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Defensive behaviours in *Bokermannohyla luctuosa* (Pombal and Haddad, 1993) (Anura, Hylidae)

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Abstract. Anurans are preyed on by a wide diversity of vertebrates and invertebrates, which use different senses and predatory strategies to capture them. For this reason, it is believed that such diversity is the main factor causing such wide defensive repertoire for anurans. Herein we described the defensive behaviour repertoire of *Bokermannohyla luctuosa*, a frog species distributed in montane areas within the Atlantic Forest domain in the states of São Paulo, Minas Gerais, and Rio de Janeiro, southeastern Brazil (Caramaschi and Verdade 2004). The study was carried out at the Parque Natural Municipal Augusto Ruschi, a conservation unit located in the municipality of São José dos Campos, southeastern Brazil. We observed the behaviours when we approached and while we were manipulating the animals. Eighteen individuals of *B. luctuosa* presented six undocumented behaviours for the species, which were: contracting, puffing up the body, crouching down, slippery secretions, immobility, and flee. Flee was the most common behaviour, observed in all individuals after being released. For all individuals, the only previously reported behaviour that we observed was odoriferous secretions. Our observations support the hypothesis that anurans may adopt different defensive strategies, according to the predation event and the predator, since we found that different defensive strategies were adopted in situations with or without manipulation. The species *B. luctuosa* has eight different defensive mechanisms, which may or may not be used simultaneously. We present the first record of the defensive behaviours of puffing up the body, slippery secretion, and contracting for the *Bokermannohyla* genus, further demonstrating that many aspects about the behaviour and natural history for these treefrogs are still unknown.

Key-words: Atlantic forest, Defence, Ethology, Natural history, PNMAR

Introduction

Predation is an important selective force for the adaptations of prey-predators (Lima and Dill 1990). The high predatory pressure exerted on anurans is considered the main factor responsible for the wide defensive repertoire of these animals (Haddad et al., 2013). When threatened, they can perform different defensive strategies, allowing them to escape from their predators in a variety of ways (Martins et al., 1993; Toledo et al., 2007, 2011). In general, all defensive behaviours act as important mechanisms for anurans to protect themselves from different predators (Ferrante et al., 2014).

The treefrog *Bokermannohyla luctuosa* (Pombal and Haddad 1993) belongs to the *Bokermannohyla circumdata* species group (Faivovich et al., 2005) which includes 19 species (Carvalho et al., 2012) that share some characteristics as: simple transverse bands on the posterior surface of thighs, well-developed prepollex, and hypertrophied forearm in adult males (Heyer 1985; Napoli and Juncá 2006; Napoli and Pimenta 2009). Within the *Bokermannohyla* genus (Frost 2017), defense behaviours are known for at least five species from different groups (Toledo et al., 2011).

The species *B. luctuosa* is distributed in montane areas within the Atlantic Forest domain in the states of São Paulo, Minas Gerais, and Rio de Janeiro, southeastern Brazil (Caramaschi and Verdade 2004). Currently known defence strategies for *B. luctuosa* include distress

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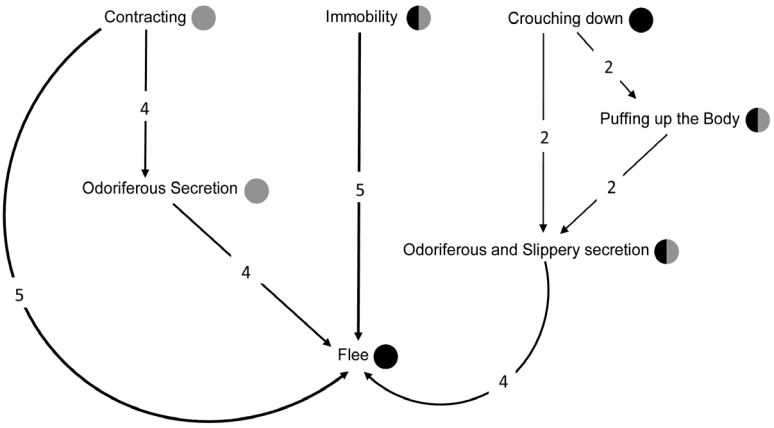

