Is the conspicuous dorsal coloration of the Atlantic forest pumpkin toadlets aposematic?

Raoni Rebouças[1], Ana Beatriz Carollo[1], Mariane de Oliveira Freitas[1], Carolina Lambertini[1], Ronaldo Mateus Nogueira dos Santos[1,2] & Luís Felipe Toledo[1,2]

[1] Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), Departamento de Biologia Animal, Universidade Estadual de Campinas, Campinas, São Paulo, 13083-862, Brazil

[2] Instituto de Biologia, Universidade Estadual de Campinas, Cidade Universitária Zeferino Vaz, Campinas, São Paulo, 13083-970, Brazil

Corresponding author: Raoni Rebouças, e-mail: raonisreboucas@gmail.com

Abstract. While crypsis is the ability of avoiding detection of an animal by another, aposematism refers to the presence of conspicuous or contrasting colour patterns, signalling the unprofitability of a prey to potential predators. Aposematic coloration is meant to advertise that the prey is dangerous in some way, for example by being aggressive, emitting loud screams, or producing toxic skin secretions. Many contrasting and colourful anuran species are considered aposematic. However, despite past studies demonstrating that a conspicuous coloration is an element of aposematism, we still lack experiments to confirm this relationship. Therefore, we conducted experimental assays to test if the fluorescent property and bright colour patterns of *Brachycephalus* are related to aposematism. We also tested the allochthonous/autochthonous hypothesis, which predicts that local (autochthonous) colour morphs would be more avoided by local predators than allochthonous colour morphs. To this end, we conducted ex situ experiments with *B. ephippium* individuals (prey) that were housed inside polycarbonate and glass boxes and presented thus to common chickens (predator) to test if their fluorescence improved predation avoidance. In situ experiments were conducted at two Atlantic forest sites, where both cryptic and conspicuously coloured *Brachycephalus* species occur. Here, we positioned trios of plasticine models, (one brown cryptic model, one yellow, and one red conspicuous model) in two situations: right on the leaf litter and on white cardboard sheets. In our ex situ experiments, we found that fluorescent dorsal colours did not influence predation, as chickens did not avoid *B. ephippium* even after experiencing their taste. In in situ experiments, we found differences in the rates of predation between cryptic and conspicuous models. In addition, we also obtained data that corroborated the autochthonous/allochthonous hypothesis. Our results support the assumption that the conspicuous coloration of *Brachycephalus* spp. is aposematic, prevents predation, and that local predator assemblages recognize autochthonous colour morphs.

Key words. Amphibia, Anura, *Brachycephalus*, aposematism, conspicuousness, coloration, predation, predator-prey interactions.

Sumário. Enquanto crípse é a habilidade de evitar detecção de um animal por outro, aposematismo é a presença de padrões de cor conspícuos ou contrastantes que são associados com a desvantagem de uma presa a predadores potenciais. A coloração aposemática sinaliza que a presa é perigosa de alguma forma, por exemplo sendo agressiva, emitindo gritos altos ou produzindo secreções cutâneas tóxicas. Muitas espécies de anuros contrastantes e coloridas são consideradas aposemáticas. Contudo, apesar de estudos passados demonstrarem que coloração conspícua é um elemento do aposematismo, ainda faltam experimentos para confirmar essa relação. Portanto, nós conduzimos ensaios experimentais para testar se a propriedade fluorescente dos escudos ósseos dorsais e os padrões coloridos das espécies de *Brachycephalus* são relacionadas com aposematismo. Nós também testamos a hipótese alóctone/autóctone, que prevê que indivíduos de cor local (autóctone) sejam mais evitados por predadores locais que indivíduos de cor de outro local (alóctones). Assim, nós conduzimos experimentos in situ e ex situ. Experimentos ex situ foram conduzidos com indivíduos de *B. ephippium* (presas) dentro de caixas de policarbonato e vidro apresentadas a galinhas comuns (predadores) para testar se a fluorescência aumenta a rejeição pela predação. Experimentos in situ foram conduzidos em dois locais de Mata Atlântica, onde tanto espécies crípticas quanto aposemáticas do gênero *Brachycephalus* ocorrem. Nós colocamos trios de modelos de massa de modelar (um modelo críptico marrom, um amarelo e um vermelho conspícuos), dispostos em dois tratamentos: diretamente na serapilheira e sobre uma folha de papel-cartão branca. Nos experimentos ex situ nós encontramos que cores dorsais não influenciam a predação, e que galinhas não evitaram *B. ephippium* mesmo depois da primeira experiência. Nos experimentos in situ nós encontramos diferenças nas taxas de predação entre modelos crípticos e aposemáticos. Além disso, também apresentamos dados que corroboram a hipótese alóctone/autóctone. Nossos resultados suportam que a coloração conspícua de *Brachycephalus* spp. é aposemática, inibindo a predação, e que assembleias locais de predadores reconhecem formas de cor autóctones.

