be) distributed over both areas, distant less than 300 km (straight-line distance), and learning/selection occurred over generations. In this case, past selective pressures may have selected the avoidance of both conspicuous colorations (GITTLEMAN *et al*., 1980; RUXTON *et al*., 2004). Furthermore, it is also possible that predators are exposed to the same warning colorations in other preys, such as beetles (GRILL & MOORE, 1998; BEZZERIDES *et al*., 2007; SEAGO *et al*., 2009), mollusks (GOSLINER, 2001), or butterflies (MALLET & SINGER, 1987; MOLLEMAN *et al*., 2010), which, in palatable forms, may also be part of their diet of mantids (BERENBAUM & MILICZKY, 1984; BOWDISH & BULTMAN, 1993), birds (COPPINGER, 1970; ROPER & WISTOW, 1986; GAMBERALE-STILLE & TULLBERG, 1999) and amphibians (HATLE & SALAZAR, 2001; SLOGGETT, 2012).

Past studies with amphibians showed that cryptic morphs were preferred against conspicuous models (KUCHTA, 2005; SAPORITO *et al*, 2007). Similarly, we found evidence that the conspicuous coloration of Atlantic Forest brachycephalids negatively influence the predation intensity. It suggests that predators avoid conspicuous simulacra and prefer cryptic simulacra probably because they associate the conspicuous coloration to defensive strategies (RETTENMEYER, 1970; SHERRATT, 2002; TOLEDO *et al*., 2011). So, here we possibly have obtained the first experimental evidence of predator's avoidance to conspicuous coloration of notoriously toxic *Brachycephalus* genus.

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Table 1. Results of the chi-square for the aposematic hypothesis and autochthonous recognition hypothesis (statistically significative result: P < 0.05).

		General experiment	**Serra do Japi**	**Núcleo Santa Virgínia**
Aposematic hypothesis	**Exposed group**	$2{,}823^{-05}$ (P < 0.001)	0,0246 (P < 0.05)	$3{,}475^{-04}$ (P <0.001)
	Cryptic group	0,0022 (P <0.01)	0,0903 (P = 0.09)	0,0055 (P <0.001)
Autochthonous recognition hypothesis	**Exposed group**	0,5175 (P = 0.52)	0,5794 (P=0.58)	0,6418 (P=0.64)
	Cryptic group	0,5854 (P=0.59)	0,1209 (P=0.12)	0,8727 (P=0.87)

Figures

Figure 1. Distribution of cryptic and exposed groups: control trio on cardboard (A) and experimental trio on litter (B). The cryptic frog *Brachycephalus hermogenesi* (C) and both conspicuous species *Brachycephalus ephippium* (D) and *B. pitanga* (E). Marks of pecks (F), stings (G) and bites (H) caused by predators in plasticine simulacra. Pictures C and D: Ivan Sazima.

Figure 2. Attacks of the simulacra in exposed and cryptic groups: total data (A) and grouped by locality: Serra do Japi, Jundiaí (B) and Núcleo Santa Virgínia, São Luis do Paraitinga (C).

Figure 1

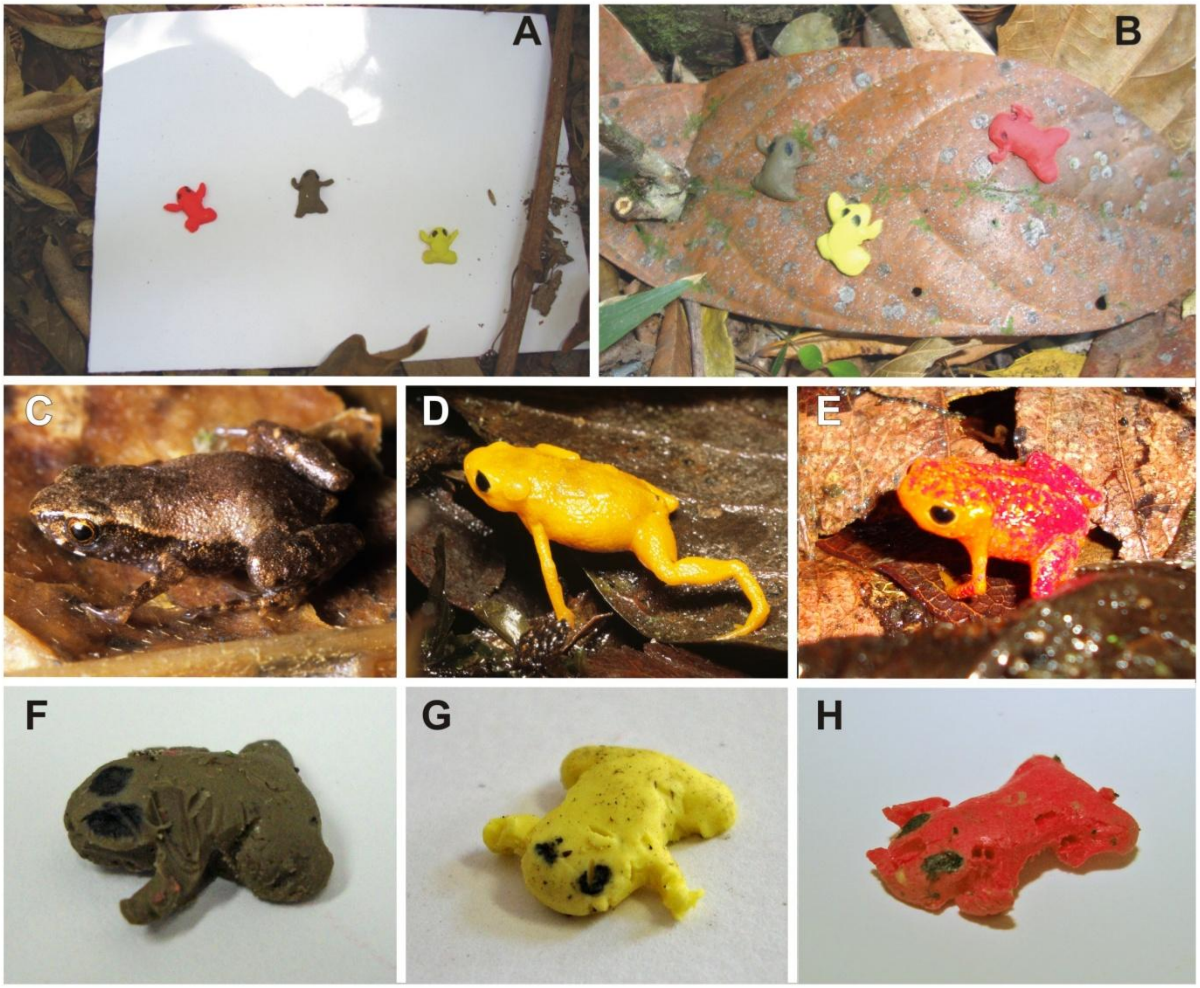
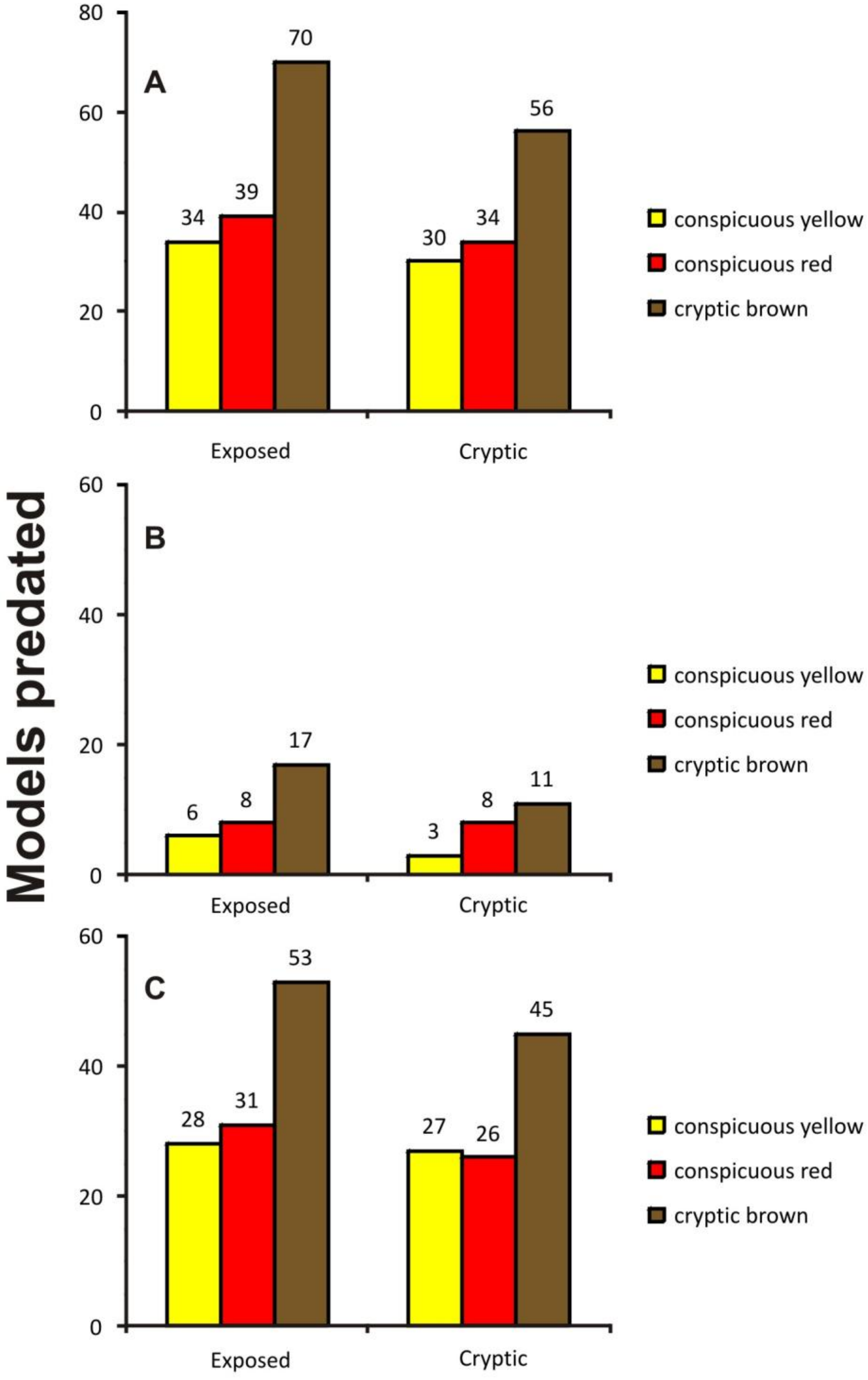


Figure 2


Models predated
A
80
60
40
20
0
70
34
39
30
34
56
Exposed
Cryptic
conspicuous yellow
conspicuous red
cryptic brown
B
60
40
20
0
17
6
8
3
8
11
Exposed
Cryptic
conspicuous yellow
conspicuous red
cryptic brown
C
60
40
20
0
53
28
31
27
26
45
Exposed
Cryptic
conspicuous yellow
conspicuous red
cryptic brown

CAPÍTULO 2

Latitudinal variation of color patterns in *Ischnocnema* species of the Atlantic Forest

Abstract

The coloration of amphibians results from natural selection acting simultaneously on different aspects of their natural history. The genus *Ischnocnema* has many polymorphic species in regard to their dorsal coloration. We intent to investigate how environmental and spatial factors can affect the patterns of coloration of species complex *Ischnocnema guentheri-henselii* and *I. parva* comparing populations at different latitudes filed in the collection of four scientific collections. Data were grouped according to locality, sex and previously established color patterns. The different patterns were plotted on maps and, through cluster analysis differences were detected in the frequency of patterns in populations located in the southern limit of the species distribution. The segregation of populations below latitude -24º coincides with morphological variations observed in other anuran species, or even with limits of distribution of sister species of frogs. Thus it is clear that this region is characterized by strong promoter diversification and climate can be one of the explanations for such pattern.

Key Words: Anura, Brachycephalidae, Polymorphism, Crypsis, Predation, Latitudinal gradients

Introduction

Chromatic polymorphism can be defined as the simultaneous occurrence of two or more coloration phenotypes, from genetic basis in populations, in which the frequency of the rarest type is greater than can be maintained by recurrent mutation (FORD, 1975; HOFFMAN & BLOUIN, 2000). Polymorphism is very common in vertebrates (e.g., SKULASON & SMITH, 1995; HOEKSTRA *et al*., 2004; GRAY & MCKINNON, 2007; REICHARD *et al*., 2009; FARALLO & FORSTNER, 2012) and especially abundant and diversified in anuran amphibians (e.g., HOFFMAN *et al*., 2006; HOOGMOED & AVILA-PIRES, 2012). In anurans variation may occur in the dorsum of the body, typically varying between presence or absence of vertebral stripes (HEYER, 1970; BROWN *et al*., 2006; KAKAZU *et al*., 2010) or variation of spots and dorsal variegated (NASCIMENTO *et al*., 2005; PADIAL & DE LA RIVA, 2009; RYAN *et al*., 2010; BELL & ZAMUDIO, 2012). Regarding the dorsal coloration is common to observe variation among red, green, brown or gray, and combinations of these (BULL, 1975; BLOUIN, 1989; HOFFMAN & BLOUIN, 2000; HOFFMAN *et al*., 2006).

The anuran coloration results of natural selection acting simultaneously on different aspects of natural history, such as the defensive strategies (e.g., COTT, 1940; WICKLER, 1968; EDMUNDS, 1974; TOLEDO & HADDAD, 2009) or reproductive strategies (e.g., ENDLER, 1978; BELL & ZAMUDIO, 2012), in thermoregulation (e.g., TATTERSALL *et al*., 2006), intraspecific communication (ENDLER, 1978) or processes related to the radiation protection (e.g., KAUL & SHOEMAKER, 1989), osmoregulation and nitrogen metabolism (e.g., SCHMUCK & LINSENMAIR, 1988). Among all these possible selective pressures, predation has been described as a main, however little studied (e.g.,VASCONCELLOS-NETO & GONZAGA, 2000; SAPORITO *et al*., 2007; TOLEDO & HADDAD, 2009; KAKAZU *et al*., 2010). The differential (or apostatic) selection may be responsible for the origin and maintenance of color polymorphisms: the perceptive specialization of predators (search image formation) can induce to maintenance of polymorphisms, once prey with rare phenotypes would be little influenced by predators that seeking the most common type (POULTON, 1884; TINBERGEN, 1960; ALLEN & GREENWOOD, 1988; VASCONCELLOS-NETO & GONZAGA, 2000; GRAY & MCKINNON, 2007). Thus, the polymorphism may benefit anurans such that one or more of the phenotypes are not included in the search image of the predator (TINBERGEN, 1960; AYALA & CAMPBELL, 1974; HOFFMAN & BLOUIN, 2000; TOLEDO & HADDAD, 2009; KAKAZU *et al*., 2010).

The Brachycephalidae family includes two genera and 52 species (FROST, 2013). The *Ischnocnema* genus has many species polymorphic as their dorsal coloration (HEYER, 1984; HOFFMAN & BLOUIN, 2000). In their revision work on polymorphism, Hoffman & Blouin (2000) lists 80 species of the *Eleutherodactylus* genus that are polymorphic. Of these, four are currently allocated in the *Ischnocnema* genus: *I. guentheri*, *I. parva*, *I. nasuta* and *I. randorum*. Besides these, of the other 28 species of the genus, we found that at least another 15 are polymorphic (Carollo, personal observation).

Ischnocnema guentheri, I. henselii and *I. parva* presents a highly variable dorsal pattern, and the dorsal pattern of *I. guentheri* and *I. henselii* ranges between uniformly dark or light to a complex pattern including dark spots on the scapular region or dark waves and vertebral or lateral light longitudinal stripes (HEYER, 1984; HEYER *et al*., 1990). *Ischnocnema parva* also displays a large number of morphotypes, including individuals uniformly light or dark, with or without vertebral or dorsolateral stripes; with flank lighter or darker than the back, or striped (pers. obs.). This species inhabit the forested areas of the Atlantic Forest and have larger females than males. They presents direct development and crepuscular and nocturnal activity (HEYER *et al.*, 1990; HADDAD *et al.*, 2013; MARTINS *et al.*, 2010).

Heyer (1984) described the *I. guentheri* complex as taxa that probably were identified as *I. gualteri*, *I. guentheri* or *I. nasuta* based on limited comparative material. A recent study has found that several populations traditionally considered as *I. guentheri* in fact forms a complex of species including *I. henselii* (in most populations) and other populations that probably these are undescribed species (GEHARA *et al.*, 2013). In this context, *I. guentheri* is restricted to the city of Rio de Janeiro, while *I. henselii* and other possible species of the complex are distributed from the state of Rio de Janeiro and Minas Gerais to Rio Grande do Sul and Argentina (GEHARA *et al.*, 2013). *Ischnocnema parva* occurs only in southeastern Brazil (FROST, 2013). The species of the *Ischnocnema guentheri-henselii* complex have arboreal habit, often vocalize from leaves or twigs on low vegetation (20-60 cm), usually from late afternoon to early evening (HEYER, 1984; HEYER *et al.*, 1990; HADDAD *et al.*, 2013). Their defense strategies are thanatosis and visual camouflage eucrypsis, e.g., imitating a random part of the substrate (HADDAD *et al.*, 2013; TOLEDO & HADDAD, 2009). Evidence suggests that they can live more than a year (HEYER *et al.*, 1990). *Ischnocnema parva* is common in the forest floor, inhabiting the leaf litter as *I. guentheri-henselii* complex. It is camouflaged and preys mainly arthropods, such as ants and woodlouses (HADDAD *et al.*, 2013; MARTINS *et al.*, 2010).