Figure 1. Ethogram of the defensive behaviours displayed by *Bokermannohyla luctuosa* individuals. The circles presented on the ethogram indicate the condition in which each behaviour was displayed while the numbers indicate the number of individuals that performed the defensive display. The grey circles indicate that the behaviour occurred during handling, the black circles indicate that the behaviour occurred during approximation, and merged circles indicate that the behaviour occurred during handling and approximation. All individuals flee after release.

calls (Toledo and Haddad 2009), odoriferous secretions (Toledo et al., 2011), mouth-gaping and spine aggression (Pombal and Haddad 1993). However, aspects about the behavioural ecology and defensive displays of *B. luctuosa* still need further investigation. Herein, in order to better understand the defensive mechanisms of *B. luctuosa*, we study and describe the defensive behaviour repertoire observed for this species.

Materials and methods

Field observations took place on 17 January 2016 at the Parque Natural Municipal Augusto Ruschi (23°04'S, 45°55'W, datum WGS-84), a conservation unit located in the municipality of São José dos Campos, São Paulo State, southeastern Brazil. We observed all behaviours at night from 18:00 h to 23:30 h. In order to standardize the observations, they were always realized by the same person.

We visually observed the defensive behaviour when we first found the individuals, from the moment they were manually captured, and right after they were released. The classification of defensive behaviours followed Toledo et al. (2011). We measured temperature and air humidity during the observations with a thermohygrometer (Instrutemp ITHT 2250). The air temperature ranged from 19.7 ºC to 26.8 ºC (mean = 23.25°C), and the relative air humidity from 80% to 86% (mean = 83%). We observed a total of 17 males and one juvenile of *B. luctuosa*. Two individuals were collected as voucher specimens (capture permit from Authorization System and Information on Biodiversity-SISBio 48620-2) and were deposited in the herpetological collection of Universidade Estadual de Campinas, São Paulo State, Brazil (accession numbers ZUEC 23318 and 23319).

Results

When spotted or handled, all individuals presented different defensive strategies (Fig. 1). We recorded six undocumented behaviours for the species. The strategies were: contracting (n=9), puffing up the body (n=2), crouching down (n=4), slippery secretions (n=4),

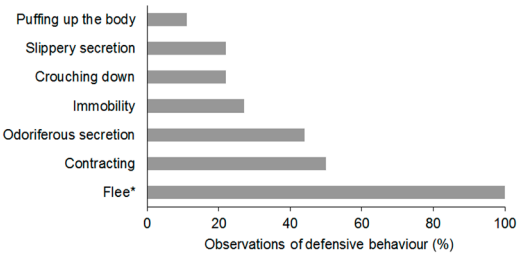

Figure 2. Defensive behaviours and the respective frequencies observed for the treefrog *Bokermannohyla luctuosa*, municipality of São José dos Campos, southeastern Brazil. The * indicates flee after release.