Introduction

Anurans are remarkably variable as to their colour patterns, ranging from cryptic to highly conspicuous and contrasting against the natural background (Hoffman & Blouin 2000, Schaefer et al. 2002, Toledo & Haddad 2009). Such coloration 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), radiation protection, thermoregulation, osmoregulation, nitrogen metabolism, intra- and interspecific communication, and protection against predators (Cott 1940, Edmunds 1974, Tattersall et al. 2006, Toledo & Haddad 2009). Thus, several selective pressures may result in the emergence of different colour morphs within the same or different species. Among these possible selective pressures, predation seems to play a major, although still understudied role (e.g., Allen 1988, Owen & Whiteley 1989, Hoffman & Blouin 2000, Vasconcellos-Neto & Gonzaga 2000, Bond & Kamil 2002, Toledo & Haddad 2009).

Colours, as a defensive strategy, can reduce the probability of detection by enhancing crypsis, or reduce predation risks by being conspicuous and aposematic (Mappes et al. 2005). Aposematism can be related to several aspects, for example, to individual unpalatability (D'Heursel & Haddad 1999), presence of toxins (e.g., Saporito et al. 2007, Santos & Cannatella 2011), or indicate that the animal may be dangerous in other ways, like having a strong defensive bite (Toledo et al. 2011). In anurans, aposematic colour patterns may protect frogs from visually-orientated predators (Cott 1940, Gittleman et al. 1980, Endler & Mappes 2004, Ruxton et al. 2004, Toledo & Haddad 2009) and are present in a wide variety of taxa (Endler & Mappes 2004, Ruxton et al. 2004, Toledo & Haddad 2009). Although a multitude of species feature conspicuous colours, like those from the Dendrobatidae, Mantellidae and Brachycephalidae families (e.g., Summers & Clough 2001, Hagman & Forsman 2003), only few groups of anurans can be classified as genuinely aposematic (Schaefer et al. 2002). Saporito et al. (2007) employed cryptic and conspicuous clay models of the poison frog *Oophaga pumilio* to demonstrate that predation occurred almost twice as often in cryptic models than in conspicuous ones. However, a study with red-bellied toads, *Melanophryniscus cambaraensis*, that has a conspicuous behaviour in addition to conspicuous colours, showed that these characteristics do not prevent attacks by predators (Bordignon et al. 2018). Thus, as there is contradicting evidence of conspicuous colours being sometimes avoided (e.g., Kuchta 2005, Saporito et al. 2007) and not in other cases (e.g., Bordignon et al. 2018), further investigation is warranted.

In the Brazilian Atlantic forest, the genus *Brachycephalus* comprises direct-developing, remarkably colourful toadlets. Most of these species are small, measuring less than 2 cm in snout–vent length (SVL). They have diurnal habits, forage and reproduce on and in forest leaf litter, and have body colorations varying from bright green, yellow, orange, pink or red (conspicuous morphs) to cryptic brownish (Haddad et al. 2013). Some of the conspicuous species have highly toxic skin compounds, such as tetrodotoxin (TTX) and ephippiotoxin (Sebben et al. 1986, Pires Jr et al. 2002). Therefore, the presence of bright contrasting colorations may be aposematic, given that they are related to the presence of strong toxins (Pombal Jr et al. 1994). However, this hypothesis has never been tested under laboratory or natural conditions.

The colours of some anuran species may include the reflection of wavelengths invisible to the naked human eye. Some anuran species have been reported to reflect infrared wavelengths (400–700 nm), like representatives of the families Centrolenidae and Phyllomedusidae (Schwalm et al. 1977). Some other species present fluorescent colours by modifying wavelengths and in this manner reflecting colours that will be distinct from the incident light on the frog's skin. For example, individuals of *Boana punctata* convert original invisible UV light (< 400 nm) into visible blue (390–430 nm) with shoulders in visible green (505–515 nm) (Taboada et al. 2017). Some *Brachycephalus* species also have dorsal bones (Clemente-Carvalho et al. 2009), which reflect light as whitish blue light and therefore differently than the rest of the dorsum (S. Goutte, M. J. Mason, D. Merle & L. F. Toledo, unpubl. data; Fig. 1). Although there are several reports indicating that body coloration may be an important property for intraspecific recognition in anurans (e.g., Ries et al. 2008), studies about the function of colour fluorescence are still incipient. While some studies showed that fluorescence can increase predation avoidance (Kloock 2005), others suggested that a fluorescent pattern may not be related to predation avoidance (Lyytnen 2001). For anurans, there is no evidence of fluorescence having any ecological function, especially not an aposematic one, although some studies have demonstrated that it may enable some specialised frogs to see better in low-light conditions, which could be related to intraspecific recognition (King et al. 1993, Taboada et al. 2017).