Studies conducted in altitudinal gradients (FAUTH *et al.*, 1989; STEVENS, 1992; GIARETTA *et al.*, 1999; LOMOLINO, 2001; NAVAS, 2003; MCCAIN, 2004; HU *et al.*, 2011) and latitudinal gradients (PIANKA, 1966; STEVENS, 1989; GASTON, 2000; WILLIG *et al.*, 2003) suggest that the geographical distribution is related to regional differences. In the case of anurans, most of these studies aimed to compare differences between the composition and species richness along gradients (e.g., STEVENS, 1989; GIARETTA *et al*., 1999; LOMOLINO, 2001; MCCAIN, 2004; HU *et al*., 2011). Eventually the studies approach interpopulation morphological variation (DALY & MYERS, 1967; BENITEZ-DIAZ, 1993; ROSSO *et al*., 2004; MARCELINO *et al*., 2009; TAZZYMAN & IWASA, 2010), and few studies were conducted dedicated to spatial variation in color (e.g., NEVO, 1973; GALEOTTI *et al*., 2003; HOEKSTRA *et al*., 2004; HOFFMAN *et al*., 2006; FORSMAN *et al*., 2008; BATALHA-FILHO *et al*., 2009; ROBERTSON & ZAMUDIO, 2009; ALHO *et al*., 2010). In these studies it was observed a large interpopulational variation, usually related to species with conspicuous coloration, often considered aposematic (DALY & MYERS, 1967; ROBERTSON & ZAMUDIO, 2009; TAZZYMAN & IWASA, 2010; HOOGMOED & AVILA-PIRES, 2012). Although there is a considerable number studies of coloration in anurans (e.g., KING *et al*., 1994; HOFFMAN & BLOUIN, 2000; SUMMERS & CLOUGH, 2001; SCHAEFER *et al*., 2002; TATTERSALL *et al*., 2006; ROBERTSON & ZAMUDIO, 2009; TOLEDO & HADDAD, 2009; BELL & ZAMUDIO, 2012; HOOGMOED &AVILA-PIRES, 2012) and many other studies of anurans in gradients, we did not find studies correlated the variation of chromatic polymorphism with latitudinal gradients.

Our goal in this study was to evaluate if there is a correlation of different phenotypes of *Ischnocnema guentheri-henselii* complex and of *I. parva* with geographical location and climatic factors comparing the coloration of populations of different latitudes. In this work we tested the following hypotheses: (1) there are frequency differences in the color pattern of *I. guentheri-henselii* complex and *I. parva* related to different latitudes, (2) there are frequency differences in the color pattern of the *I. guentheri-henselii* complex and *I. parva* in different localities, and (3) differences between latitudes and localities are related to climatic factors. To test these hypotheses, we analyze the distribution of different color patterns in two species of Brachycephalidae anurans of the Atlantic rainforest.

Methods

The data of the *I. guentheri-henselii* complex and *I. parva* were obtained in scientific collections and subsequently analyzed statistically.

Visits to museums

Between January and June 2012 were examined specimens of *I. guentheri-henselii* complex and *I. parva* in four scientific collections: Museu de Zoologia "Prof. Adão José Cardoso" (ZUEC), Universidade de Campinas (UNICAMP); Coleção Célio F. B. Haddad (CFBH), Universidade Estadual Paulista (UNESP); Museu de Zoologia, Universidade de São Paulo (MZUSP); and Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ). All specimens were photographed and the following data were collected: snout-vent length (SVL, measured with a digital caliper), sex (when it was possible to identify: male or female), municipality, specific locality in the municipality, and when present, also latitude, longitude and altitude, year of collection and color pattern (explained below).

The specimens were sorted and eliminated those: 1) collected previously to 1940, because of lack of a very distinct coloration; 2) whose locality were not consistent with the distribution of species or whose morphology suggested some misidentification; and 3) whose locality data were not precise to make impracticable the determination of latitude. This sorting allowed us to use 1590 specimens of the *I. guentheri-henselii* complex and 846 of *I. parva*.

Color patterns

The color pattern of individuals was initially determined based on four morphological characteristics and their states: dorsal stripe – white (B) or absent (A); dorsolateral stripes – white (B), black (P) or absent (A); general pattern – generally slick and may be presence of dirt or eyespots (L) or brindle dorsum, characterized by the predominance of more than one color (R); flank – equal or slightly lighter than the dorsum, not characterizing a dorsoconcolor pattern (I), lighter than the dorsum (C) or darker than the dorsum (E). The combination of these four state variables resulted in different patterns of coloration for each species, and here we call this methodology "4var pattern". In *I. guentheri-henselii* complex, 20 morphotypes were identified for 4var pattern (Figure 1, Table S1). In *I. parva*, 22 morphotypes were recognized for 4var pattern (Figure 2, Table S2).

Alternatively, Heyer (1984) suggested other color patterns, which we also considered, with adaptations to classify specimens of *I. guentheri* (currently needing redetermination). This classification system herein is referred to as "color patterns according to Heyer (1984)". Since the aim of this study was to compare color patterns in gradients, we considered only the dorsal general patterns, because it is the most exposed surface of the anuran and hence it is more related to predators and climatic variables. Heyer (1984) found three main general dorsal patterns: (1) uniform or spotted: A, (2) dorsoconcolor: B, and (3) wavy stripes: C. Furthermore, he subdivided into discrete states, totaling 14 dorsal color patterns: A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, B-1, B-2 and C. In the present work, the following patterns were not considered: A-3, because they were not located specimens that corresponded to it, and A-10, because in our understanding is very similar to A-1. It also included the B-3 pattern, which was not provided in the work by Heyer (1984) and characterized by having the flank lighter than the dorsum.

Therefore, in our work we use only 13 color patterns according to Heyer (1984) (Figure 3, Table S3).

Data Analysis

The sexual dimorphism was analyzed by a chi-square test between the color patterns and the specimens that was possible to determine the sexes (1281 of *I. guentheri-henselii* complex and 614 of *I. parva*). We prepared proportion graphics for females and males for 4var pattern to *I. guentheri-henselii* complex, 4var pattern to *I. parva* and color patterns according to Heyer (1984) to *I. guentheri-henselii* complex.

The latitude of the specimens that did not contain this information was determined according to the centroid of the municipality and subsequently grouped on gradients of 0.5 to 0.5 latitude degrees, since there was a large quantity of localities and few specimens in each. To *I. guentheri-henselii* complex, we analyzed the following 12 latitude gradients, which had between 19 and 476 individuals: -20.5 to -21; -21,5 to -22; -22 to -22,5; -22,5 to -23; -23 to -23,5; -23,5 to -24; -24 to -24,5; -24,5 to -25; -25 to -25,5; -25,5 to -26; -26 to -26,5. To *I. parva* we analyzed following 4 latitude gradients, which had between 52 and 535 individuals: -22 to -22,5; -22,5 to -23; -23 to -23,5; -23,5 to -24.

We also performed a sampling with localities that had 20 or more specimens. To *I. guentheri-henselii* complex 21 localities had 20 or more specimens for comparison and were identified by the following abbreviations: Rio de Janeiro, RJ (RJ-RIJ) (*I. guentheri*); São Paulo, SP (SP-SAP) (*I. henselii*); Teresópolis, RJ (RJ-TER) (*Ischnocnema* sp.); Santo André, SP (SP-STA); São Bernardo do Campo, SP (SP-SBC) (*I. henselii*); Resende, RJ (RJ-RES); São José do Barreiro, SP (SP-SJB) (*Ischnocnema* sp.); Campos do Jordão, SP (SP-CJD) (*Ischnocnema* sp.); Cachoeiras de Macacu, RJ (RJ-CMC) (*Ischnocnema* sp.); Mogi das Cruzes, SP (SP-MGC);

Boracéia, São Sebastião, SP (SP-BOR); Guaratuba, PR (PR-GRT) (*Ischnocnema* sp.); São Luís do Paraitinga, SP (SP-SLP) (*Ischnocnema* sp.); Petrópolis, RJ (RJ-PTP) (*Ischnocnema* sp.); Novo Horizonte, SC (SC-NVR); Piedade, SP (SP-PDD) (*Ischnocnema* sp.); Jundiaí, SP (SP-JUD) (*Ischnocnema* sp.); Ilhabela, SP (SP-IBL) (*Ischnocnema* sp.); Pilar do Sul, SP (SP-PLS); São Miguel Arcanjo, SP (SP-SMA); and Ribeirão Grande, SP (SP-RGD) (*I. henselii*). For *I. parva* 13 localities had 20 or more specimens: Ilhabela, SP (SP-IBL); São Paulo, SP (SP-SAP); Caraguatatuba, SP (SP-CRG); Rio de Janeiro, RJ (RJ-RIJ); Mogi das Cruzes, SP (SP-MGC); Teresópolis, RJ (RJ-TER); Ubatuba, SP (SP-UBT); Santo André, SP (SP-STA); Bertioga, SP (SP-BTO); Salesópolis, SP (SP-SLS); São Luís do Paraitinga, SP (SP-SLP); Bananal, SP (SP-BNN); and Santana do Parnaíba, SP (SP-STP).

The color patterns were analyzed statistically using a G test, which assessed whether there were differences in the proportion of each morphotype for 1) latitudes and 2) localities.

From the latitude gradients, we performed a spatial analysis through proportion graphics by latitude plotted on maps in DIVA-GIS® software. Spatial analysis was made for the three predefined patterns: 4var pattern to *I. guentheri-henselii* complex, 4var pattern to *I. parva* and color patterns according to Heyer (1984) to *I. guentheri-henselii* complex. For the same patterns we also prepared comparative graphics between the proportional frequency variation and latitude (Figure S1) and between the proportional frequency variation and locality (Figure S2).

For localities with more than 20 individuals, the color patterns were subjected to a cluster analysis and to canonical correspondence analysis (CCA), both made in the Past® software. The cluster analysis and the CCA were made for the three predefined patterns. CCA evaluated the patterns according to six environmental predictor variables, obtained by Diva-GIS BIOCLIM® software: annual mean (A), maximum temperature in the warmest month (B), minimum temperature in the coldest month (C) gradient annual temperature (D), mean temperature in the warmest quarter (E) and mean temperature in the coldest quarter (F). Both analyzes were illustrated to correlating locality with latitude. For the same localities we also prepared bar graphs comparing the proportional frequency variation and the locality.

Sample rarefaction curves were performed in software Past® to standardize the sample size to 21 individuals in every locality; every specimen corresponded to one sample. T test was too conducted in the Past® software to verify differences in the number of patterns between the localities that spatial analyzes (cluster and CCA) showed how divergent.

Results

In the comparison of the frequency for color patterns between sexes (Figure 4) we found difference for the 4var pattern of the *I. guentheri-henselii* complex (chi-square p=0.0424) and we found no difference for the color patterns according to Heyer (1984) of *I. guentheri-henselii* complex (chi-square p=0.2815) and for the 4var pattern of the *I. parva* (chi-square p=0.1179).

We found difference (G test P<0.05) in the distribution of each morphotype at different latitudes of the following patterns: 4var of the *I. guentheri-henselii* complex, morphotypes ABRI, APLC, APRI, BPLE and BPRI; 4var of *I. parva*, morphotypes ABLI, ABRC, ABRI, BBLC and BPRI and color patterns according to Heyer (1984) of *I. guentheri-henselii* complex, morphotypes A-6, A-7, B-3 and C. For other morphotypes there was no difference (P>0.05, Table 1). The G test has detected differences in the following morphotypes by locality in the 4var pattern of the *I. guentheri-henselii* complex: ABRI, APLC, APRC, APRI, BARI, BBRI, BPLE and BPRI. In the 4var pattern of *I. parva*, one of morphotypes did not occurred in any of the selected localities (BPLE), so that was not included in the analysis by localities; therefore were analyzed 21 patterns in localities. Frequency differences by latitude were detected in the following morphotypes: AARC, AARE, AARI, ABLI, ABRC, ABRI, APLE and BBRC. In the color patterns according to Heyer (1984) of *I. guentheri-henselii* complex the following morphotypes have had difference: A-1, A-4, A-5, A-7, B-1, B-2, B-3 and C (Table 2). The G test showed significant difference in the frequency distribution the part of the color patterns by latitude and practically half of the color patterns by locality.

The maps showed no clear trend in the increase or decrease in the frequency of the patterns in either case (Figure 5), although they can be noted variations in the frequency of all patterns between the latitudes for both species (Figure S1).

Cluster analysis and CCA showed a tendency to grouping together for 4var pattern of the *I. guentheri-henselii* complex (Figure 6) and color patterns according to Heyer (1984) of *I. guentheri-henselii* complex (Figure 7) in the localities of highest latitude (SP-RGD, -24º; PR-GRT, -25º; and SC-NVR,-26º). The considered localities are graphically represented in Figure 8. To *I. parva*, analyzes showed no tendency (Figure 9). In all analyzes, the CCA indicated that the climate variable annual temperature gradient (D) is divergent from the other variables.

The graphics of the sample rarefaction curves indicated differences in standardized N between localities for the 4var pattern of *I. guentheri-henselii* complex (Fig. S3) for color patterns according to Heyer (1984) of *I. guentheri-henselii* complex (Figure S4) and for the 4var pattern of *I. parva* (Figure S5). The T test indicated a significant difference in the amount of patterns of the *I. guentheri-henselii* complex between the localities of higher latitudes (SP-RGD, -24º; PR-GRT, -25º; and SC-NVR,-26º) and the others in the 4var pattern for *I. guentheri-henselii* complex (t=3.12, gl=19, two-tailed t =2.09), however indicated no difference in the color patterns according to Heyer (1984) for *I. guentheri-henselii* complex (t=1.45, gl=19, two-tailed t =2.09).To *I. parva*, the test also revealed no differences (t=-0.631, gl=11, two-tailed t =2.20).

Discussion

Heyer (1984) found no difference between the frequencies of dorsal color patterns for the sexes in *I. guentheri*, and attributed the differences in pattern among juveniles, males and females to any selection or ontogenetic change. However, our study revealed that there are differences between the sexes, at least for 4var pattern of *I. guentheri-henselii* complex. However, there is no evidence that the coloration of *I. guentheri-henselii* complex has any implication on sexual selection, as this species reproduces at night (HEYER *et al,*, 1990; HADDAD *et al.* 2013) and this reduces the possibility of sexual selection of color or pattern characteristics (HOFFMAN & BLOUIN, 2000). Sexual dimorphism in *I. guentheri-henselii* complex, as well as in *I. parva*, is evidenced only by differences in size (CRC females larger than CRC males) and the presence of vocal slits in males (HEYER, 1984). These differences in the patterns frequency observed between populations can be related to the existence of a large species complex, as shown recently (GEHARA *et al.*, 2013). However, since we do not have a good resolution to delimit species, we cannot separate the patterns between different evolutionary units. Regarding *I. parva*, whose analysis for sexual dimorphism resulted in no difference, we believe, since this is a species of more restricted distribution, is subject to a lower climatic variation and probably is better resolved in terms of taxonomy.

Although the maps of this work does not clearly indicate differences between the frequency of the patterns, can be perceived subtle differences in frequency between some patterns in the graphics of latitude and locality. It is possible that the difference found in frequency of the patterns is due to the fact that distinct species are distributed in different localities, as seen with the same species and some of the same specimens in the work of Gehara et al. (2013), who obtained evidence that *I. guentheri*, *I. henselii* and four new species presents distribution including simpatrys and sintopys.

The south of the state of São Paulo is a region that should have a significant geographical barrier that impacts the distribution of several groups of animals, such as bees (BATALHA-FILHO *et al*., 2009; BATALHA-FILHO *et al*., 2010; BATALHA-FILHO & MIYAKI, 2011), birds (CABANNE *et al*., 2007; CABANNE *et al*., 2008) and snakes (GRAZZIOTIN *et al*., 2006). The same is also observed for amphibians such as the *Rhinella crucifer* group (THOMÉ *et al*., 2010), the *Aparasphenodon* genus (NETO & TEIXEIRA-JR, 2012), and species of the genus *Brachycephalus*, *Cycloramphus*, *Proceratophrys*, *Aplastodiscus*, *Dendropsophus*, *Scinax*, *Hylodes*, *Physalaemus*, *Leptodactylus,* and *Ischnocnema* (HADDAD *et al*., 2013). This is also the case with some newly identified cryptic species in *I. guentheri-henselii* complex (GEHARA *et al*., 2013). This barrier not only affects the distribution limits of the species, or higher taxa, but also is well known cases of morphological differentiation between populations above and below this region. For example, populations of *Hypsiboas bischoffi* differs by having stripes to the north and does not have stripes to the south of this region (MARCELINO *et al*., 2009). Most of these work attributes the existence of this limit to the theory of forest refuges (HAFFER, 1969), which justifies the differentiation occurred in the tropics by climatic fluctuations that have caused successive retractions of forest environments during the Pleistocene. However, Rodríguez *et al*. (2012) attributed the diversity of the *Eleutherodactylus limbatus* group in eastern Cuba to the environmental heterogeneity of the region, characterized by mountain massifs of divergent geological history interrupted by lowlands, with high bioclimatic variation between individual sites. Heyer (1984) affirmed that there was no evidence of a geographic component to intraspecific variation, but based on the results obtained in this study and reports in the literature, it is plausible that the geological history and the resulting climate change are the cause for the difference in frequency of patterns found in this study. This barrier can be related to an interruption that the Serra do Mar suffers between the states of São Paulo and Paraná, mostly caused by erosion due to the rivers Ribeira de Iguape, Juquiá and, more recently, Cubatão river (DE ALMEIDA & CARNEIRO, 1998). It caused, among other factors, a discontinuity in altitude between the portion of the Serra do Mar in São Paulo, which has altitudes between 800 and 1200 m, and Paraná, which reaches up to 1800 m (DE ALMEIDA & CARNEIRO, 1998), and the altitude is a remarkable barrier to amphibian populations, causing reproductive isolation between upper and lower populations (e.g. RODRÍGUEZ *et al.*, 2012).

Rodríguez *et al.* (2012) observed that the temperature, in seven different modalities, is the most significant climatic variable in their analysis of PCA. In the present work, the climatic variable annual temperature gradient showed that differs from the others in the CCA, which may be an explanation for the difference found in the frequency patterns, since the localities of highest latitude tend to have lower annual temperature than localities of lower latitudes.

Rodríguez *et al.* (2012), in their work, concluded that the color patterns are not stable between the species of the *E. limbatus* group. Hoffman *et al.* (2006) compared loci spatial and temporal in samples to detect selection, but found no evidence for the existence of selection on the locus of color. In the present work, it was not possible to verify the occurrence of fluctuations in the frequency of morphotypes along time, because we did not obtain many samples from the same locality at different times. We suggest that future studies may experimentally test the effects of predation on the frequency of polymorphic species color patterns in order to check the fluctuation morphotype over time.

Hoogmoed & Avila-Pires (2012), in their work with *Adelphobates galactonotus*, reported deforestation and urbanization as some of the major threats to the species and as a factor limiting the distribution of this species. According to Heyer (1984), the pattern of local differentiation indicates that historical factors are crucial to understanding the current patterns of distribution and differentiation of the species. Given that the loss and fragmentation of habitat constitutes the main factors that affect amphibians (CUSHMAN, 2006; HADDAD *et al*., 2013; BECKER *et al*., 2007) and color patterns may not be stable (e.g. RODRÍGUEZ *et al.*, 2012), we cannot ignore that the intensive urban occupation in southeastern and southern Brazil, such as any kind of barrier, may have contributed to the emergence of the frequency differences in color patterns between populations due to reduced gene flow, but this hypothesis also needs further investigation.