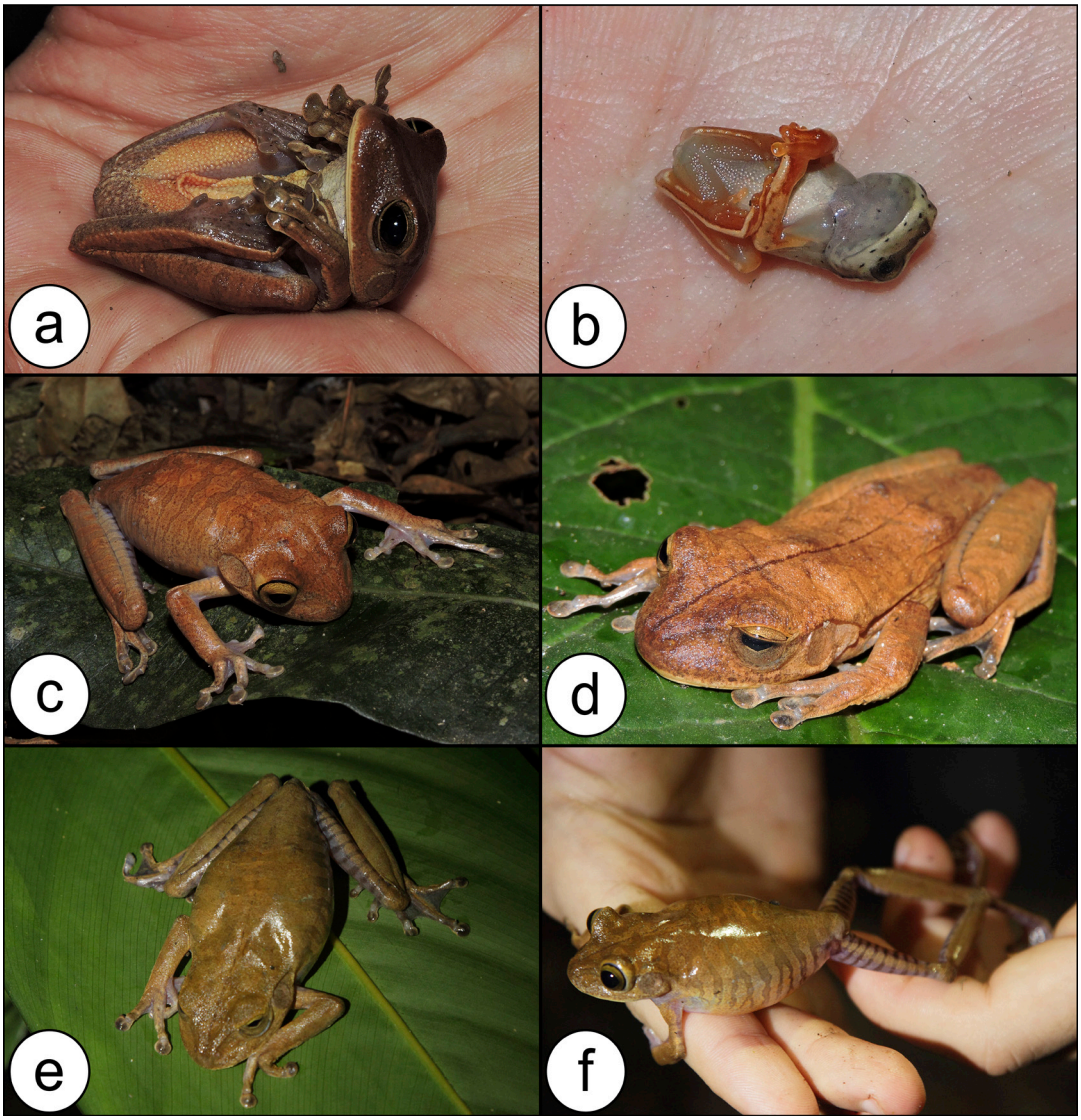

Figure 3. Some defensive behaviours of *Bokermannohyla luctuosa* observed in the municipality of São José dos Campos, state of São Paulo, Brazil. (a) Contracting; (b) Contracting in juvenile individual; (c) and (d) Crouching down; (e) Puffing up the body with crouching down; (f) Puffing up the body when handled. Photographs: Matheus Moroti (a-d) and Rodrigo Dela Rosa (e-f).

immobility (n=5), and flee (n=18). The most frequent behaviour recorded was "flee" when individuals were released (100%), followed by "contracting" (50%) (Fig. 2), including a juvenile (Fig. 3b), and odoriferous secretions (44%). Four individuals synergistically presented contracting behaviour followed by odoriferous secretions.

Four individuals presented crouching down behaviour as soon as they were spotted on the vegetation (22%; Fig. 3c). Two individuals synergistically presented the behaviour of puffing up their body (11%), one before it was captured (Fig. 3e), and another one when it was captured (Fig. 3f). When handled, both individuals produced an odoriferous secretion with a sweetish smell followed by a slippery secretion (22%), which facilitated the escape of the individual. When handled, two individuals presented only crouching down behaviour, followed by odoriferous and slippery secretion. Five

individuals presented immobility behaviour when spotted and handled (27%). All individuals presented escape behaviour after released.