Figure 1. *Brachycephalus ephippium* individual photographed with a UV flashlight, revealing the contrasting fluorescent whitish/bluish reflection of its dermal bones.

Finally, most conspicuous morphs of genus *Brachycephalus* have a unique coloration and are micro-endemic (CONDEZ et al. 2014). Thus, if these species are aposematic, it is possible that predators recognize autochthonous, but not allochthonous colour morphs. Therefore, we tested three hypotheses: (i) the presence of the contrasting fluorescent colours of *Brachycephalus ephippium* reduces the risk of predation by visually-orientated predators; (ii) conspicuous morphs of *Brachycephalus* spp. are more avoided than cryptic ones; and (iii) natural predators avoid autochthonous conspicuous morphs more often than conspicuous allochthonous ones.

Material and methods
Ex situ experiment

To describe the dorsal coloration of *Brachycephalus ephippium* in the ex situ experiment (including the fluorescent component), all collected individuals were photographed with a Canon G10 digital camera with an ultra-violet (UV) flash light (λ = 365 nm) in a dark room. After that, five points on the dorsal dermal bones and five points on the dorsum without dermal bones were randomly selected. The colour tone of each of the 10 selected points was analysed with Adobe Photoshop CC's histogram tool, using the RGB colour system, and we considered for analysis the average value of the red (255, 0, 0), green (0, 255, 0) and blue (0, 0, 255) levels.

We used common chickens (*Gallus gallus*) to test the function of fluorescent colours as a defensive signal due to their ability to see colours from the visible spectrum to ultraviolet wavelengths (e.g., PRESCOTT & WATHES 1999). They were chosen for their diurnal habits and being unfamiliar with anurans native to the Atlantic forest, which makes them a good model for the development of this experiment. We used 17 chickens raised since hatching until day 21 on a diet of chicken feed, to guarantee no previous exposure to either *B. ephippium* or corn grains. We selected this species for the ex situ trials because it has relatively large dorsal bone plates (Fig. 1). All chickens were kept unfed for 12 to 16 hours prior to experimental trials as to avoid the possible influence of previous feeding. After the experiment, these chickens were returned to the farms or sellers where they had been acquired.

For the ex situ experiment, live individuals of *B. ephippium* were collected from within the municipality of Mogi das Cruzes in the state of São Paulo, Brazil (23.5248 S, 46.1871 W), individually housed in plastic boxes outfitted with leaf litter, and kept at controlled temperatures (17°C) and a natural daylight cycle. This experiment was conducted in four sequential phases: (i) *G. gallus* individuals were fed with whole corn grains, which are similar in colour and size to *B. ephippium* (Fig. S1) inside the experimental predation arena (cardboard box: 70.5 × 83 × 61 cm, without roof and under daylight conditions, Fig. S2); (ii) one *B. ephippium* individual was placed inside a glass cube (5 × 5 × 5 cm), which is permeable to UV-light. Another *B. ephippium* individual was placed inside a polycarbonate cube (same dimensions as the glass cube), which reflects UV-light, preventing the differential reflection from the dorsal bones of the toadlets (Fig. S3). These cubes were spaced at 30 cm from each other, and a chicken was released 70 cm from the cubes inside the predation arena (Fig. 2); (iii) a toadlet
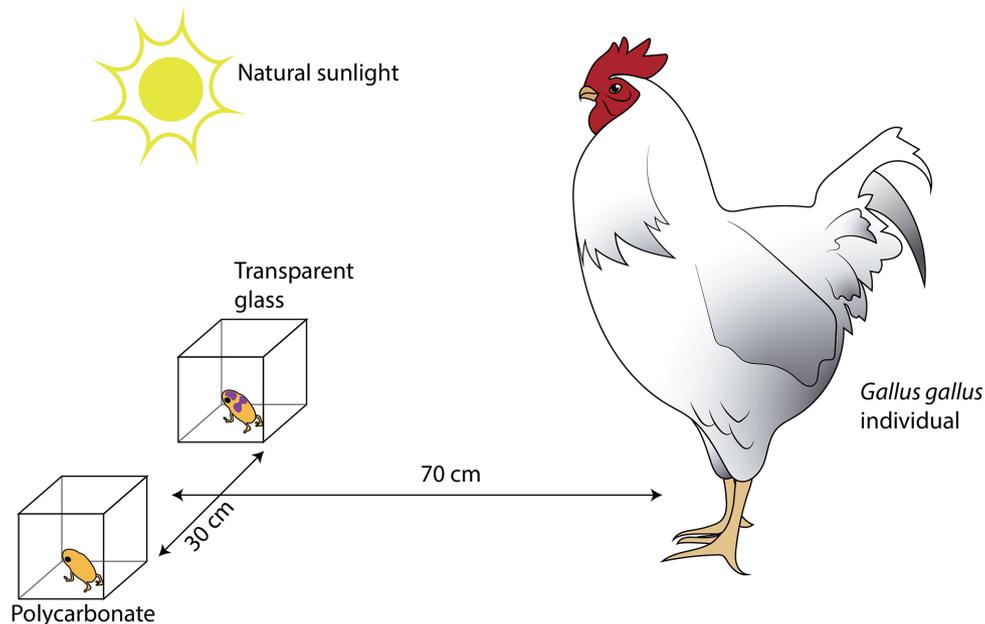