The coloration may be taxonomically relevant in the identification and diagnosis of amphibian species. In anurans of the *Eleutherodactylus* genus in Cuba, the taxonomy is almost entirely based on differences in color pattern (RODRÍGUEZ *et al*., 2012). In the present study, using only the coloration was possible to identify a barrier in the distribution of *I. guentheri-henselii* complex into the south of the State of São Paulo. Comparing the results obtained in this study with those of Gehara *et al*. (2013), we can suggest that the changes in the color patterns at the beginning of Serra de Paranapiacaba may be due to the limit distribution of a not described species from the work of Gehara *et al*. (2013), CS3 species, which not extending beyond the south of São Paulo. The absence of this species further south may cause a shift in the observed patterns. Furthermore, we can suggest that color patterns may be used to limit recognition of the distribution to the taxa of *Ischnocnema* genus.

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Table 1. Results of the G test for the latitudes in the 4var pattern and color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex and 4var pattern of *I. parva*.

4var pattern of *I. guentheri-henselii* complex				**Color patterns according to Heyer (1984) of *I. guentheri-henselii* complex**				**4var pattern of *I. parva***			
AALI	0,7979	**BALI**	0,9829	**A1**	0,4836	**B2**	0,0963	**AALC**	0,0793	**APRI**	0,2343
AARI	0,1781	**BARI**	0,2524	**A2**	0,3610	**B3**	0,0069	**AALI**	0,2276	**BALC**	0,3071
ABLI	0,6933	**BBLI**	0,3424	**A4**	0,4552	**C**	$4,69^{-04}$	**AARC**	0,3074	**BALI**	0,8260
ABRI	0,0083	**BBRI**	0,2993	**A5**	0,1950			**AARE**	0,2462	**BARC**	0,8260
APLC	0,0057	**BPLC**	0,5735	**A6**	0,0018			**AARI**	0,2912	**BBLC**	0,0303
APLE	0,3348	**BPLE**	0,0344	**A7**	0,0066			**ABLC**	0,4204	**BBLI**	0,0720
APLI	0,6039	**BPLI**	0,4207	**A8**	0,0862			**ABLI**	0,0261	**BBRC**	0,0505
APRC	0,0750	**BPRC**	0,5394	**A9**	0,9658			**ABRC**	0,0323	**BBRI**	0,2346
APRE	0,0732	**BPRE**	0,8435	**A11**	0,0841			**ABRI**	0,0059	**BPLE**	0,0986
APRI	0,0145	**BPRI**	0,0078	**B1**	0,0909			**APLE**	0,0751	**BPRE**	0,5759
								APLI	0,8996	**BPRI**	0,0353

Table 2. Results of the G test for the localities in the 4var pattern and color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex and 4var pattern of *I. parva*.

4var pattern of *I. guentheri-henselii* complex				**Color patterns according to Heyer (1984) of *I. guentheri-henselii* complex**				**4var pattern of *I. parva***			
AALI	0,1542	**BALI**	0,8328	**A1**	0,0434	**B2**	0,0080	**AALC**	0,1648	**APRI**	0,9613
AARI	0,4337	**BARI**	0,0124	**A2**	0,0960	**B3**	$1,24^{-06}$	**AALI**	0,1883	**BALC**	0,8996
ABLI	0,1871	**BBLI**	0,4197	**A4**	0,0221	**C**	$1,22^{-04}$	**AARC**	$5,87^{-04}$	**BALI**	0,9254
ABRI	0,0052	**BBRI**	0,0387	**A5**	0,0021			**AARE**	0,0454	**BARC**	0,9667
APLC	$2,44^{-05}$	**BPLC**	0,9243	**A6**	0,0742			**AARI**	0,0018	**BBLC**	0,3347
APLE	0,2174	**BPLE**	0,0201	**A7**	$4,08^{-04}$			**ABLC**	0,0612	**BBLI**	0,0855
APLI	0,4381	**BPLI**	0,8737	**A8**	0,9921			**ABLI**	0,0137	**BBRC**	0,0480
APRC	0,0028	**BPRC**	0,9648	**A9**	0,2468			**ABRC**	$1,34^{-04}$	**BBRI**	0,6318
APRE	0,0527	**BPRE**	0,0843	**A11**	0,0895			**ABRI**	$8,26^{-07}$	**BPLE**	---
APRI	0,0057	**BPRI**	$1,39^{-04}$	**B1**	0,0046			**APLE**	0,0263	**BPRE**	0,8345
								APLI	0,6208	**BPRI**	0,1727

Figures

Figure 1. 4var color patterns of *Ischnocnema guentheri-henselii* complex: AALI (A); AARI (B); ABLI (C); ABRI (D); APLC (E); APLE (F); APLI (G); APRC (H); APRE (I); APRI (J); BALI (K); BARI (L); BBLI (M); BBRI (N); BPLC (O); BPLE (P); BPLI (Q); BPRC (R); BPRE (S); and BPRI (T).

Figure 2. 4var color patterns of *Ischnocnema parva*: AALC (A); AALI (B); AARC (C); AARE (D); AARI (E); ABLC (F); ABLI (G); ABRC (H); ABRI (I); APLE (J); APLI (K); APRI (L); BALC (M); BALI (N); BARC (O); BBLC (P); BBLI (Q); BBRC (R); BBRI (S); BPLE (T); BPRE (U); and BPRI (V).

Figure 3. Color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex: A-1 (A); A-2 (B); A-4 (C); A-5 (D); A-6 (E); A-7 (F); A-8 (G); A-9 (H); A-11 (I); B-1 (J); B-2 (K); B-3 (L); and C (M).

Figure 4. Diagrams representing, for both sexes: the proportion of males and females for the 4var color pattern to *I. guentheri-henselii* complex (A); the proportion of males and females for the color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex (B); the proportion of males and females for the 4var color pattern to *I. parva* (C).

Figure 5. Latitudinal distribution of patterns: latitudinal distribution for the 4var color pattern of *I. guentheri-henselii* complex (A); latitudinal distribution for the color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex (B); latitudinal distribution for the 4var color pattern of *I. parva* (C).

Figure 6. Dendrogram of cluster analysis (A) and canonical correspondence analysis (B) of the 4var color pattern for *I. guentheri-henselii* complex by locality. Cophenetic correlation coefficient of the cluster analysis: r=0,70. Abbreviations of localities: SP-CJD: Campos do Jordão, SP; SP-PLS: Pilar do Sul, SP; SP-IBL: Ilhabela, SP; SC-NVR: Novo Horizonte, SC; PR-

GRT: Guaratuba, PR; SP-RGD: Ribeirão Grande, SP; SP-SJB: São José do Barreiro, SP; SP-SBC: São Bernardo do Campo, SP; SP-JUD: Jundiaí, SP; SP-SAP: São Paulo, SP; SP-SMA: São Miguel Arcanjo, SP; RJ-TER: Teresópolis, RJ; SP-BOR: Boracéia, São Sebastião, SP; RJ-RIJ: Rio de Janeiro, RJ; RJ-PTP: Petrópolis, RJ; SP-STA: Santo André, SP; SP-SLP: São Luís do Paraitinga, SP; RJ-CMC: Cachoeiras de Macacu, RJ; SP-MGC: Mogi das Cruzes, SP; SP-PDD: Piedade, SP; and RJ-RES: Resende, RJ. Abbreviation of climatic variables of canonical correspondence analysis (CCA): annual mean (A), maximum temperature in the warmest month (B), minimum temperature in the coldest month (C), annual temperature gradient (D), mean temperature in warmest quarter (E) and mean temperature in coldest quarter (F).

Figure 7. Dendrogram of cluster analysis (A) and canonical correspondence analysis (B) of the color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex by locality. Cophenetic correlation coefficient of the cluster analysis: r=0,78. Abbreviations of localities: RJ-CMC: Cachoeiras de Macacu, RJ; SP-JUD: Jundiaí, SP; SP-SLP: São Luís do Paraitinga, SP; SP-BOR: Boracéia, São Sebastião, SP; RJ-PTP: Petrópolis, RJ; RJ-TER: Teresópolis, RJ; SP-SBC: São Bernardo do Campo, SP; SP-SAP: São Paulo, SP; SP-SMA: São Miguel Arcanjo, SP; RJ-RIJ: Rio de Janeiro, RJ; SP-PDD: Piedade, SP; SP-STA: Santo André, SP; SP-MGC: Mogi das Cruzes, SP; SP-PLS: Pilar do Sul, SP; SP-IBL: Ilhabela, SP; PR-GRT: Guaratuba, PR; SP-RGD: Ribeirão Grande, SP; RJ-RES: Resende, RJ; SP-CJD: Campos do Jordão, SP; SC-NVR: Novo Horizonte, SC; and SP-SJB: São José do Barreiro, SP. Abbreviation of climatic variables of canonical correspondence analysis (CCA): annual mean (A), maximum temperature in the warmest month (B), minimum temperature in the coldest month (C), annual temperature gradient (D), mean temperature in warmest quarter (E) and mean temperature in coldest quarter (F).

Figure 8. Localities analyzed to *I. guentheri-henselii* complex. The hatched line shows the region in which we observed the differentiation of patterns, the triangles represent the localities between latitudes -22 ° and -23 °, and the squares represent the localities between -24 ° and -26 °.

Figure 9. Dendrogram of cluster analysis (A) and canonical correspondence analysis (B) of the 4var color pattern for *I. parva* by locality. Cophenetic correlation coefficient of the cluster

analysis: r=0,87. Abbreviations of localities: SP-STP: Santana do Parnaíba, SP; SP-MGC: Mogi das Cruzes, SP; RJ-RIJ: Rio de Janeiro, RJ; RJ-TER: Teresópolis, RJ; SP-IBL: Ilhabela, SP; SP-BTO: Bertioga, SP; SP-SLP: São Luís do Paraitinga, SP; SP-SAP: São Paulo, SP; SP-CRG: Caraguatatuba, SP; SP-SLS: Salesópolis, SP; SP-BNN: Bananal, SP; SP-STA: Santo André, SP; and SP-UBT: Ubatuba, SP. Abbreviation of climatic variables of canonical correspondence analysis (CCA): annual mean (A), maximum temperature in the warmest month (B), minimum temperature in the coldest month (C), annual temperature gradient (D), mean temperature in warmest quarter (E) and mean temperature in coldest quarter (F).