Discussion

The variation in the degree of stress that is generated by a predator upon approaching or handling a prey may trigger different defensive behaviours for the latter (Lourenço-de-Moraes et al., 2016). Of the 18 individuals observed in this study, none presented the previously registered behaviours for the species (mouth gaping, spine aggression, and distress call), except for odoriferous secretions (Fig. 1) (Pombal and Haddad 1993; Toledo et al., 2011). It is possible that *B. luctuosa* did not present those behaviours due to the degree of stress generated by handling.

The adoption of different behaviours combined may increase the defensive efficiency for anurans (Toledo et al., 2011). In the study area we observed potential predators of *B. luctuosa*, such as the snakes *Bothrops jararaca* (Wied, 1824) and *Xenodon neuwiedii* (Günther, 1863), which are documented as predators for other hylid species (Hartmann et al., 2009), as well as the bird *Attila rufus* (Vieillot, 1819), which was previously recorded preying on an individual of *B. luctuosa* in the study area (Souza et al., 2017). Our observations support the hypothesis that anurans may adopt different defensive strategies, according to the predation event and the predator, since different defensive strategies were adopted in situations with or without manipulation (Menin and Rodrigues 2007; Ferrante et al., 2014).

For contracting behaviour, the individual remains motionless to avoid visual detection by predators or to prevent further damage during capture caused by struggling (Miyatake et al., 2004; Toledo et al., 2010). Similar to adult individuals, the juvenile individual we recorded presented this contracting defensive behaviour, suggesting that this defensive strategy does not present ontogenetic variation. However, we found only one juvenile individual, needing more observations to support this hypothesis

During our observations, we recorded that *B. luctuosa* is able to use odoriferous and slippery secretions, which act as chemical warnings of harmfulness to predators (Smith et al., 2004). This associated behaviour may indicate that the individuals can change behaviours after failed physical defences, such as contracting and puffing up the body, or by mouth-gaping and spine aggression. Usually, odoriferous and slippery secretions are a source of volatile and bioactive compounds that can control and prevent predation events (Ehrlichman and Bastone 1992).

The slippery secretion produced by *Bokermannohyla luctuosa* may make the individual unpalatable, while the odour could be functionally associated with the memory of unpalatability, preventing future predation events for individuals that produce such odour, including nocturnal predators or those with limited vision (Williams et al., 2000; Smith et al., 2004). The yellowish colour found on the ventral region of adults of *B. luctuosa*, as well as the reddish legs and toes in the juveniles (Fig. 3b), may have an aposematic function, i.e., they may visually indicate toxicity or unpalatability to predators (Toledo et al., 2010).

The species *B. luctuosa* displays eight different defensive mechanisms, which may or may not be used simultaneously. We present the first records of the defensive behaviours puffing up the body, slippery secretion, and contracting for the *Bokermannohyla* genus, supporting that many aspects about the behaviour and natural history of these treefrogs are still unknown. Based on the defensive behaviours observed here, we postulate that different populations of *B. luctuosa* may display different defence strategy responses, since they could be adapted to the different predators at each location.

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References

Caramaschi, U., Verdade, V. (2004): *Bokermannohyla luctuosa*. The IUCN Red List Of Threatened Species. http://www.iucnredlist.org/13/03/17/1. Accessed 13 March 2017.

Carvalho, T.R., Giaretta, A.A., Magrini, L. (2012): A new species of the *Bokermannohyla circumdata* group (Anura: Hylidae) from southeastern Brazil, with bioacoustic data on seven species of the genus. Zootaxa **68**: 57–68.

Ehrlichman, H., Bastone, L. (1992): Science of Olfaction. In: Serby MJ, Chobor KL (Eds.) Olfaction and Emotion, (1st ed). Springer-Verlag Inc, New York, pp 410–433.

Faivovich, J., Haddad, C.F.B., Garcia, P.C.A., Frost, D.R., Campbell, J.A., Wheeler, W.C. (2005): Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. Bulletin of the American Museum of natural History **294**: 1–240.

Ferrante, L., Sacramento, M., Angulo, A. (2014): Defensive behaviour in *Aplastodiscus leucopygius* (Cruz and Peixoto, 1985) (Anura: Hylidae). Herpetology Notes **7**: 135–138.