Figure 2. Experimental design with two live *Brachycephalus ephippium* individuals (prey) in two boxes (a polycarbonate box that reflects UV radiation and a transparent glass box that is permeable by UV light) and one domestic *Gallus gallus* (visually-orientated predator).

was made openly available to a chicken for five minutes or until a predation attempt was observed; and (iv) we repeated the second step with two toadlets being made available inside polycarbonate and glass boxes to one chicken for five minutes (Fig. 2). Avoidance was judged by observing the attack performed by the chicken: if a chicken attacked the glass box, we surmised that the individual avoided the polycarbonate box. Also, if the chicken attacked the uncovered individual *B. ephippium* and, after that, stopped attacking the boxes, we concluded that the chicken would not attack these toadlets again.

In situ experiment

Experimental trials with plasticine models (in situ experiments, see below) were conducted at two different sites in the Brazilian Atlantic forest: Serra do Japi, municipality of Jundiaí, São Paulo, Brazil, where *Brachycephalus ephippium* naturally occurs in syntopy with some cryptic brachycephalid species (Haddad & Sazima 1992); and in the Parque Estadual da Serra do Mar (PESM) Núcleo Santa Virgínia, municipality of São Luís do Paraitinga, São Paulo, Brazil, where *Brachycephalus pitanga* is endemic and occurs syntopic with other cryptic brachycephalid species (Silva et al. 2017). At Serra do Japi, the experiment was performed in a forested area near a swamp (23.24141 S, 046.93776 W, 1058 m a.s.l.). At Núcleo Santa Virgínia, the experiment was conducted on the Pirapitinga trail, next to a stream (23.33555 S, 45.146235 W, 902 m a.s.l.).