Figure 1

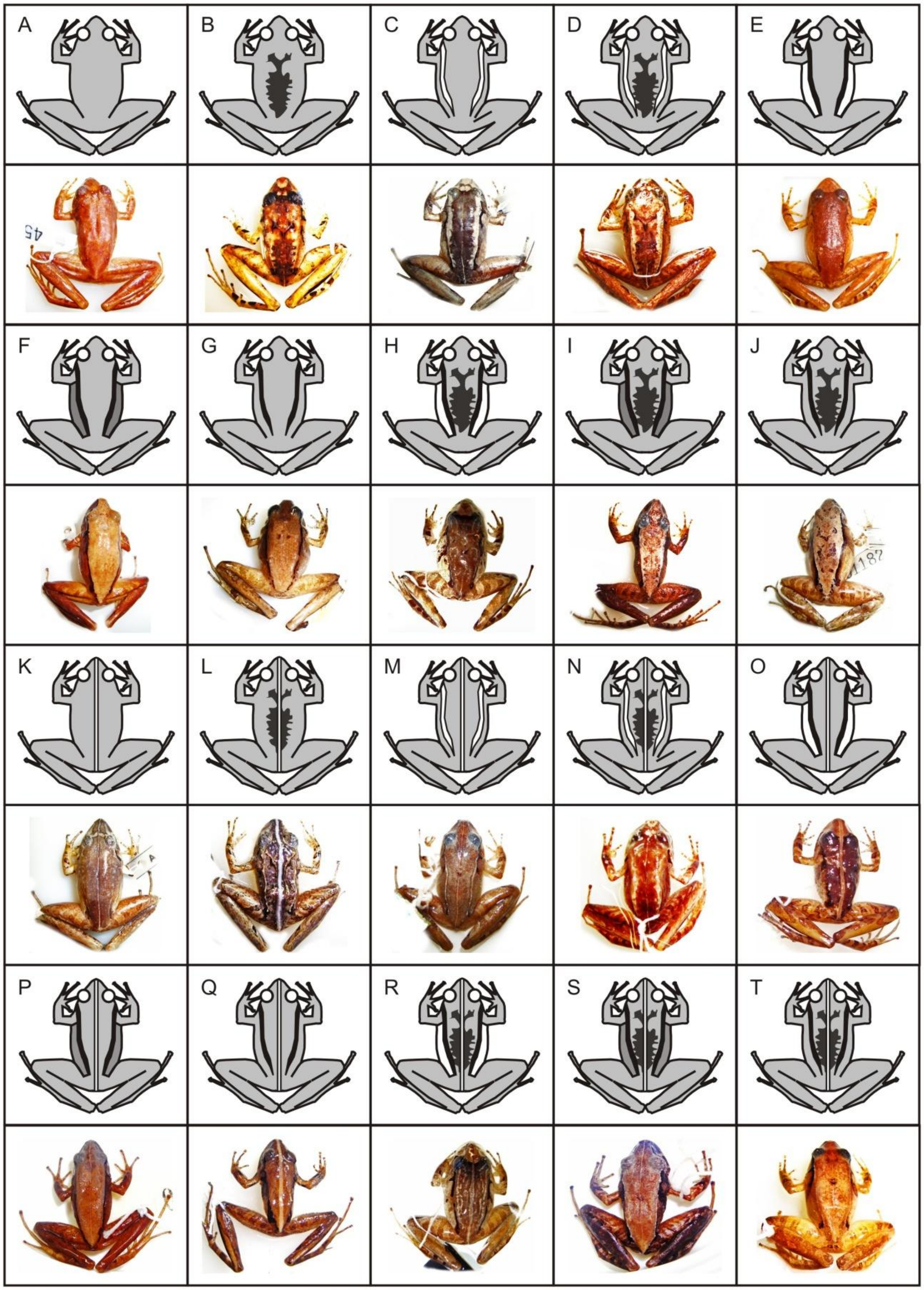


Figure 2

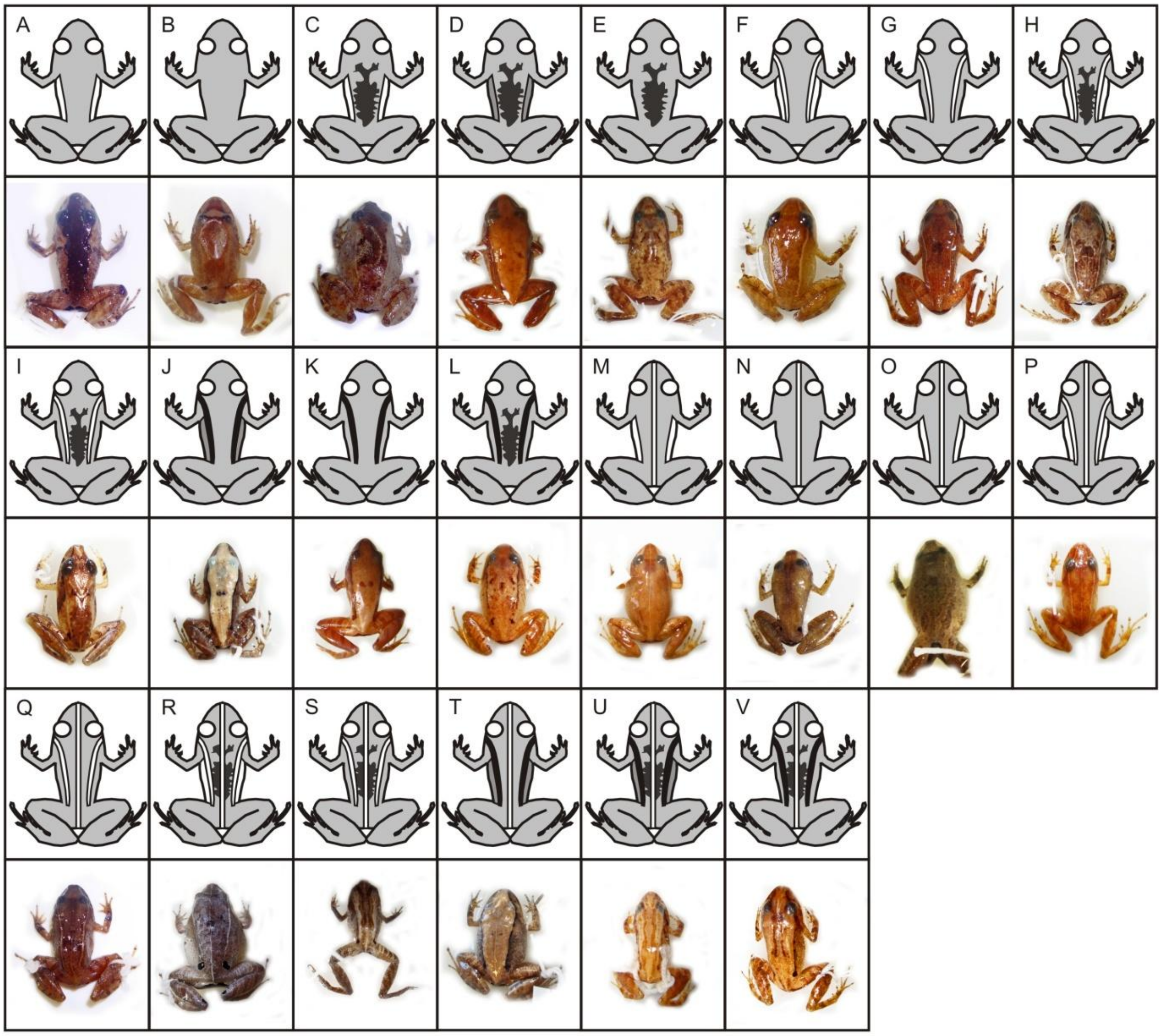


Figure 3

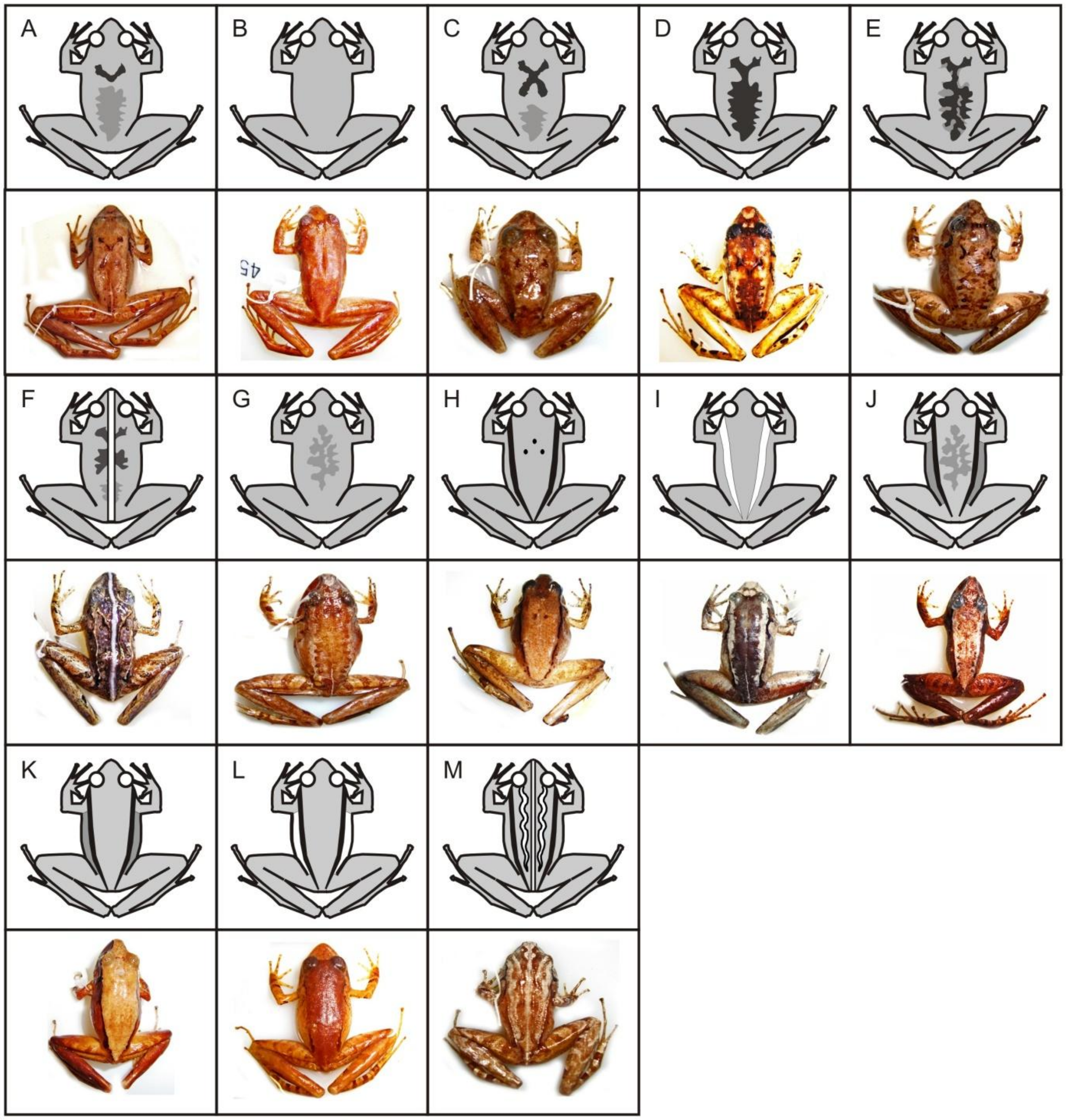


Figure 4

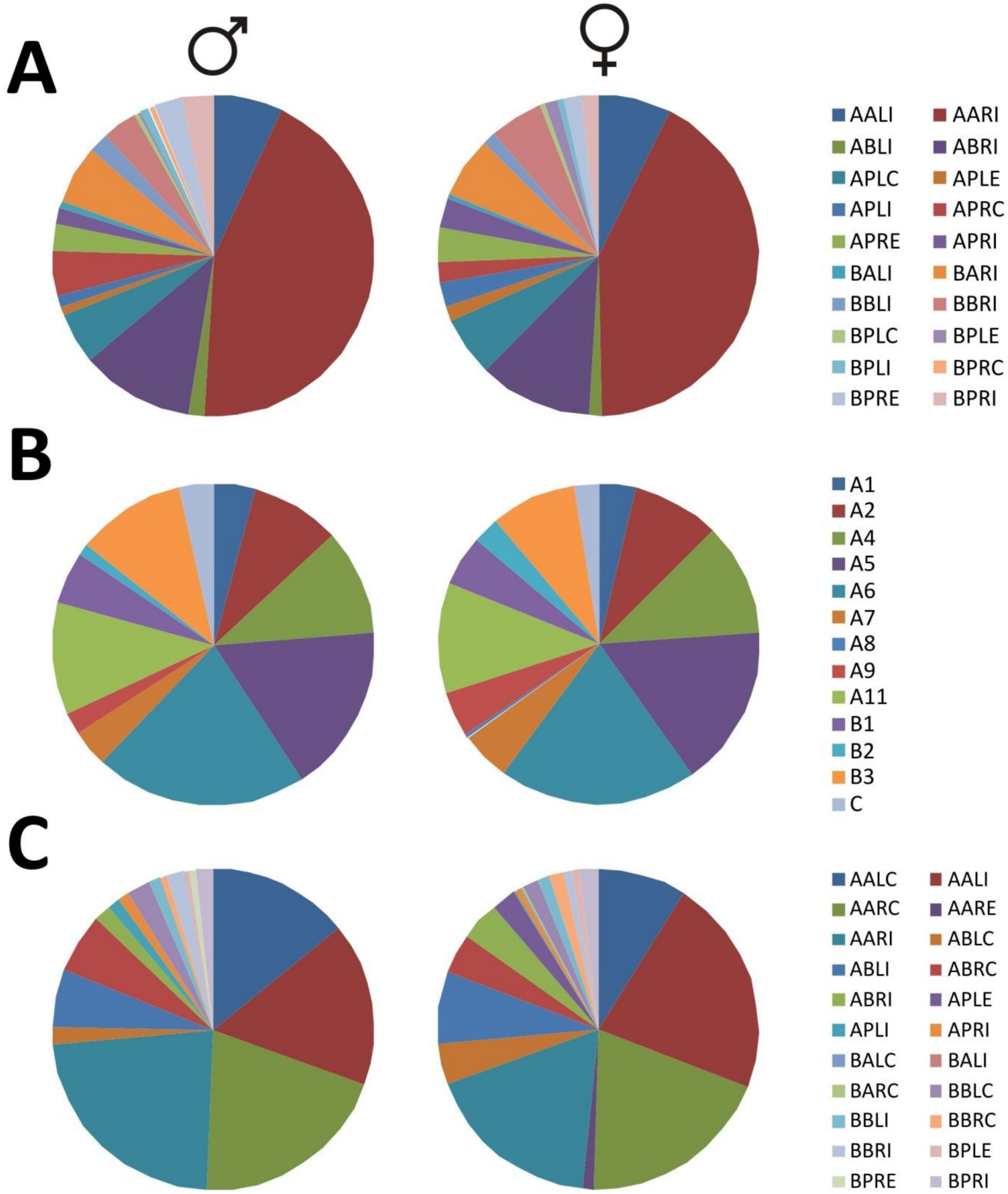

A
♂
♀
AALI
AARI
ABLI
ABRI
APLC
APLE
APLI
APRC
APRE
APRI
BALI
BARI
BBLI
BBRI
BPLC
BPLE
BPLI
BPRC
BPRE
BPRI
B
A1
A2
A4
A5
A6
A7
A8
A9
A11
B1
B2
B3
C
C
AALC
AALI
AARC
AARE
AARI
ABLC
ABLI
ABRC
ABRI
APLE
APLI
APRI
BALC
BALI
BARC
BBLC
BBLI
BBRC
BBRI
BPLE
BPRE
BPRI

Figure 5

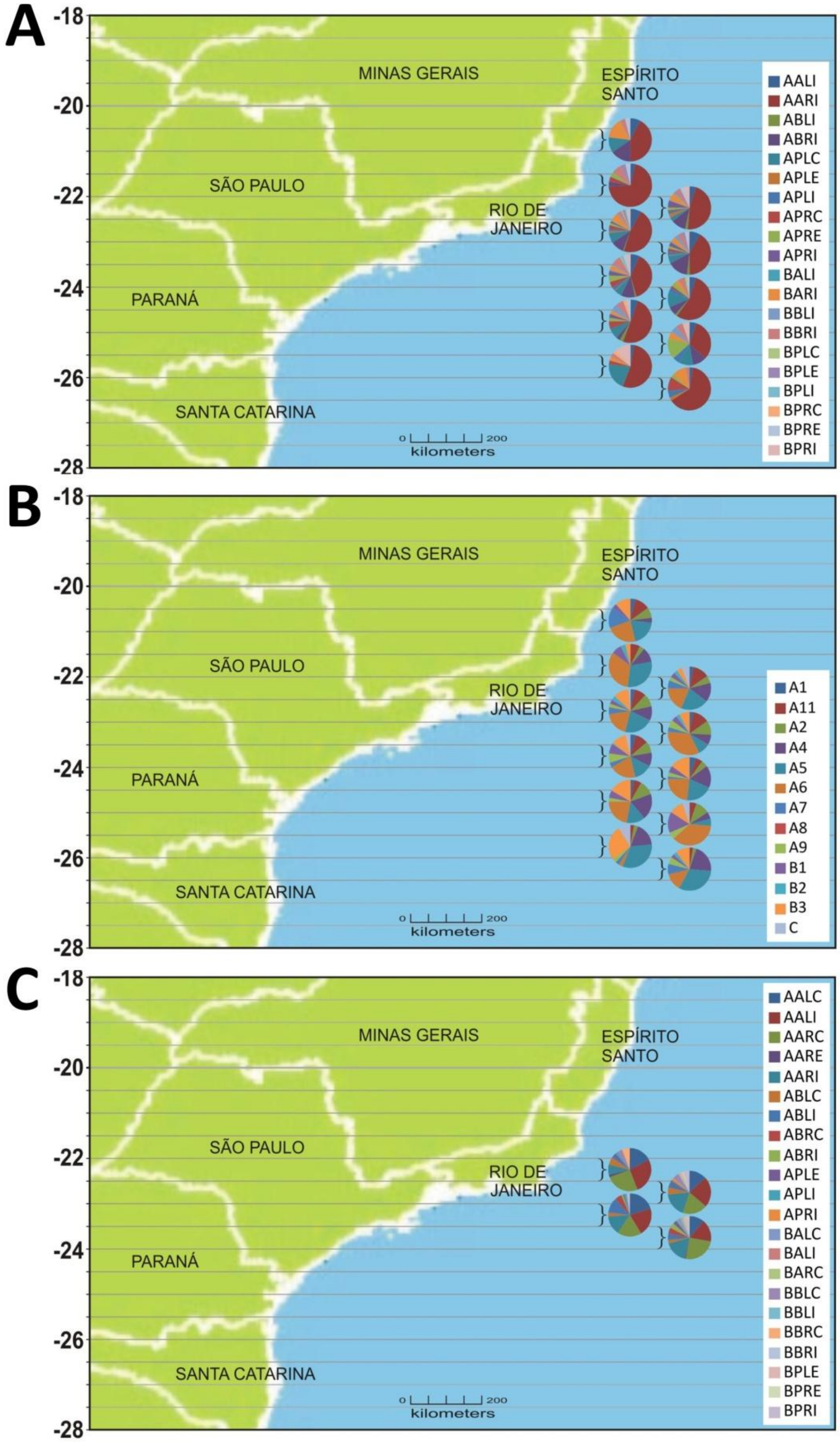

A
B
C
-18
-20
-22
-24
-26
-28
MINAS GERAIS
ESPÍRITO SANTO
SÃO PAULO
RIO DE JANEIRO
PARANÁ
SANTA CATARINA
0 200
kilometers
AALI
AARI
ABLI
ABRI
APLC
APLE
APLI
APRC
APRE
APRI
BALI
BARI
BBLI
BBRI
BPLC
BPLE
BPLI
BPRC
BPRE
BPRI
A1
A11
A2
A4
A5
A6
A7
A8
A9
B1
B2
B3
C
AALC
AALI
AARC
AARE
AARI
ABLC
ABLI
ABRC
ABRI
APLE
APLI
APRI
BALC
BALI
BARC
BBLC
BBLI
BBRC
BBRI
BPLE
BPRE
BPRI

Figure 6

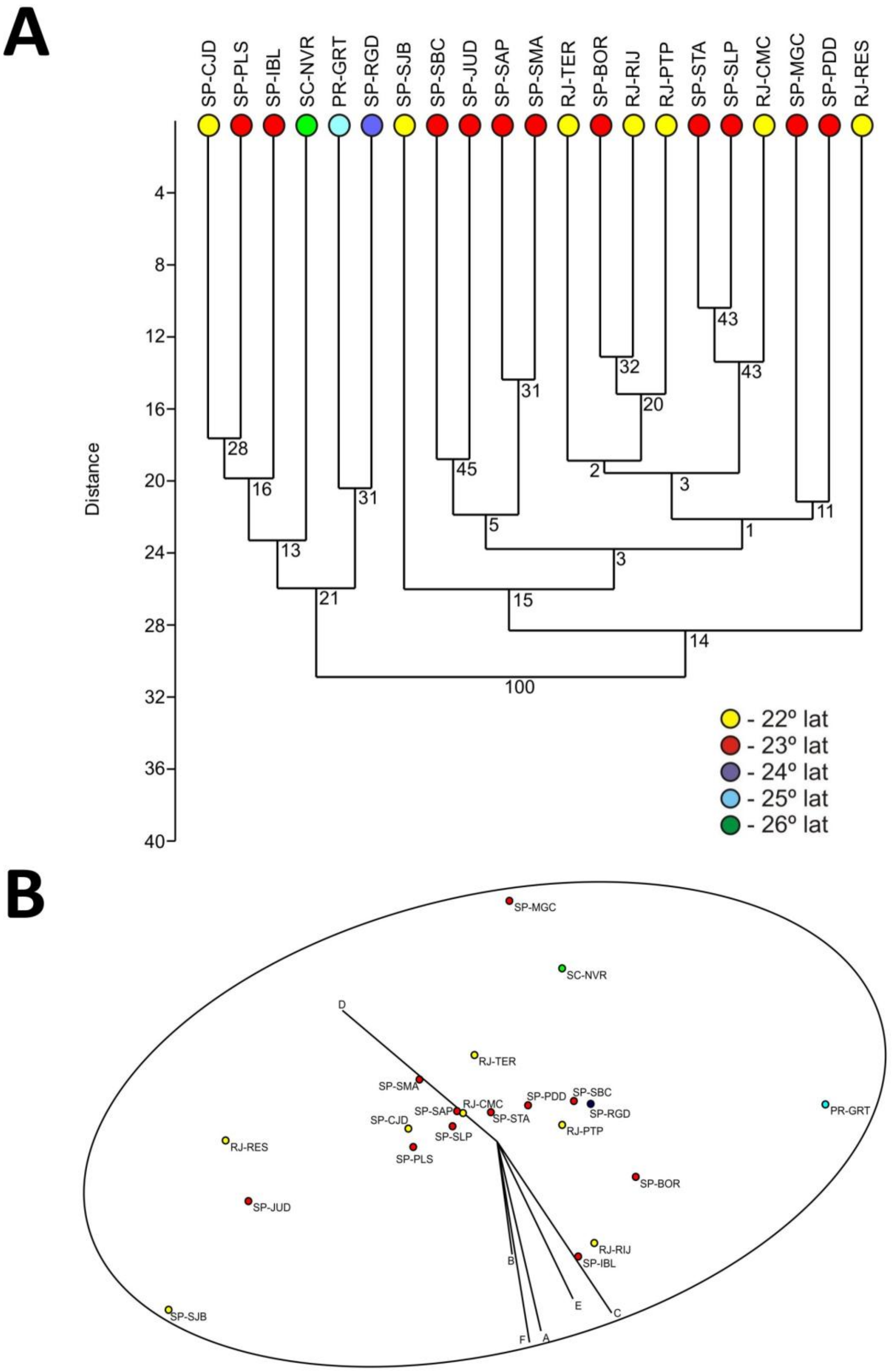

A
SP-CJD
SP-PLS
SP-IBL
SC-NVR
PR-GRT
SP-RGD
SP-SJB
SP-SBC
SP-JUD
SP-SAP
SP-SMA
RJ-TER
SP-BOR
RJ-RIJ
RJ-PTP
SP-STA
SP-SLP
RJ-CMC
SP-MGC
SP-PDD
RJ-RES
Distance
4
8
12
16
20
24
28
32
36
40
28
16
13
31
21
45
31
5
32
20
43
43
2
3
1
11
3
15
14
100
🟡 - 22º lat
🔴 - 23º lat
🟣 - 24º lat
🔵 - 25º lat
🟢 - 26º lat
B
SP-MGC
SC-NVR
D
RJ-TER
SP-SMA
SP-PDD
SP-SBC
SP-RGD
RJ-CMC
SP-SAP
SP-STA
PR-GRT
SP-CJD
RJ-PTP
SP-SLP
RJ-RES
SP-PLS
SP-BOR
SP-JUD
RJ-RIJ
B
SP-IBL
E
C
SP-SJB
F
A