Frost, D.R. (2017): Amphibian Species of the World: an Online Reference. Version 6.0. http://research.amnh.org/herpetology/amphibia/index.html/13/03/17/1. Accessed 13 March 2017.

Haddad, C.F.B., Toledo, L.F., Prado, C.P.A., Loebmann, D., Gasparini, J.L., Sazima, I. (2013): Guia dos anfíbios da Mata Atlântica: diversidade e biologia. Anolis Books, São Paulo.

Hartmann, P.A., Hartmann, M.T., Martins, M. (2009): Ecologia e história natural de uma taxocenose de serpentes no Núcleo Santa Virgínia do Parque Estadual da Serra do Mar, no sudeste do Brasil. Biota Neotropica, **9**: 173–184.

Heyer, W.R. (1985): New species of frogs from Boracéia, São Paulo, Brazil. Proceedings of the Biological Society of Washington **98**: 657–671.

Lima, S.L., Dill, L.M. (1990): Behavioral decisions made under the risk of predation: a review and prospectus. Canadian journal of zoology **68**: 619–640.

Lourenço-de-Moraes, R., Ferreira, R.B., Mira-Mendes, C.V., Zocca, C.Z., Medeiros, T., Ruas, D.S., Solé, M. (2016): Escalated antipredator mechanisms of two neotropical marsupial treefrogs. The Herpetological Journal **26**: 237–244.

Martins, M., Sazima, I., Egler, S.G. (1993): Predators of the nest building gladiator frog *Hyla faber*, in southeastern Brazil. Amphibia-Reptilia **14**: 307–309.

Menin, M., Rodrigues, D.J. (2007): *Ctenophryne geayi* (Brow Egg Frog). Behavior. Herpetological Review. **38**(2): 182.

Miyatake, T., Katayama, K., Takeda, Y., Nakashima, A., Sugita, A., Mizumoto, M. (2004): Is death-feigning adaptive? Heritable variation in fitness difference of death-feigning behaviour. Proceedings of the Royal Society of London B: Biological Sciences **271**: 2293–2296.

Napoli, M.F., Juncá, F.A. (2006): A new species of the *Bokermannohyla circumdata* group (Amphibia: Anura: Hylidae) from Chapada Diamantina, State of Bahia, Brazil. Zootaxa **1244**: 57–68.

Napoli, M.F., Pimenta, B.V.S. (2009): A new species of the *Bokermannohyla circumdata* group (Anura: Hylidae) from the coastal forests of Bahia, northeastern Brazil. Copeia **4**: 674–683.

Pombal, J.P., Haddad, C.F.B. (1993): *Hyla luctuosa*, a new treefrog from southeastern Brazil (Amphibia, Hylidae). Herpetologica **49**: 16–21.

Souza, R.D.R., Moroti, M.T., Briet, J., Machado, I.F. (2017): *Bokermannohyla luctuosa*. Predation. Herpetological Review **48**: 158.

Smith, B.P.C., Williams, C.R., Tyler, M.J., Williams, B.D. (2004): A survey of frog odorous secretions, their possible functions and phylogenetic significance. Applied Herpetology **2**: 47–82.

Toledo, L.F., Ribeiro, R.S., Haddad, C.F.B. (2007): Anurans as prey: an exploratory analysis and size relationships between predators and their prey. Journal of Zoology **271**: 170–177.

Toledo, L.F., Haddad, C.F.B. (2009): Defensive vocalizations of Neotropical anurans. South American Journal of Herpetology **4**: 25–42.

Toledo, L.F., Sazima, I., Haddad, C.F.B. (2010): Is it all death feigning? Case in anurans. Journal of Natural History **44**: 1979–1988.

Toledo, L.F., Sazima, I., Haddad, C.F.B. (2011): Behavioural defences of anurans: an overview. Ethology Ecology & Evolution **23**: 1–25.

Williams, C.R., Brodie Jr, E.D., Tyler, M.J., Walker, S.J. (2000): Antipredator mechanisms of Australian frogs. Journal of Herpetology **34**: 431–443.

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