We prepared plasticine models as to represent three similarly sized species of genus *Brachycephalus*: *B. hermogenesi*, a cryptic brownish toadlet (Fig. 3C); *B. ephippium*, a conspicuous, bright yellowish toadlet (Fig. 3D); and
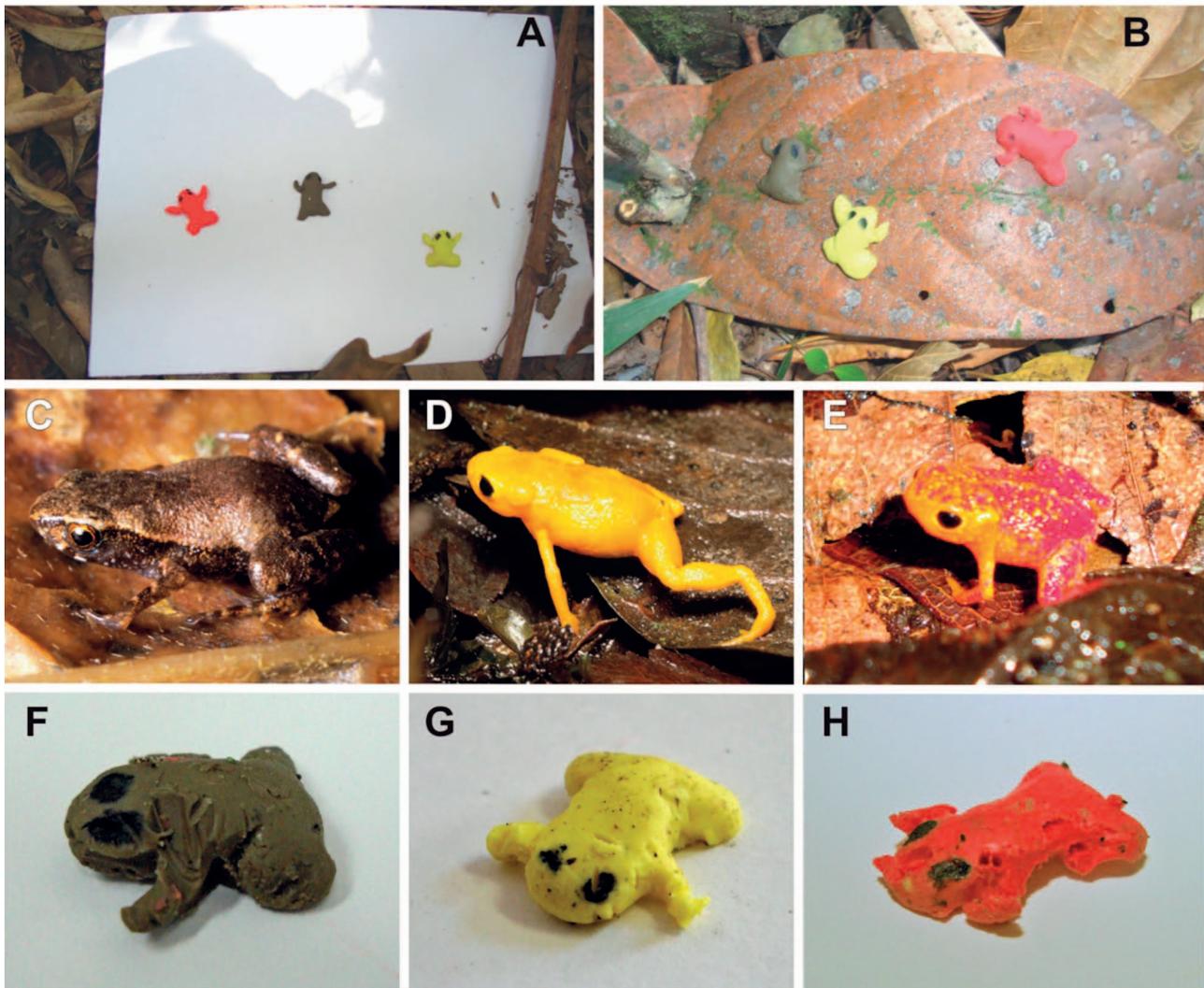

Figure 3. Distribution of cryptic and exposed trial setups: exposed on a white cardboard (A) and cryptic on natural leaf litter (B). A cryptic *Brachycephalus hermogenesi* (C), the conspicuous species *Brachycephalus ephippium* (D) and *B. pitanga* (E). Marks of pecks (F), stings (G), and bites (H) left by potential predators on plasticine models. Photographs C and D by Ivan Sazima.

B. pitanga (Fig. 3E), also a conspicuous but reddish coloured toadlet. As for the last species, the model also mimicked species of the genus *Ischnocnema* (Brachycephalidae), which occurs syntopically with both mimicked conspicuous *Brachycephalus* species. Models were crafted from odourless modelling plasticine in a plaster mould coated with a layer of enamel painttaken from a preserved specimen of *B. ephippium*, and covered with nail varnish to cover layering imperfections.

Models were arranged in trios of a brown, a yellow, and a red model each (Fig. 3). A total of 200 trios were distributed in each location (Núcleo Santa Virgínia and Serra do Japi), so that predators could freely choose between one or more models. In each location, these trios were divided in two groups: (i) "exposed group", where the trios were placed on a piece of white cardboard (15 × 21 cm), ensuring maximum visual exposure of all models; and (ii) "cryptic group", where the trios were distributed on the leaf litter, where the brown models were cryptic (Figs 3A–B). 50 model trios each were distributed at approximately 2 m from each other along four transects at both study sites. We checked for predation activity every day, in the afternoon, for four consecutive days, and the whole trio was removed when we found evidence of predation. We considered a predation event having taken place when models had disappeared, or when there were "marks" such as pecks, bites and stings. A photographic record was made of all predation marks to possibly identify the types of predators.

Legal and ethics statements

Collection permits were issued by the Chico Mendes National Institute for Biodiversity Conservation (SISBio #59199) and work permits by the Secretaria do Meio Ambiente de Jundiaí and Instituto Florestal (#012.192/2011). This study was approved by the university's (Unicamp) Ethics Committee (CEUA #4635-1/2017) and is in accordance with the requirements by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen # A92578A).