Figure 7

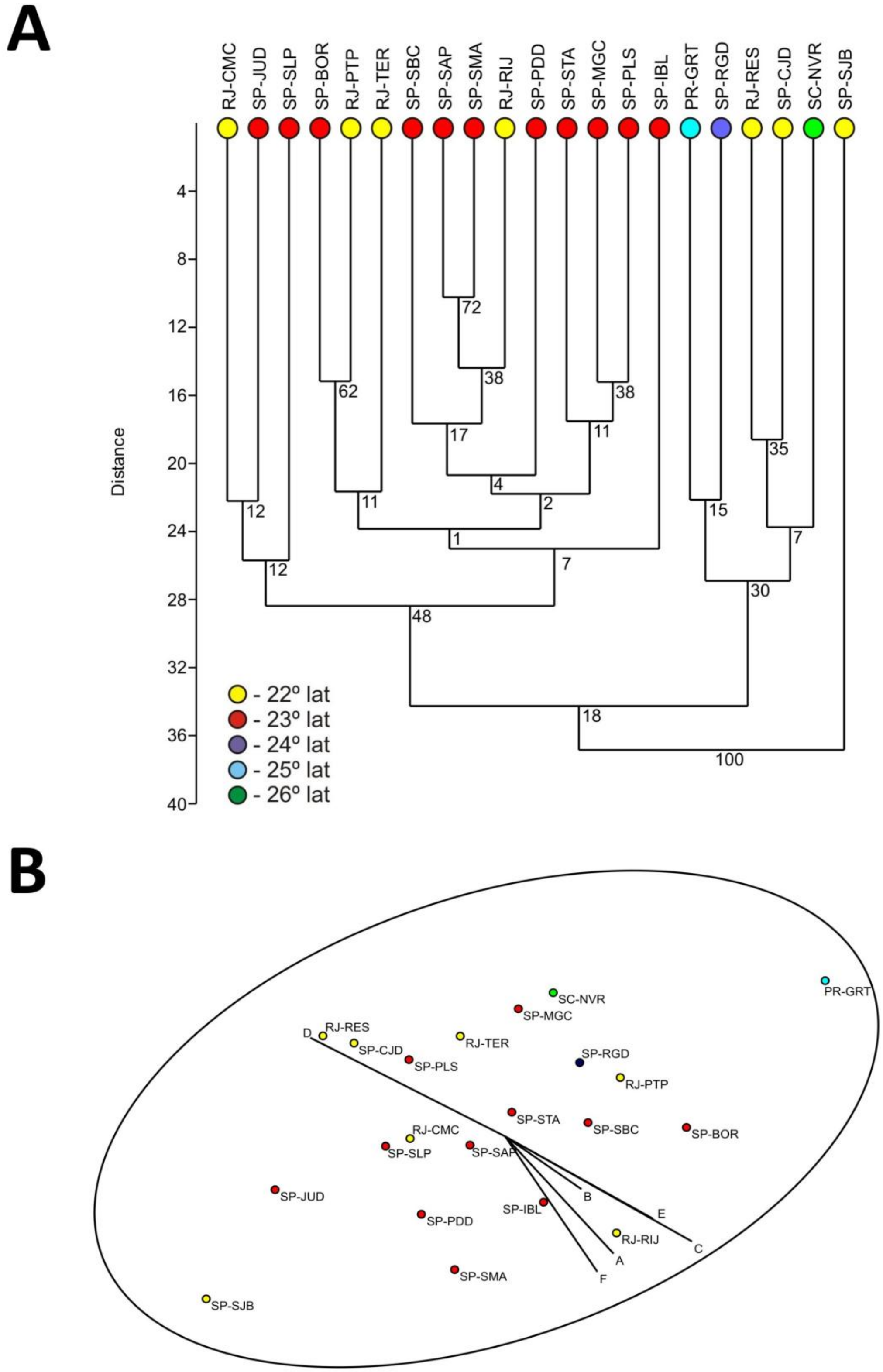

A
RJ-CMC
SP-JUD
SP-SLP
SP-BOR
RJ-PTP
RJ-TER
SP-SBC
SP-SAP
SP-SMA
RJ-RIJ
SP-PDD
SP-STA
SP-MGC
SP-PLS
SP-IBL
PR-GRT
SP-RGD
RJ-RES
SP-CJD
SC-NVR
SP-SJB
Distance
4
8
12
16
20
24
28
32
36
40
72
38
62
38
17
11
35
4
2
12
11
15
1
7
12
7
30
48
18
100
● - 22º lat
● - 23º lat
● - 24º lat
● - 25º lat
● - 26º lat
B
SC-NVR
PR-GRT
SP-MGC
D
RJ-RES
SP-CJD
RJ-TER
SP-PLS
SP-RGD
RJ-PTP
SP-STA
SP-SBC
SP-BOR
RJ-CMC
SP-SLP
SP-SAP
B
E
SP-JUD
SP-IBL
SP-PDD
RJ-RIJ
C
A
F
SP-SMA
SP-SJB

Figure 8

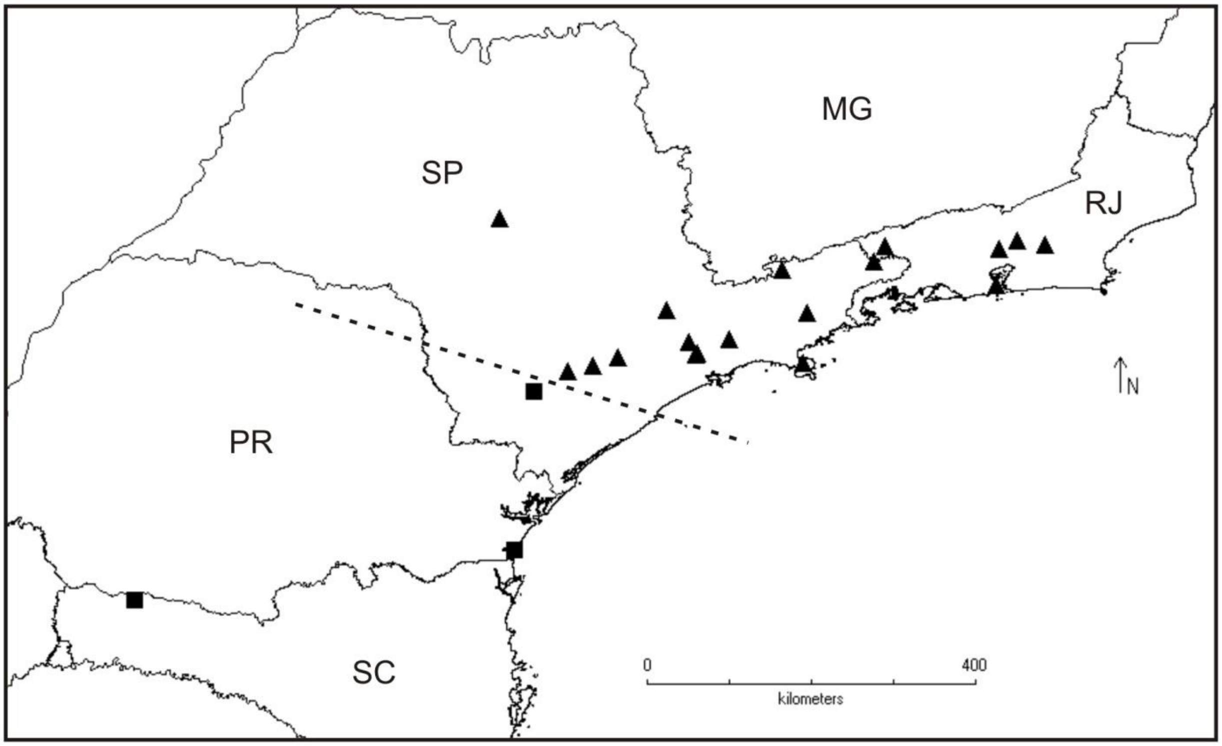
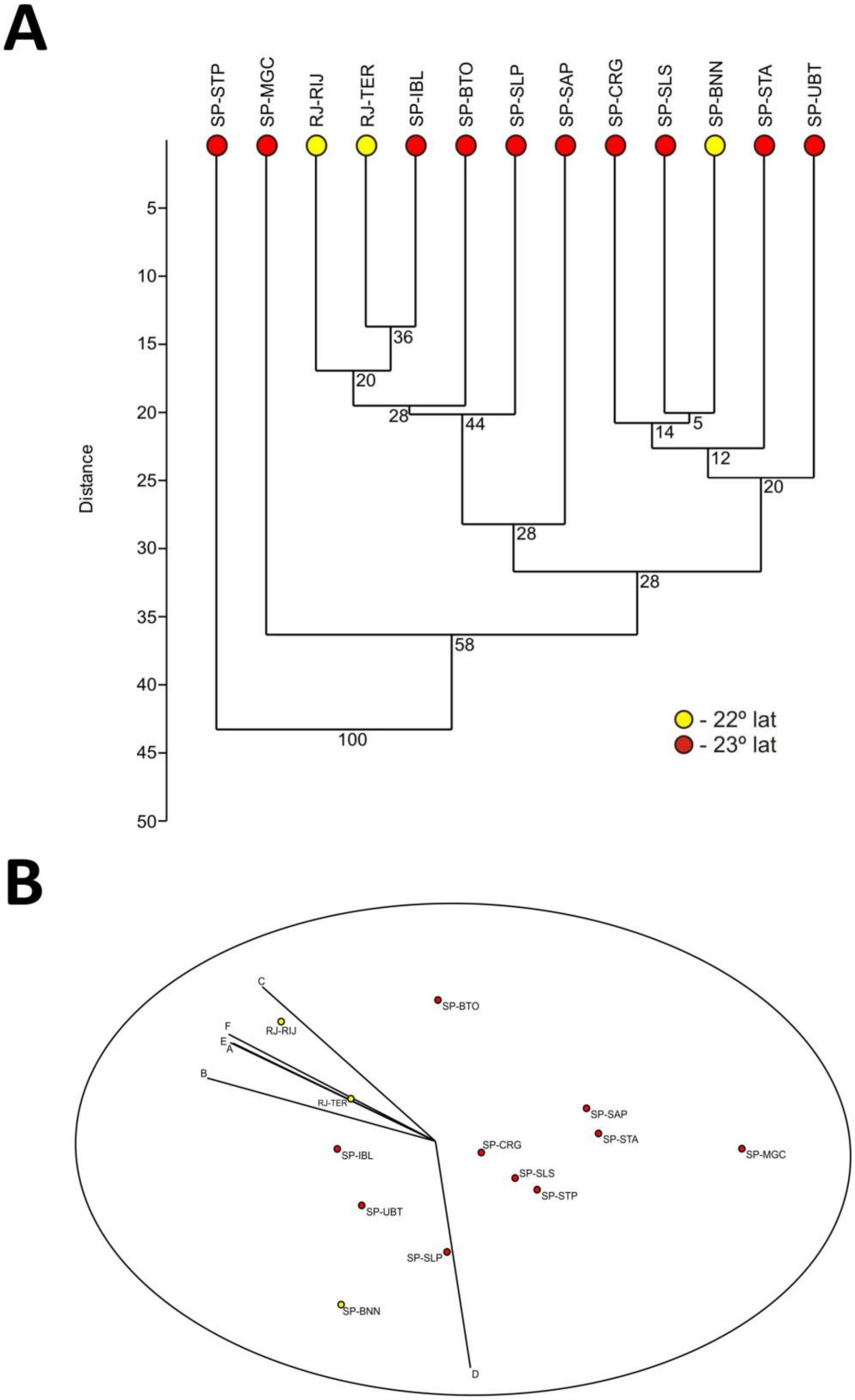


Figure 9


A
SP-STP
SP-MGC
RJ-RIJ
RJ-TER
SP-IBL
SP-BTO
SP-SLP
SP-SAP
SP-CRG
SP-SLS
SP-BNN
SP-STA
SP-UBT
Distance
5
10
15
20
25
30
35
40
45
50
36
20
28
44
5
14
12
20
28
28
58
100
🟡 - 22º lat
🔴 - 23º lat
B
C
SP-BTO
F
RJ-RIJ
E
A
B
RJ-TER
SP-SAP
SP-STA
SP-CRG
SP-MGC
SP-IBL
SP-SLS
SP-STP
SP-UBT
SP-SLP
SP-BNN
D

APPENDIX

Table S1. Description of the 4var color patterns of *I. guentheri-henselii* complex.

Pattern	Dorsal stripe	Dorsolateral stripes	General pattern	Flank
AALI	Absent	Absent	Slick	Equal
AARI	Absent	Absent	Brindle	Equal
ABLI	Absent	White	Slick	Equal
ABRI	Absent	White	Brindle	Equal
APLC	Absent	Black	Slick	Lighter
APLE	Absent	Black	Slick	Darker
APLI	Absent	Black	Slick	Equal
APRC	Absent	Black	Brindle	Lighter
APRE	Absent	Black	Brindle	Darker
APRI	Absent	Black	Brindle	Equal
BALI	White	Absent	Slick	Equal
BARI	White	Absent	Brindle	Equal
BBLI	White	White	Slick	Equal
BBRI	White	White	Brindle	Equal
BPLC	White	Black	Slick	Lighter
BPLE	White	Black	Slick	Darker
BPLI	White	Black	Slick	Equal
BPRC	White	Black	Brindle	Lighter
BPRE	White	Black	Brindle	Darker
BPRI	White	Black	Brindle	Equal

Table S2. Description of the 4var color patterns of *I. parva*.

Pattern	Dorsal stripe	Dorsolateral stripes	General pattern	Flank
AALC	Absent	Absent	Slick	Lighter
AALI	Absent	Absent	Slick	Equal
AARC	Absent	Absent	Brindle	Lighter
AARE	Absent	Absent	Brindle	Darker
AARI	Absent	Absent	Brindle	Equal
ABLC	Absent	White	Slick	Lighter
ABLI	Absent	White	Slick	Equal
ABRC	Absent	White	Brindle	Lighter
ABRI	Absent	White	Brindle	Equal
APLE	Absent	Black	Slick	Darker
APLI	Absent	Black	Slick	Equal
APRI	Absent	Black	Brindle	Equal
BALC	White	Absent	Slick	Lighter
BALI	White	Absent	Slick	Equal
BARC	White	Absent	Brindle	Lighter
BBLC	White	White	Slick	Lighter
BBLI	White	White	Slick	Equal
BBRC	White	White	Brindle	Lighter
BBRI	White	White	Brindle	Equal
BPLE	White	Black	Slick	Darker
BPRE	White	Black	Brindle	Darker
BPRI	White	Black	Brindle	Equal

Table S3. Description of the color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex and correspondence with 4var color patterns.

Pattern	Description	Correspondent in 4var
A-1	With a "V" spot in the anterior part of the dorsum	AARI, ABRI, APRI, BARI or BBRI
A-2	Slick and uniform, clear, intermediate or dark tonality	AALI, BALI or BBLI
A-4	With a "X" spot in dorsum	AARI, ABRI, APRI, BARI, BBRI or BPRI
A-5	With a "X" spot in dorsum, continuing diamond-shaped to the back	AARI, ABRI, APRI, BARI or BBRI
A-6	According to Heyer (1984), not so distinct as A-5	AARI, ABRI, APRI, BARI or BBRI
A-7	With dorsal spot interrupted by white vertebral stripe	BARI or BBRI
A-8	According to Heyer (1984), completely variegated	AARI
A-9	With dorsolateral black stripes that delimit the dorsum from the flank	APLI, APRI, BPLI or BPRI
A-11	With dorsolateral white stripes marking the dorsum side ends	ABLI, ABRI, BBLI or BBRI
B-1	Brindle dorsum lighter than the flank, with the presence of dorsolateral black stripes	APRE or BPRE
B-2	According to Heyer (1984), like a B-1 without dark spots	APLE or BPLE
B-3	With dorsum slick or brindle, darker than the flank, with the presence of dorsolateral black stripes	APLC, APRC, BPLC or BPRC
C	With wavy stripes	BBRI, BPRE or BPRI

Figure S1. Comparison of proportional frequency of the patterns by latitude: 4var color pattern of *I. guentheri-henselii* complex (A); 4var color pattern of *I. parva* (B); and color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex (C).

Figure S2. Comparison of proportional frequency of the patterns by locality: 4var color pattern of *I. guentheri-henselii* complex (A); 4var color pattern of *I. parva* (B); and color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex (C).

Figure S3. Sample rarefaction **c**urve with standard deviation for the 4var color pattern of *I. guentheri-henselii* complex for localities that had more than 21 specimens: Rio de Janeiro, RJ (A); São Paulo, SP (B); Teresópolis, RJ (C); Santo André, SP (D); São Bernardo do Campo, SP (E); Resende, RJ (F); São José do Barreiro, SP (G); Campos do Jordão, SP (H); Cachoeiras de Macacu, RJ (I); Mogi das Cruzes, SP (J); Boracéia, São Sebastião, SP (K); Guaratuba, PR (L); São Luís do Paraitinga, SP (M); Petrópolis, RJ (N); Novo Horizonte, SC (O); Piedade, SP (P); Jundiaí, SP (Q);Ilhabela, SP (R); Pilar do Sul, SP (S); São Miguel Arcanjo, SP (T); and Ribeirão Grande, SP (U). The solid line and the number correspond to the expected number of patterns for n = 21, whereas the hatched line corresponds to the standard deviation.

Figure S4. Sample rarefaction **c**urve with standard deviation for the color patterns according to Heyer (1984) of *Ischnocnema guentheri-henselii* complex for localities that had more than 21 specimens: Rio de Janeiro, RJ (A); São Paulo, SP (B); Teresópolis, RJ (C); Santo André, SP (D); São Bernardo do Campo, SP (E); Resende, RJ (F); São José do Barreiro, SP (G); Campos do Jordão, SP (H); Cachoeiras de Macacu, RJ (I); Mogi das Cruzes, SP (J); Boracéia, São Sebastião, SP (K); Guaratuba, PR (L); São Luís do Paraitinga, SP (M); Petrópolis, RJ (N); Novo Horizonte, SC (O); Piedade, SP (P); Jundiaí, SP (Q); Ilhabela, SP (R); Pilar do Sul, SP (S); São Miguel Arcanjo, SP (T); and Ribeirão Grande, SP (U). The solid line and the number correspond to the expected number of patterns for n = 21, whereas the hatched line corresponds to the standard deviation.

Figure S5. Sample rarefaction **c**urve with standard deviation for the 4var color pattern of *I. parva* for localities that had more than 21 specimens: Ilhabela, SP (A); São Paulo, SP (B); Caraguatatuba, SP (C); Rio de Janeiro, RJ (D); Mogi das Cruzes, SP (E); Teresópolis, RJ (F); Ubatuba, SP (G); Santo André, SP (H); Bertioga, SP (I); Salesópolis, SP (J); São Luís do Paraitinga, SP (K); Bananal, SP (L); and Santana do Parnaíba, SP (M). The solid line and the number correspond to the expected number of patterns for n = 21, whereas the hatched line corresponds to the standard deviation.