Statistical analysis

We calculated the average values of the three colour levels (Red, Green and Blue) of the analysed points to obtain a singular "colour value" of dorsal bone and body for each individual. After that, we used Pearson's product-moment correlation test to evaluate if the fluorescent coloration was related to the general dorsal colour. We performed a two-way Fisher's test between the two types of boxes (polycarbonate and glass) and exposure to *Brachycephalus ephippium* individuals (pre or post exposure) to check for dependencies between pre- and post-exposure trials and types of boxes, and after that evaluated if fluorescence was a factor influencing a predation attempt. Our null hypothesis was that fluorescence induces no avoidance. We used a

GLM, with binomial family and "logit" as link function, to evaluate how the experience of predation of *B. ephippium* individuals may influence a subsequent predation attempt, in other words, GLM was used in this case to estimate the avoidance to individuals of *Brachycephalus* spp. To evaluate the influence of colours, place and trial on the predation of models, we also performed a GLM, but used Poisson's distribution and "log" as link function. Preference and avoidance in the model experiment were evaluated using Ivlev's electivity index, which ranges from -1 (avoidance) to 1 (preference) (IVLEV 1961). We performed all analyses in R 3.5.0 (R Core Team 2018) and with a 95 % confidence interval.

Results

A total of 35 *Brachycephalus ephippium* individuals were analysed to their colour. Their dorsal skin coloration ranged from yellow to orange, and dorsal bone colours ranged from orange to pink or blue (Fig. 4). Pearson's product-moment correlation test suggested a correlation between the natural and fluorescent colour tones of *B. ephippium* individuals (t = 5.35, P < 0.001). The two-way chi-squared test did not reveal a relationship between type of box (polycarbonate and transparent glass) and the type of experiment (before and after exposure to *B. ephippium*) (χ^2 = 1.88, P = 0.93). The GLM analysis indicated no effect of *B. ephippium* exposure and avoidance of new predation attempts (Table 1, Fig. S4). However, some chickens cleaned their
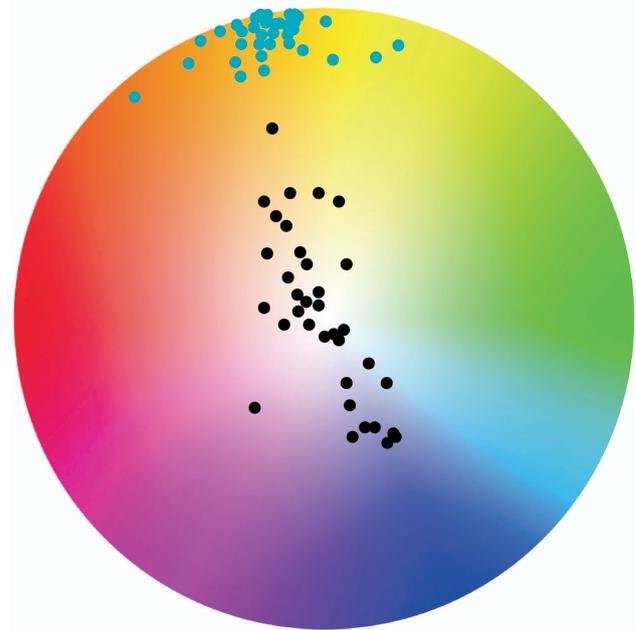
Figure 4. Colour map of the *Brachycephalus ephippium* dorsum, indicating the colours of regions without dermal bones, yellowish (blue dots), and of the region with dermal bones, whitish/bluish (black dots).

Table 1. Coefficients of the Generalized Linear Model analysis, using binomial distribution and "logit" as link function, of the ex situ experiment's data, illustrating the trend of avoidance of *Brachycephalus ephippium* after it was first made available.

	estimate	std. error	z value	P
interception	0.25	0.5	0.49	0.62
after exposure	-1.04	0.74	-1.41	0.16

Table 2. Coefficients of the Generalized Linear Model analysis, using Poisson's distribution and "log" as link function, of the in situ experiment's data, illustrating the influence of each parameter on the predation of the *Brachycephalus* spp. models (interaction between explanation variables are represented by ":"). Significant values are in bold.

	estimate	std. error	z value	P
interception	2.42	0.18	13.36	**<0.001**
site	1.37	0.15	8.95	**<0.001**
red colour morph	-0.5	0.22	-2.3	**0.02**
yellow colour morph	-0.62	0.23	-2.76	**0.006**
exposed group	0.22	0.18	1.24	0.21
red colour morph: trial	-0.08	0.29	-0.29	0.77
yellow colour morph: trial	-0.1	0.3	-0.32	0.75

beaks after sampling a *B. ephippium* individual, which is probably related to their unpalatability (Videos S1, S2).