Figure S1

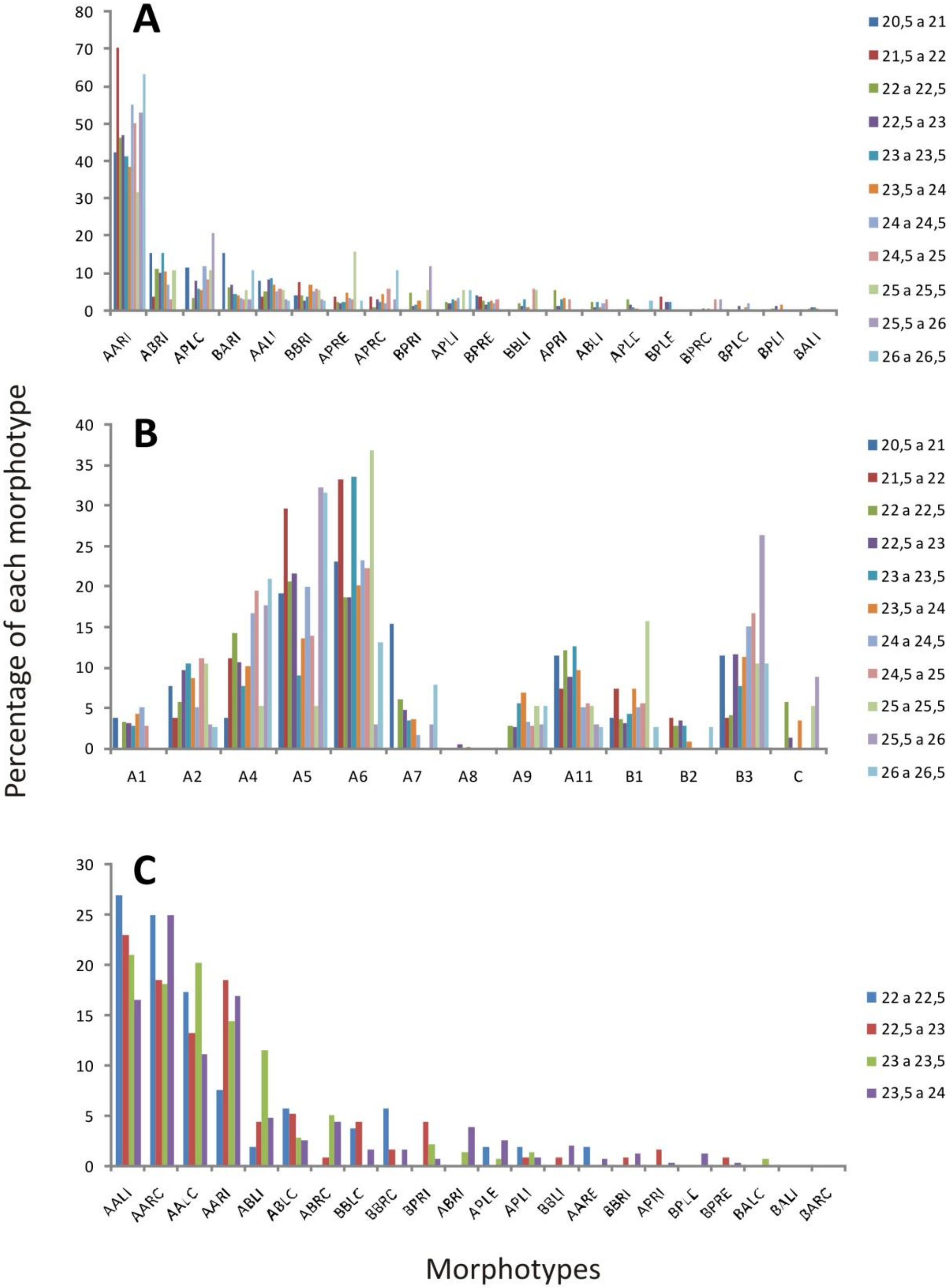

A
80
70
60
50
40
30
20
10
0
AARI
ABRI
APLC
BARI
AALI
BBRI
APRE
APRC
BPRI
APLI
BPRE
BBLI
APRI
ABLI
APLC
BPLE
BPRC
BPLC
BPLI
BALI
20,5 a 21
21,5 a 22
22 a 22,5
22,5 a 23
23 a 23,5
23,5 a 24
24 a 24,5
24,5 a 25
25 a 25,5
25,5 a 26
26 a 26,5
Percentage of each morphotype
B
40
35
30
25
20
15
10
5
0
A1
A2
A4
A5
A6
A7
A8
A9
A11
B1
B2
B3
C
20,5 a 21
21,5 a 22
22 a 22,5
22,5 a 23
23 a 23,5
23,5 a 24
24 a 24,5
24,5 a 25
25 a 25,5
25,5 a 26
26 a 26,5
C
30
25
20
15
10
5
0
AALI
AARC
AALC
AARI
ABLI
ABLC
ABRC
BBLC
BBRC
BPRI
ABRI
APLE
APLI
BBLI
AARE
BBRI
APRI
BPLE
BPRE
BALC
BALI
BARC
22 a 22,5
22,5 a 23
23 a 23,5
23,5 a 24
Morphotypes

Figure S2

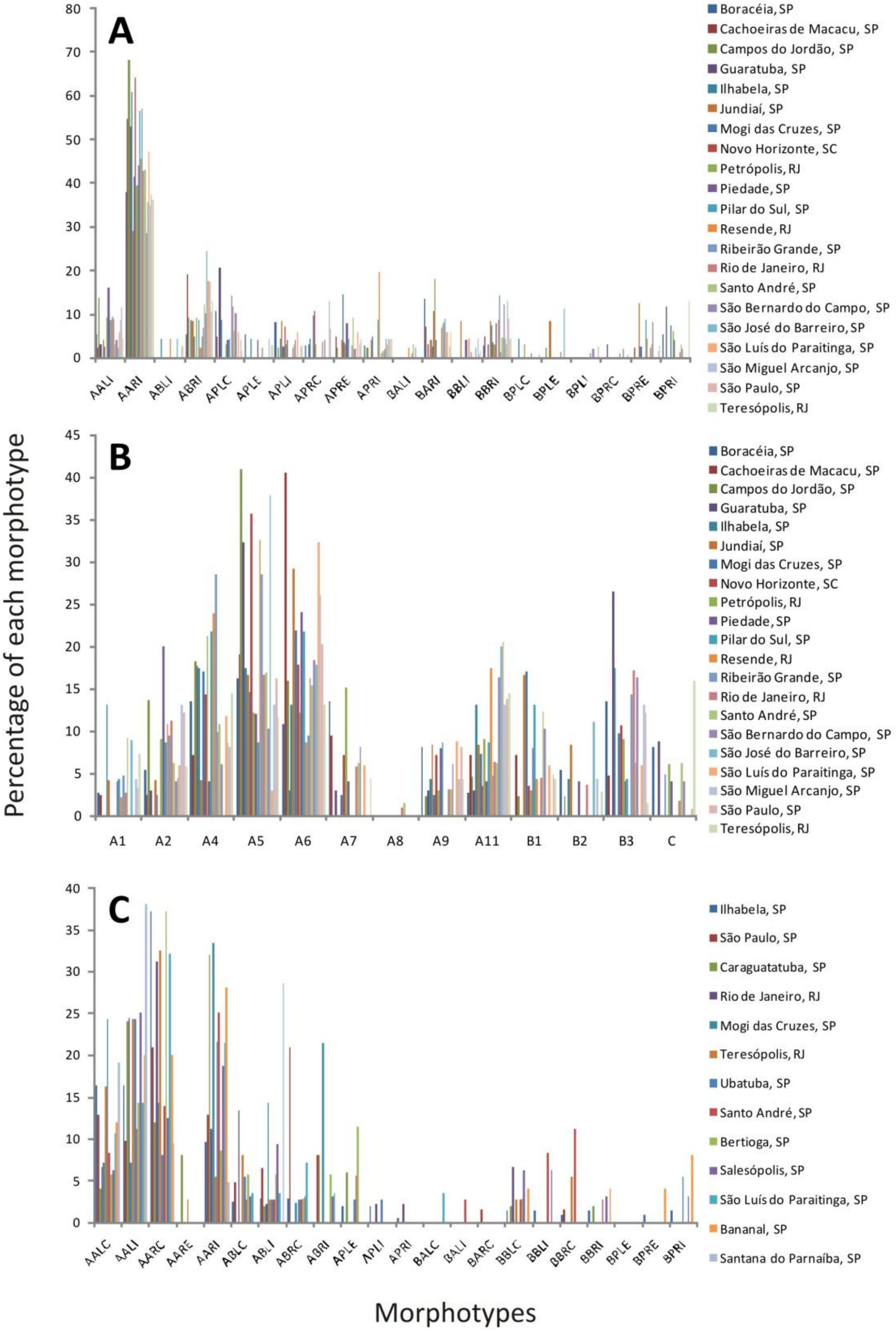
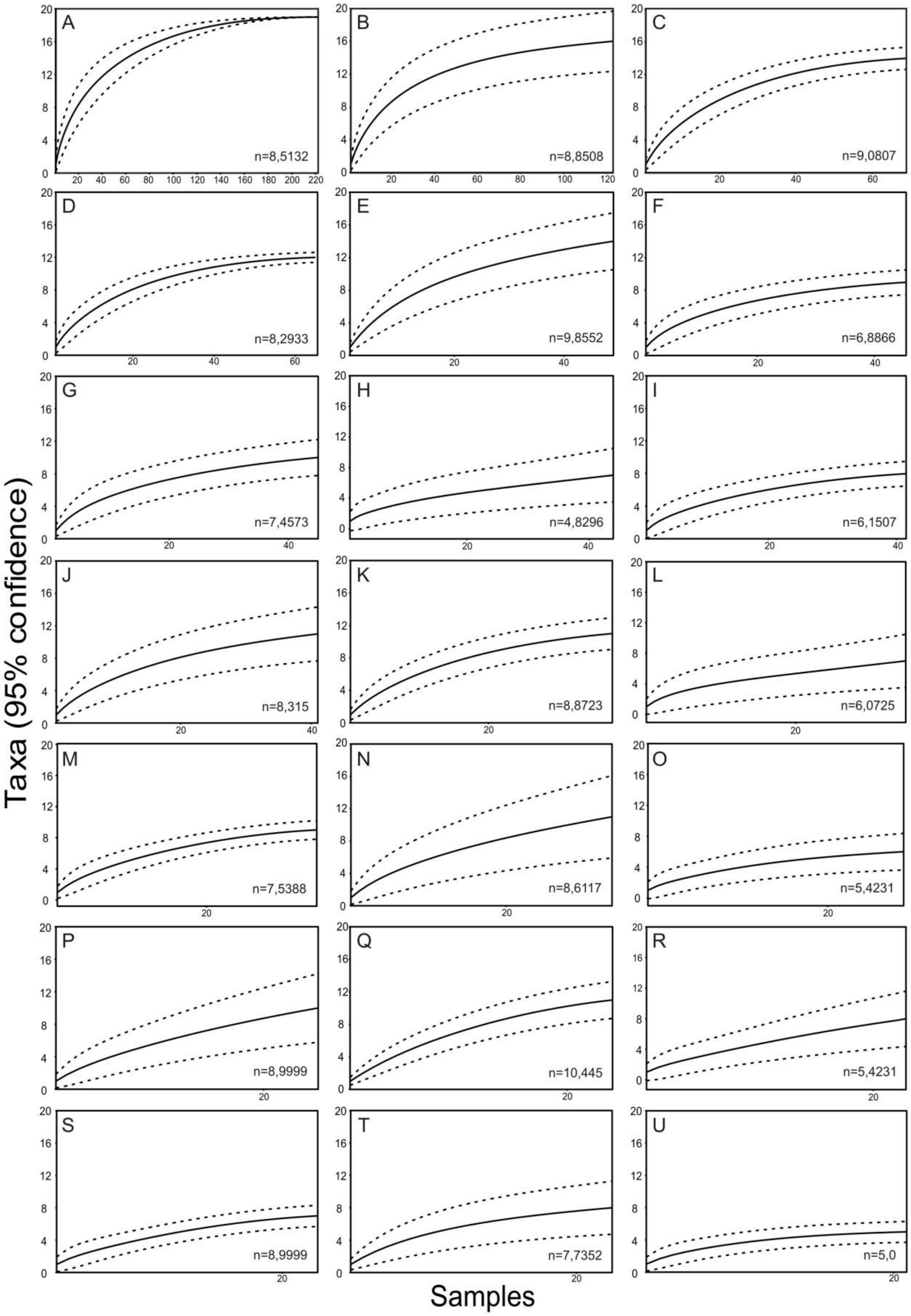

A
80
70
60
50
40
30
20
10
0
AALI
AARI
ABLI
ABRI
APLC
APLE
APLI
APRC
APRE
APRI
BALI
BARI
BBLI
BBRI
BPLC
BPLE
BPLI
BPRC
BPRE
BPRI
Boracéia, SP
Cachoeiras de Macacu, SP
Campos do Jordão, SP
Guaratuba, SP
Ilhabela, SP
Jundiaí, SP
Mogi das Cruzes, SP
Novo Horizonte, SC
Petrópolis, RJ
Piedade, SP
Pilar do Sul, SP
Resende, RJ
Ribeirão Grande, SP
Rio de Janeiro, RJ
Santo André, SP
São Bernardo do Campo, SP
São José do Barreiro, SP
São Luís do Paraitinga, SP
São Miguel Arcanjo, SP
São Paulo, SP
Teresópolis, RJ
Percentage of each morphotype
B
45
40
35
30
25
20
15
10
5
0
A1
A2
A4
A5
A6
A7
A8
A9
A11
B1
B2
B3
C
Boracéia, SP
Cachoeiras de Macacu, SP
Campos do Jordão, SP
Guaratuba, SP
Ilhabela, SP
Jundiaí, SP
Mogi das Cruzes, SP
Novo Horizonte, SC
Petrópolis, RJ
Piedade, SP
Pilar do Sul, SP
Resende, RJ
Ribeirão Grande, SP
Rio de Janeiro, RJ
Santo André, SP
São Bernardo do Campo, SP
São José do Barreiro, SP
São Luís do Paraitinga, SP
São Miguel Arcanjo, SP
São Paulo, SP
Teresópolis, RJ
C
40
35
30
25
20
15
10
5
0
AALC
AALI
AARC
AARE
AARI
ABLC
ABLI
ABRC
ABRI
APLE
APLI
APRI
BALC
BALI
BARC
BBLC
BBLI
BBRC
BBRI
BPLE
BPRE
BPRI
Ilhabela, SP
São Paulo, SP
Caraguatatuba, SP
Rio de Janeiro, RJ
Mogi das Cruzes, SP
Teresópolis, RJ
Ubatuba, SP
Santo André, SP
Bertioga, SP
Salesópolis, SP
São Luís do Paraitinga, SP
Bananal, SP
Santana do Parnaíba, SP
Morphotypes

Figure S3


Taxa (95% confidence)
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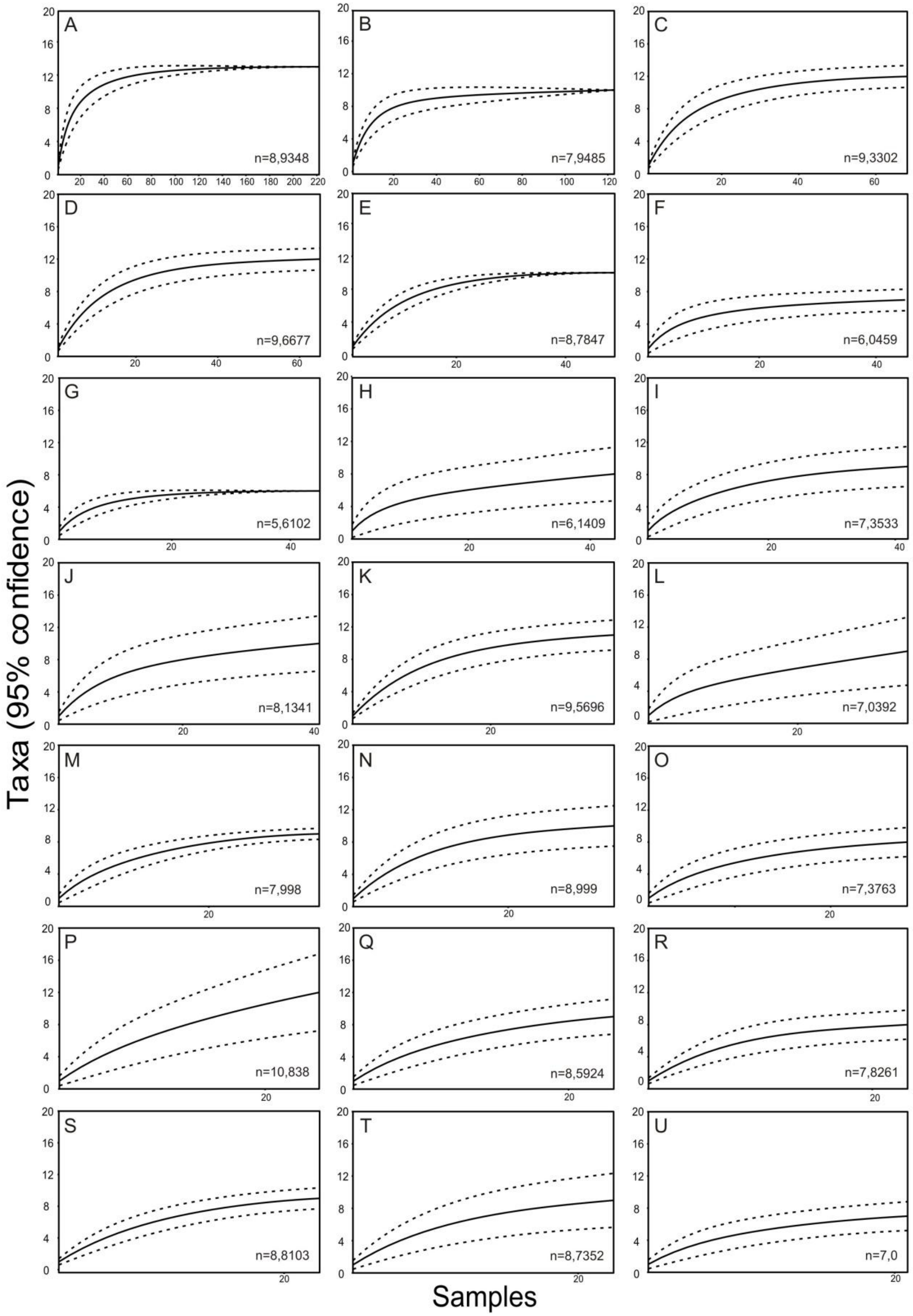
B
n=8,8508
C
n=9,0807
D
n=8,2933
E
n=9,8552
F
n=6,8866
G
n=7,4573
H
n=4,8296
I
n=6,1507
J
n=8,315
K
n=8,8723
L
n=6,0725
M
n=7,5388
N
n=8,6117
O
n=5,4231
P
n=8,9999
Q
n=10,445
R
n=5,4231
S
n=8,9999
T
n=7,7352
U
n=5,0
0
4
8
12
16
20
20
40
60
80
100
120
140
160
180
200
220