We found models with three different types of marks (Figs 3F–H): V-shaped marks, which were associated with birds (pecking); dotted or crumbly marks, which were associated with arthropod predation; and U-shaped or parallel depressions (II-shaped) with tooth indentations, which were associated with small mammal or lizard bites. In addition, we registered instances of disappeared models, which thus could not be associated with any type of predator.

In general, out of the 200 trios arranged on cardboard (exposed group), 64 conspicuous models (30 yellow, 34 red), and 56 brown cryptic models were attacked (Fig. 3A). Out of the 200 trios arranged on leaf litter (cryptic group), 73 conspicuous models (34 yellow and 39 red), and 70 brown cryptic models were attacked (Fig. 3B). In Serra do Japi, out of the 100 trios of the exposed group, six yellow conspicuous, eight red conspicuous, and 17 brown cryptic models were predated upon. Out of the 100 trios of the cryptic group, three yellow conspicuous, eight red conspicuous, and 11 brown cryptic models were predated upon (Fig. 3B). At Núcleo Santa Virgínia, out of the 100 trios from the exposed group, 28 yellow conspicuous, 31 red conspicuous, and 53 brown cryptic models were predated upon. Out of the 100 trios of the cryptic group, 27 yellow conspicuous, 26 red conspicuous, and 45 brown cryptic models were predated upon (Fig. 3C).

GLM analysis showed a combined influence of locality and colour morphs on predation attempts. Conspicuous colours morphs were less often predated upon than cryptic

Table 3. Coefficients of Ivlev's electivity index for each plasticine model's colour and each site where the plasticine models were exposed to natural predators. Values range from -1 (avoided) to 1 (preferred).

	Serra do Japi		Núcleo Santa Virgínia	
	exposed	cryptic	exposed	cryptic
yellow	-0.26	-0.42	-0.14	-0.09
red	-0.12	0.04	-0.09	-0.11
brown	0.24	0.2	0.17	0.16

models (Fig. 5). However, there was no influence of trial type (exposed models, on white cardboard, and cryptic, on leaf litter) on the predated models (Table 2). Ivlev's electivity index revealed that the yellow models were avoided in Serra do Japi in both exposed and cryptic trials. The opposite was observed in Núcleo Santa Virgínia, where the red models were avoided more than the yellow models. The brown cryptic models were preferentially predated upon relative to the conspicuous models in all trial setups and at all sites (Table 3).
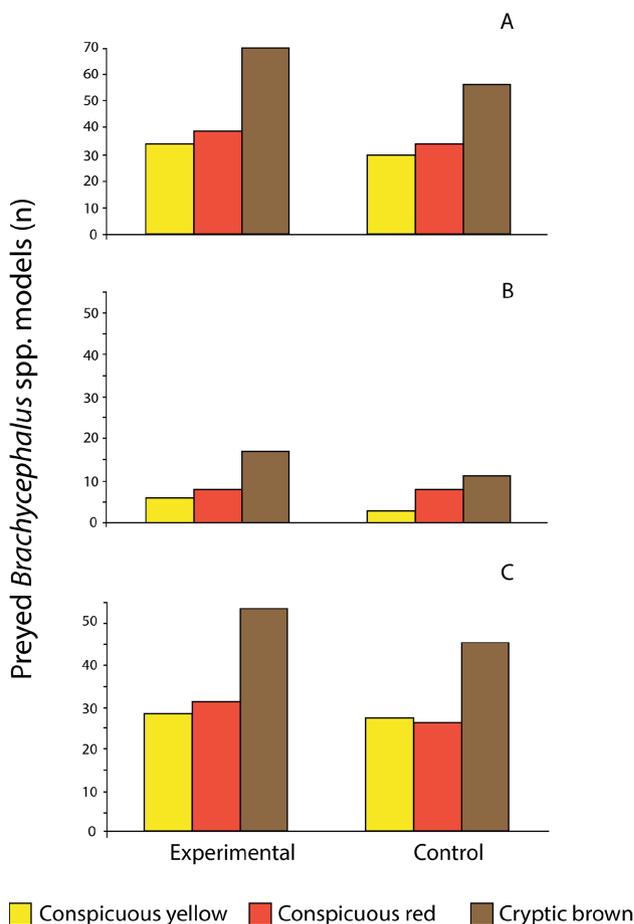

Figure 5. Attacks on plasticine models in the exposed and cryptic groups: pooled data (A) and data by locality: Serra do Japi (B) and Núcleo Santa Virgínia (C).

Discussion

Our results revealed no preference of *Gallus gallus* for a specific type of box (glass or polycarbonate). Also, we observed that experience with predation on *Brachycephalus ephippium* was not related to new predation attempts. *Brachycephalus ephippium* individuals have a high concentration of tetrodotoxin in several organs, especially in the skin (PIRES JR et al. 2002). This substance is present in several taxa (e.g., KODAMA et al. 1985), including amphibians (e.g., DALY et al. 1987) and its neurotoxic effect may be lethal (e.g., ABITA et al. 1977, KIERNAN et al. 2005). However, our data do not support a claim that the toxin concentration in *B. ephippium*'s skin promotes avoidance of further predation attempts by chickens.

The pattern of dorsal bone plates may play a role in interspecific recognition, since the form of these plates varies between species (e.g., ALVES et al. 2009, CLEMENTE-CARVALHO et al. 2009, CONDEZ et al. 2014), and in intraspecific sexual selection, as these plates also vary between individuals of the same species (POMBAL & IECKSOHN 2011, CONDEZ et al. 2014). Therefore, further studies are necessary to evaluate the functions of the fluorescence of *Brachycephalus* spp. dorsal bone plates.

Our results demonstrate that *B. ephippium* and *B. pitanga* are aposematic species (POULTON 1887, COTT 1940, RETTENMEYER 1970). Despite this being hypothesized in several studies (e.g., PIRES JR et al. 2002, DORIGO et al. 2012, BORNSCHEIN et al. 2016), we here present the first experimental evidence of aposematism in the genus *Brachycephalus*. We observed that attacks on conspicuous models were less intense in both trials (control and experimental setups) and at both trial sites (Serra do Japi and Núcleo Santa Virgínia). Brown models were preferred in predation attempts over yellow and red ones. Based on the aposematism hypothesis (COTT 1940, EDMUNDS 1974, ENDLER 1978), it was expected that attacks upon cryptic models would be less intense when they were placed on leaf litter, than when they were placed on contrasting backgrounds (e.g., KUCHTA 2005, SAPORITO et al. 2007, FARALLO & FORSTNER 2012). However, we did not detect differences in the attack frequency on cryptic models between contrasting and cryptic backgrounds. Instead, predators preferred cryptic models even when these were made available on matching backgrounds, suggesting that the predator's image searching may already be overcoming their cryptic function.

Our observation that autochthonous conspicuous colour morphs were more avoided than allochthonous colour morphs in Serra do Japi corroborates our autochthonous recognition hypothesis. However, for Núcleo Santa Virgínia, both conspicuous colour morphs were predated upon to a similar extent. This can be explained by *B. pitanga* individuals being endemic to this locality, but individuals of other *Brachycephalus* species, which have colour patterns similar to that of *B. ephippium*, occur near this locality: *B. toby* is known to occur just 8 km from Núcleo Santa Virgínia (HADDAD et al. 2010) and *B. crispus* about 20 km distant (CONDEZ et al. 2014). Therefore, it is possible that predators in this area may be in contact not only with *B. pitanga*, but also with other bright yellow species, which could be mimicked by our yellow model. We also demonstrated that there was avoidance of both yellow and red models, in contrast to brown models, indicating that these species are also aposematic in Núcleo Santa Virgínia, and also corroborating the autochthonous recognition hypothesis.

We demonstrated in this study that predator experience, and not prey fluorescence, may be a factor inducing avoidance in prey selection. Similarly, we found evidence that the conspicuous coloration of brachycephalids native to the Brazilian Atlantic forest negatively influence predation intensity. It suggests that predators avoid conspicuous and prefer cryptic models, probably because they associate the conspicuous coloration with defensive strategies (RETTENMEYER 1970, SHERRATT 2002, TOLEDO et al. 2011). The evidence obtained from our experimental study suggests that aposematism in these species is geographically related, effectively deterring only syntopic (probably experienced) predators. Therefore, we may have obtained the first experimental evidence of aposematism being employed as a defensive strategy by the notoriously toxic *Brachycephalus* genus and our study provides additional data highlighting the importance of considering syntopy or sympatry in predator-prey relationships.

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Supplementary material

Supplementary Figure S1. Comparison between an individual of *Brachycephalus ephippium* and a corn grain.

Supplementary Figure S2. Experiment with chicken and individuals of *Brachycephalus* encased in two boxes.

Supplementary Figure S3. Two individuals of *Brachycephalus ephippium* under UV light in a box that reflects UV radiation, and a transparent glass box, permeable by UV light.

Supplementary Figure S4. Percentage of *Gallus gallus* individuals that attempted or did not attempt to predate upon *Brachycephalus ephippium*.

Supplementary Video S1. Footage of the ex situ experiment.

Supplementary Video S2. Footage of the ex situ experiment.