Figure S4


Taxa (95% confidence)
Samples
A
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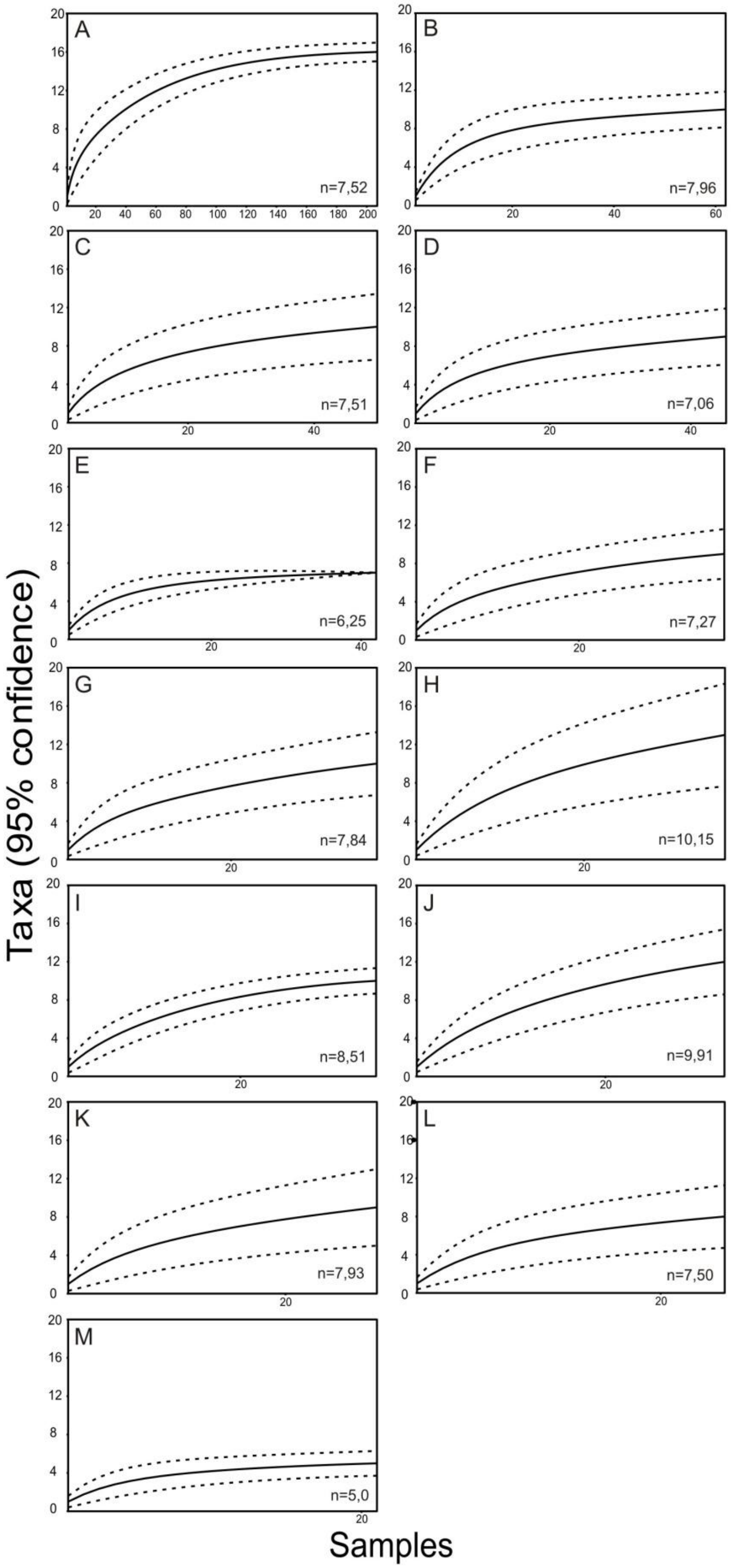
B
n=7,9485
C
n=9,3302
D
n=9,6677
E
n=8,7847
F
n=6,0459
G
n=5,6102
H
n=6,1409
I
n=7,3533
J
n=8,1341
K
n=9,5696
L
n=7,0392
M
n=7,998
N
n=8,999
O
n=7,3763
P
n=10,838
Q
n=8,5924
R
n=7,8261
S
n=8,8103
T
n=8,7352
U
n=7,0

Figure S5


Taxa (95% confidence)
Samples
A
n=7,52
0
4
8
12
16
20
20
40
60
80
100
120
140
160
180
200
B
n=7,96
20
40
60
C
n=7,51
20
40
D
n=7,06
20
40
E
n=6,25
20
40
F
n=7,27
20
G
n=7,84
20
H
n=10,15
20
I
n=8,51
20
J
n=9,91
20
K
n=7,93
20
L
n=7,50
20
M
n=5,0
20

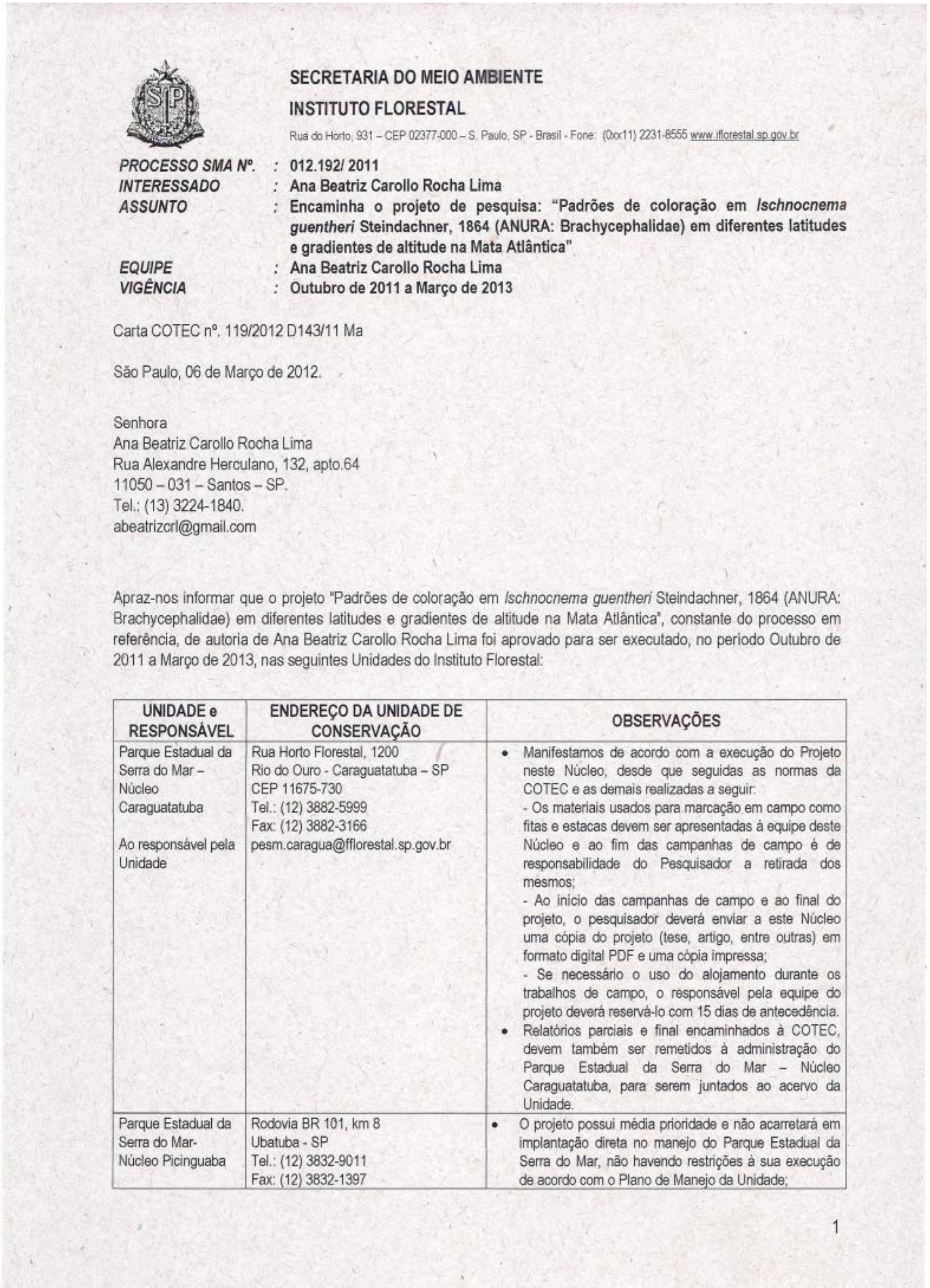
Síntese Geral

O estudo da coloração dos anfíbios é de grande relevância para a melhor compreensão deste grupo tão peculiar e tão ameaçado, dado que a coloração influencia diretamente em todos os aspectos de sua vida. Poucos são os experimentos que testam o aposematismo em espécies de anfíbios, e o experimento do primeiro capítulo contribuiu para demonstrar que a coloração conspícua dos braquicefalídeos da mata atlântica influencia negativamente a intensidade da predação, fornecendo assim a primeira evidência possível de que as espécies de *Brachycephalus* conspícuas da mata atlântica são aposemáticas. Os resultados deste capítulo possivelmente também reforçam a hipótese da evitação inata de presa conspícua, já que os modelos conspícuos alóctones também foram evitados; porém, outra explicação para a evitação dos modelos conspícuos alóctones é a possibilidade de que as mesmas espécies de predador tenham tido contato anterior com ambas as espécies em razão da proximidade das áreas. Sugerimos que estudos sejam feitos para esclarecer a razão da evitação de modelos conspícuos alóctones, como experimentos com predadores comprovadamente inexperientes com as presas e a identificação dos predadores de ambas as espécies e sua área de ocorrência.

Através da análise de padrões de coloração de espécimes fixados e identificados como *I. guentheri* em coleções científicas, detectou-se no segundo capítulo uma diferença na frequência dos padrões do complexo de *I. guentheri-henselii* entre populações situadas acima e abaixo da latitude -24º. Este fenômeno ocorre em muitos grupos de organismos e também em anuros, de modo que o limite meridional situado ao sul do Estado de São Paulo se revela uma região promotora de diversificação. Entre as explicações para este fenômeno está 1) o clima, 2) a teoria dos refúgios do quaternário e 3) uma interrupção existente na Serra do Mar que proporciona uma diversidade de altitude, gerando uma barreira capaz de interromper o fluxo gênico, causando assim isolamento reprodutivo e a consequente especiação das populações entre estas localidades ao longo do tempo. Os resultados deste trabalho permitem concluir que a coloração de espécies polimórficas pode auxiliar na taxonomia e na identificação de espécies crípticas, já que utilizando somente a coloração, foram obtidos resultados semelhantes a outro trabalho realizado com o mesmo táxon que utilizou dados moleculares e bioacústicos (GEHARA et al., 2013). Sugerimos que trabalhos futuros utilizem esta metodologia complementarmente a outras para identificação de espécies crípticas em complexos de espécies de amplas áreas de distribuição, e também que outros trabalhos testem experimentalmente o efeito da predação sobre a frequência dos padrões do complexo de *I. guentheri-henselii* e a flutuação nos morfotipos ao longo do tempo.

ANEXO

Licença do Parque Estadual da Serra do Mar



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PROCESSO SMA Nº. : **012.192/ 2011**
INTERESSADO : **Ana Beatriz Carollo Rocha Lima**
ASSUNTO : **Encaminha o projeto de pesquisa: "Padrões de coloração em *Ischnocnema guentheri* Steindachner, 1864 (ANURA: Brachycephalidae) em diferentes latitudes e gradientes de altitude na Mata Atlântica"**
EQUIPE : **Ana Beatriz Carollo Rocha Lima**
VIGÊNCIA : **Outubro de 2011 a Março de 2013**

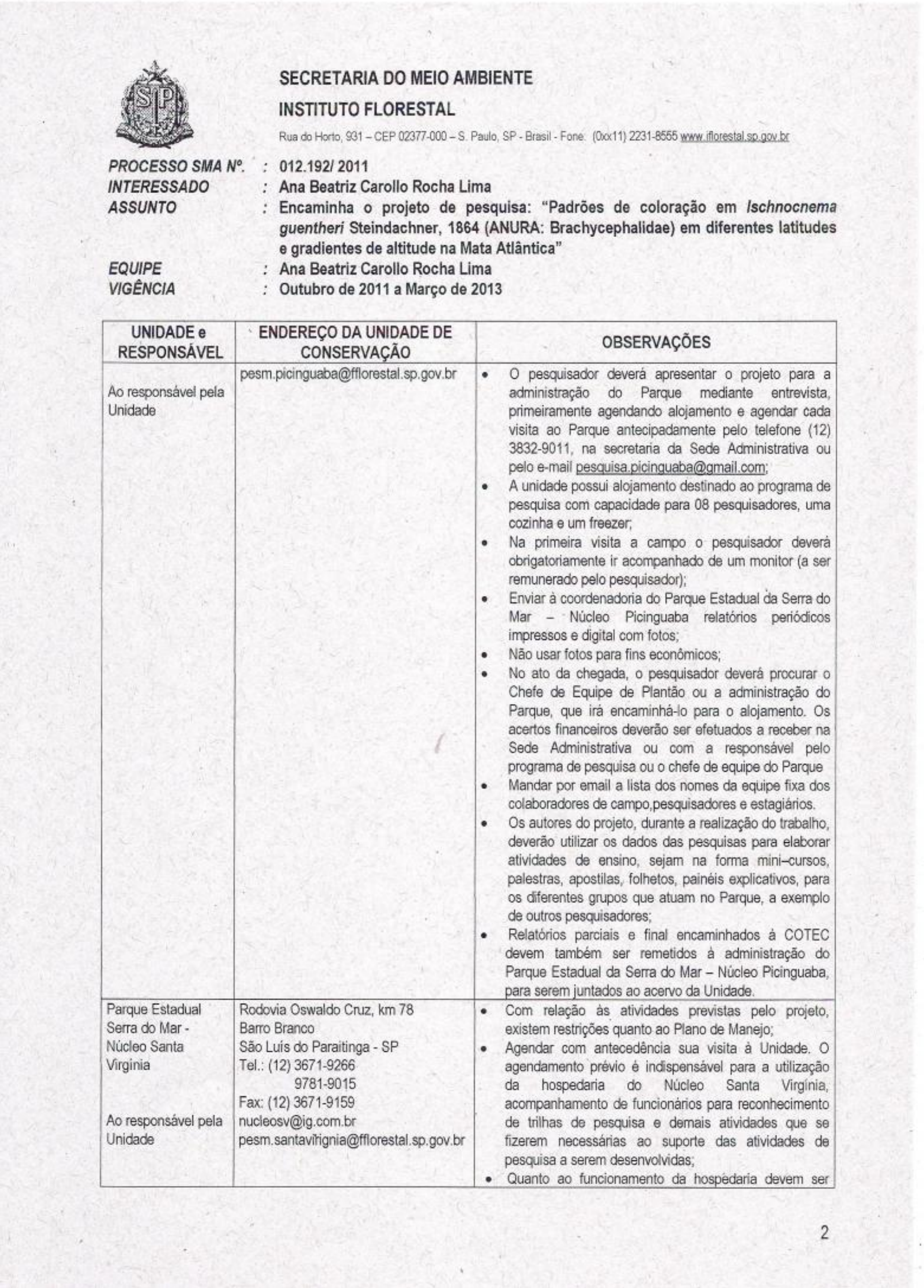
Carta COTEC nº. 119/2012 D143/11 Ma

São Paulo, 06 de Março de 2012.

Senhora
Ana Beatriz Carollo Rocha Lima
Rua Alexandre Herculano, 132, apto.64
11050 – 031 – Santos – SP.
Tel.: (13) 3224-1840.
abeatrizcrl@gmail.com

Apraz-nos informar que o projeto "Padrões de coloração em *Ischnocnema guentheri* Steindachner, 1864 (ANURA: Brachycephalidae) em diferentes latitudes e gradientes de altitude na Mata Atlântica", constante do processo em referência, de autoria de Ana Beatriz Carollo Rocha Lima foi aprovado para ser executado, no período Outubro de 2011 a Março de 2013, nas seguintes Unidades do Instituto Florestal:

UNIDADE e RESPONSÁVEL	ENDEREÇO DA UNIDADE DE CONSERVAÇÃO	OBSERVAÇÕES
Parque Estadual da Serra do Mar – Núcleo Caraguatatuba Ao responsável pela Unidade	Rua Horto Florestal, 1200 Rio do Ouro - Caraguatatuba – SP CEP 11675-730 Tel.: (12) 3882-5999 Fax: (12) 3882-3166 pesm.caragua@fflorestal.sp.gov.br	• Manifestamos de acordo com a execução do Projeto neste Núcleo, desde que seguidas as normas da COTEC e as demais realizadas a seguir: - Os materiais usados para marcação em campo como fitas e estacas devem ser apresentadas à equipe deste Núcleo e ao fim das campanhas de campo é de responsabilidade do Pesquisador a retirada dos mesmos; - Ao início das campanhas de campo e ao final do projeto, o pesquisador deverá enviar a este Núcleo uma cópia do projeto (tese, artigo, entre outras) em formato digital PDF e uma cópia impressa; - Se necessário o uso do alojamento durante os trabalhos de campo, o responsável pela equipe do projeto deverá reservá-lo com 15 dias de antecedência. • Relatórios parciais e final encaminhados à COTEC, devem também ser remetidos à administração do Parque Estadual da Serra do Mar – Núcleo Caraguatatuba, para serem juntados ao acervo da Unidade.
Parque Estadual da Serra do Mar- Núcleo Picinguaba	Rodovia BR 101, km 8 Ubatuba - SP Tel.: (12) 3832-9011 Fax: (12) 3832-1397	• O projeto possui média prioridade e não acarretará em implantação direta no manejo do Parque Estadual da Serra do Mar, não havendo restrições à sua execução de acordo com o Plano de Manejo da Unidade;



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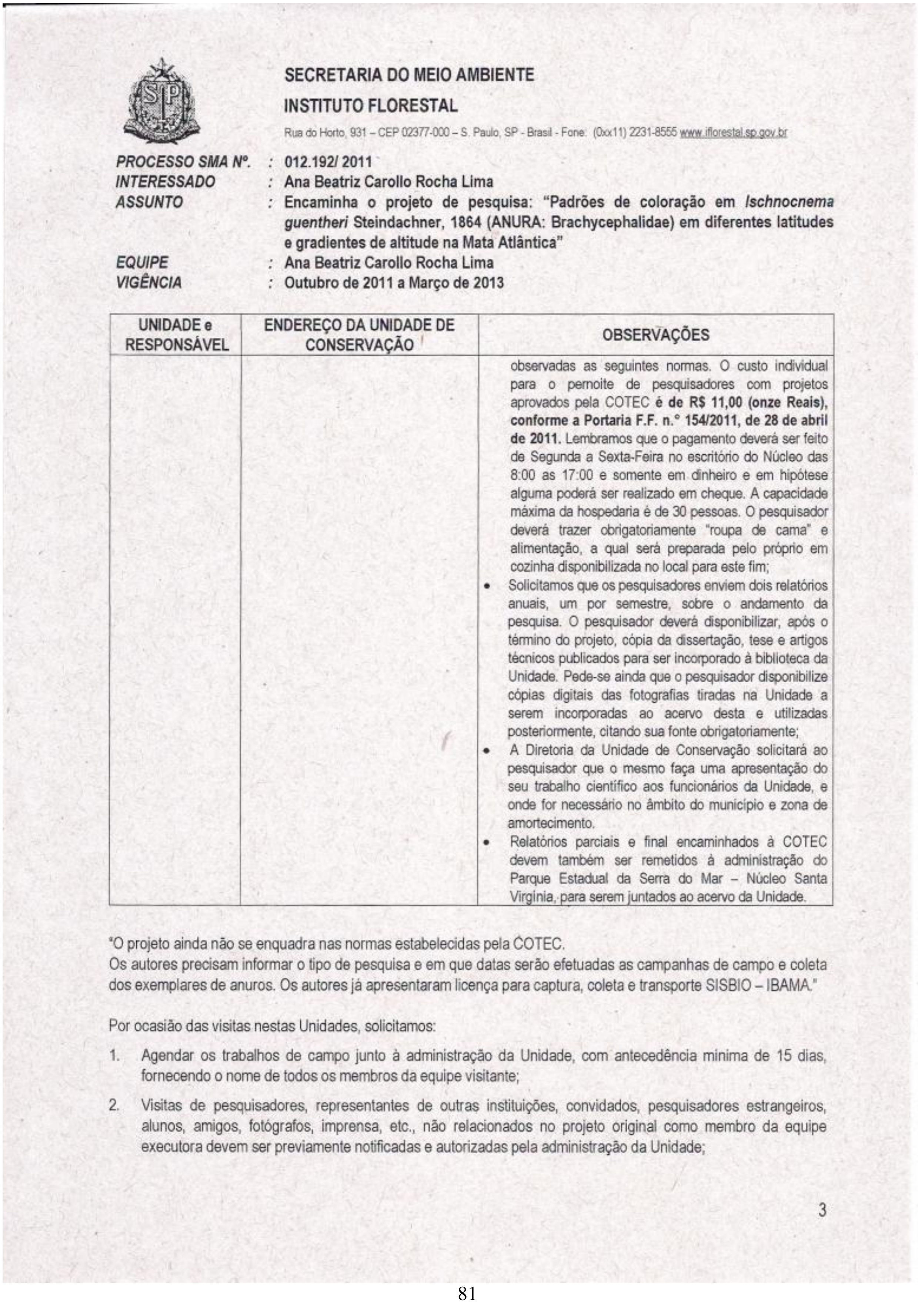
UNIDADE e RESPONSÁVEL	ENDEREÇO DA UNIDADE DE CONSERVAÇÃO	OBSERVAÇÕES
Ao responsável pela Unidade	pesm.picinguaba@fflorestal.sp.gov.br	• O pesquisador deverá apresentar o projeto para a administração do Parque mediante entrevista, primeiramente agendando alojamento e agendar cada visita ao Parque antecipadamente pelo telefone (12) 3832-9011, na secretaria da Sede Administrativa ou pelo e-mail pesquisa.picinguaba@gmail.com; • A unidade possui alojamento destinado ao programa de pesquisa com capacidade para 08 pesquisadores, uma cozinha e um freezer; • Na primeira visita a campo o pesquisador deverá obrigatoriamente ir acompanhado de um monitor (a ser remunerado pelo pesquisador); • Enviar à coordenadoria do Parque Estadual da Serra do Mar – Núcleo Picinguaba relatórios periódicos impressos e digital com fotos; • Não usar fotos para fins econômicos; • No ato da chegada, o pesquisador deverá procurar o Chefe de Equipe de Plantão ou a administração do Parque, que irá encaminhá-lo para o alojamento. Os acertos financeiros deverão ser efetuados a receber na Sede Administrativa ou com a responsável pelo programa de pesquisa ou o chefe de equipe do Parque • Mandar por email a lista dos nomes da equipe fixa dos colaboradores de campo,pesquisadores e estagiários. • Os autores do projeto, durante a realização do trabalho, deverão utilizar os dados das pesquisas para elaborar atividades de ensino, sejam na forma mini–cursos, palestras, apostilas, folhetos, painéis explicativos, para os diferentes grupos que atuam no Parque, a exemplo de outros pesquisadores; • Relatórios parciais e final encaminhados à COTEC devem também ser remetidos à administração do Parque Estadual da Serra do Mar – Núcleo Picinguaba, para serem juntados ao acervo da Unidade.
Parque Estadual Serra do Mar - Núcleo Santa Virginia Ao responsável pela Unidade	Rodovia Oswaldo Cruz, km 78 Barro Branco São Luís do Paraitinga - SP Tel.: (12) 3671-9266 9781-9015 Fax: (12) 3671-9159 nucleosv@ig.com.br pesm.santavirignia@fflorestal.sp.gov.br	• Com relação às atividades previstas pelo projeto, existem restrições quanto ao Plano de Manejo; • Agendar com antecedência sua visita à Unidade. O agendamento prévio é indispensável para a utilização da hospedaria do Núcleo Santa Virgínia, acompanhamento de funcionários para reconhecimento de trilhas de pesquisa e demais atividades que se fizerem necessárias ao suporte das atividades de pesquisa a serem desenvolvidas; • Quanto ao funcionamento da hospedaria devem ser



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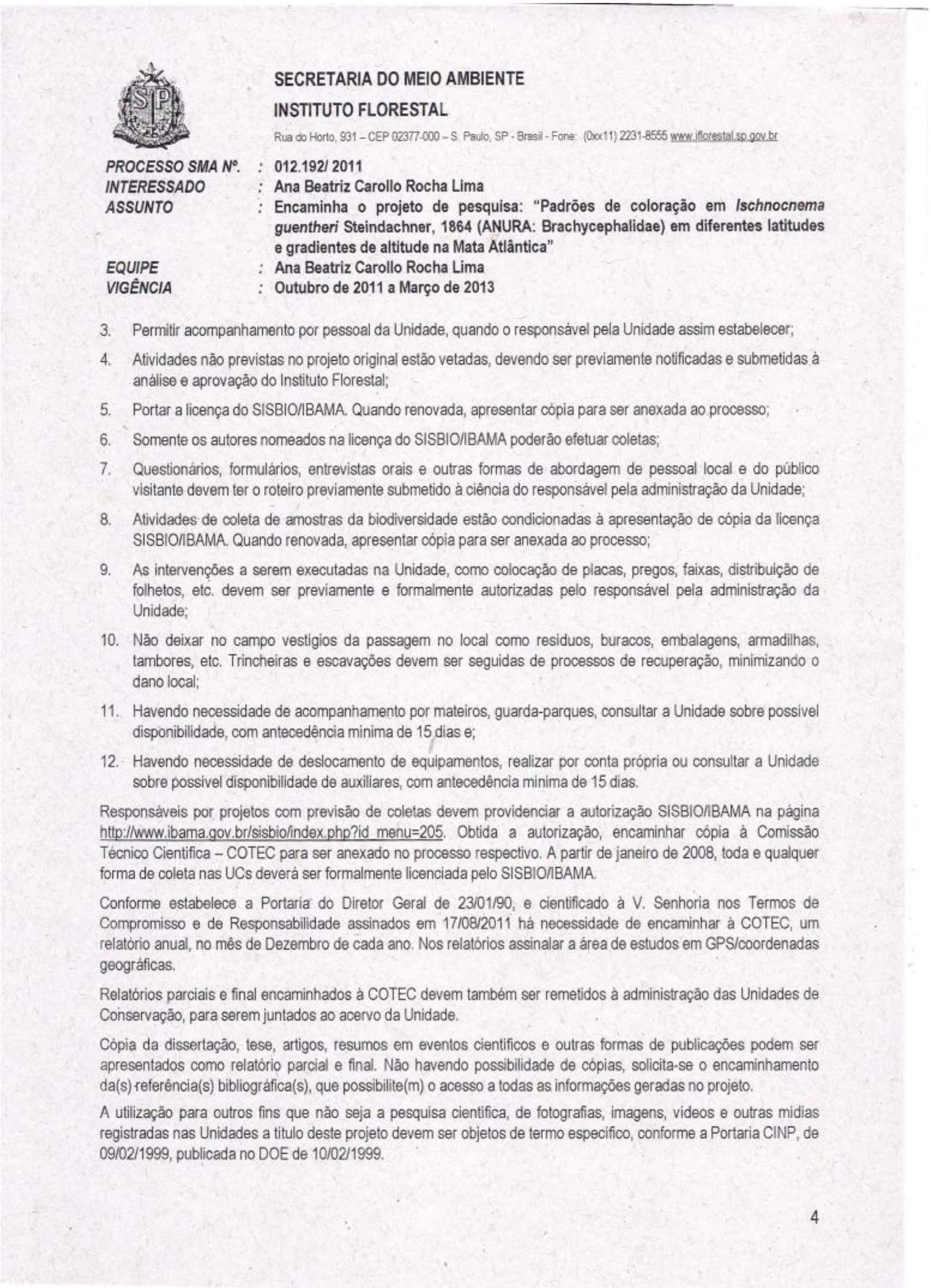
UNIDADE e RESPONSÁVEL	ENDEREÇO DA UNIDADE DE CONSERVAÇÃO	OBSERVAÇÕES
		observadas as seguintes normas. O custo individual para o pernoite de pesquisadores com projetos aprovados pela COTEC **é de R$ 11,00 (onze Reais), conforme a Portaria F.F. n.° 154/2011, de 28 de abril de 2011.** Lembramos que o pagamento deverá ser feito de Segunda a Sexta-Feira no escritório do Núcleo das 8:00 as 17:00 e somente em dinheiro e em hipótese alguma poderá ser realizado em cheque. A capacidade máxima da hospedaria é de 30 pessoas. O pesquisador deverá trazer obrigatoriamente "roupa de cama" e alimentação, a qual será preparada pelo próprio em cozinha disponibilizada no local para este fim; • Solicitamos que os pesquisadores enviem dois relatórios anuais, um por semestre, sobre o andamento da pesquisa. O pesquisador deverá disponibilizar, após o término do projeto, cópia da dissertação, tese e artigos técnicos publicados para ser incorporado à biblioteca da Unidade. Pede-se ainda que o pesquisador disponibilize cópias digitais das fotografias tiradas na Unidade a serem incorporadas ao acervo desta e utilizadas posteriormente, citando sua fonte obrigatoriamente; • A Diretoria da Unidade de Conservação solicitará ao pesquisador que o mesmo faça uma apresentação do seu trabalho científico aos funcionários da Unidade, e onde for necessário no âmbito do município e zona de amortecimento. • Relatórios parciais e final encaminhados à COTEC devem também ser remetidos à administração do Parque Estadual da Serra do Mar – Núcleo Santa Virgínia, para serem juntados ao acervo da Unidade.

"O projeto ainda não se enquadra nas normas estabelecidas pela COTEC.
Os autores precisam informar o tipo de pesquisa e em que datas serão efetuadas as campanhas de campo e coleta dos exemplares de anuros. Os autores já apresentaram licença para captura, coleta e transporte SISBIO – IBAMA."

Por ocasião das visitas nestas Unidades, solicitamos:

1. Agendar os trabalhos de campo junto à administração da Unidade, com antecedência minima de 15 dias, fornecendo o nome de todos os membros da equipe visitante;
2. Visitas de pesquisadores, representantes de outras instituições, convidados, pesquisadores estrangeiros, alunos, amigos, fotógrafos, imprensa, etc., não relacionados no projeto original como membro da equipe executora devem ser previamente notificadas e autorizadas pela administração da Unidade;



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3. Permitir acompanhamento por pessoal da Unidade, quando o responsável pela Unidade assim estabelecer;
4. Atividades não previstas no projeto original estão vetadas, devendo ser previamente notificadas e submetidas à análise e aprovação do Instituto Florestal;
5. Portar a licença do SISBIO/IBAMA. Quando renovada, apresentar cópia para ser anexada ao processo;
6. Somente os autores nomeados na licença do SISBIO/IBAMA poderão efetuar coletas;
7. Questionários, formulários, entrevistas orais e outras formas de abordagem de pessoal local e do público visitante devem ter o roteiro previamente submetido à ciência do responsável pela administração da Unidade;
8. Atividades de coleta de amostras da biodiversidade estão condicionadas à apresentação de cópia da licença SISBIO/IBAMA. Quando renovada, apresentar cópia para ser anexada ao processo;
9. As intervenções a serem executadas na Unidade, como colocação de placas, pregos, faixas, distribuição de folhetos, etc. devem ser previamente e formalmente autorizadas pelo responsável pela administração da Unidade;
10. Não deixar no campo vestígios da passagem no local como resíduos, buracos, embalagens, armadilhas, tambores, etc. Trincheiras e escavações devem ser seguidas de processos de recuperação, minimizando o dano local;
11. Havendo necessidade de acompanhamento por mateiros, guarda-parques, consultar a Unidade sobre possível disponibilidade, com antecedência mínima de 15 dias e;
12. Havendo necessidade de deslocamento de equipamentos, realizar por conta própria ou consultar a Unidade sobre possível disponibilidade de auxiliares, com antecedência mínima de 15 dias.

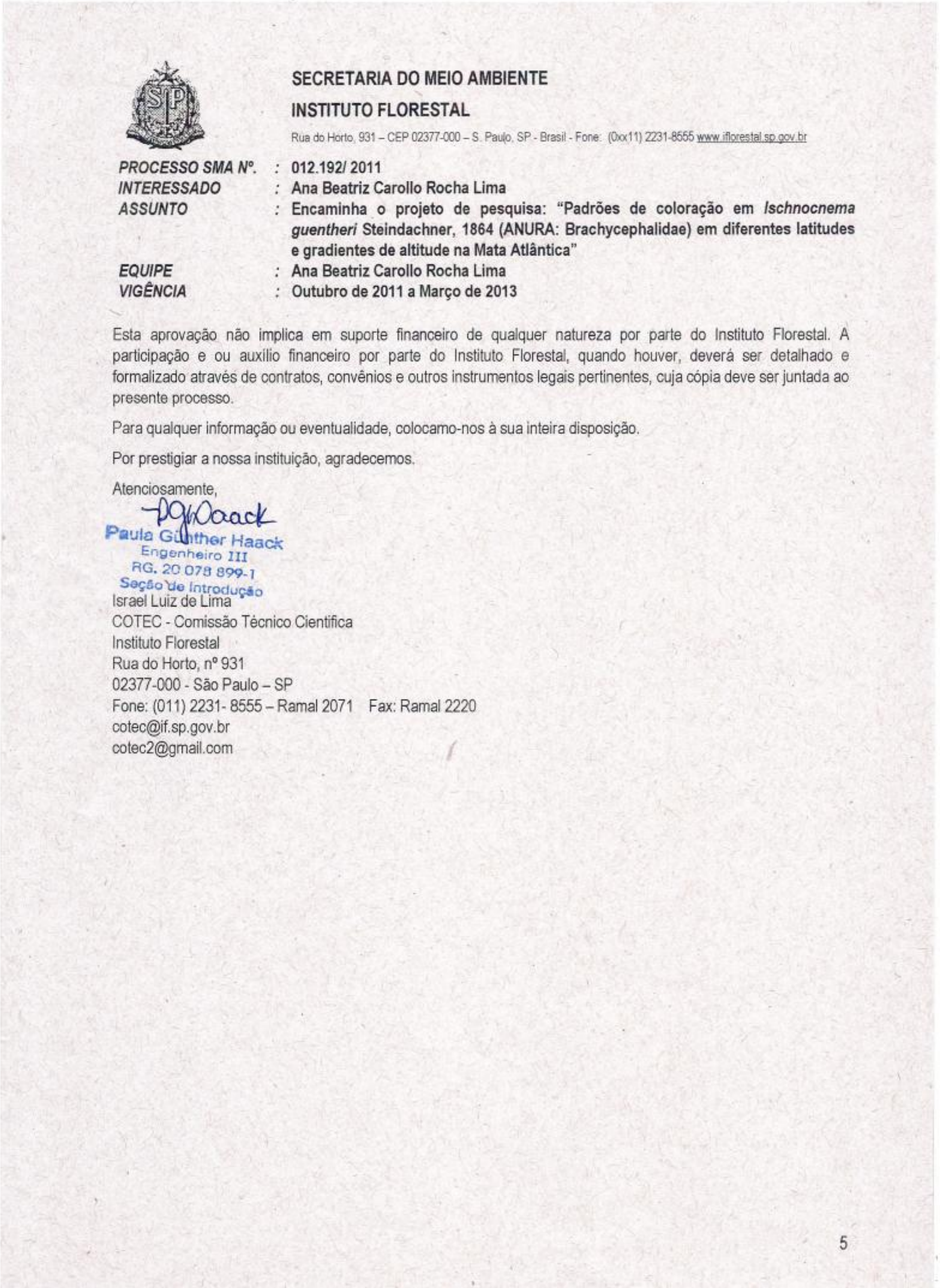
Responsáveis por projetos com previsão de coletas devem providenciar a autorização SISBIO/IBAMA na página http://www.ibama.gov.br/sisbio/index.php?id_menu=205. Obtida a autorização, encaminhar cópia à Comissão Técnico Científica – COTEC para ser anexado no processo respectivo. A partir de janeiro de 2008, toda e qualquer forma de coleta nas UCs deverá ser formalmente licenciada pelo SISBIO/IBAMA.

Conforme estabelece a Portaria do Diretor Geral de 23/01/90, e cientificado à V. Senhoria nos Termos de Compromisso e de Responsabilidade assinados em 17/08/2011 há necessidade de encaminhar à COTEC, um relatório anual, no mês de Dezembro de cada ano. Nos relatórios assinalar a área de estudos em GPS/coordenadas geográficas.

Relatórios parciais e final encaminhados à COTEC devem também ser remetidos à administração das Unidades de Conservação, para serem juntados ao acervo da Unidade.

Cópia da dissertação, tese, artigos, resumos em eventos científicos e outras formas de publicações podem ser apresentados como relatório parcial e final. Não havendo possibilidade de cópias, solicita-se o encaminhamento da(s) referência(s) bibliográfica(s), que possibilite(m) o acesso a todas as informações geradas no projeto.

A utilização para outros fins que não seja a pesquisa científica, de fotografias, imagens, vídeos e outras mídias registradas nas Unidades a título deste projeto devem ser objetos de termo específico, conforme a Portaria CINP, de 09/02/1999, publicada no DOE de 10/02/1999.



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Esta aprovação não implica em suporte financeiro de qualquer natureza por parte do Instituto Florestal. A participação e ou auxílio financeiro por parte do Instituto Florestal, quando houver, deverá ser detalhado e formalizado através de contratos, convênios e outros instrumentos legais pertinentes, cuja cópia deve ser juntada ao presente processo.

Para qualquer informação ou eventualidade, colocamo-nos à sua inteira disposição.

Por prestigiar a nossa instituição, agradecemos.

Atenciosamente,

Paula Günther Haack
Engenheiro III
RG. 20 078 899-1
Seção de Introdução

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