Polyphenism: Defensive colour behaviour of *Phasmahyla guttata* (A. Lutz, 1924) (Amphibia, Anura, Hylidae)

Ibere Farina Machado^{1*}, Rafael Costabile Mengucci², Humberto Fonseca Mendes², Matheus Toledo Moroti³

Amphibians are a link between the terrestrial and the aquatic environment in reproductive events or as part of prey-predator interactions. To avoid being eventually consumed by potential predators such as other vertebrates or invertebrates (Tilman, 1986), amphibians developed defensive strategies (Duellman and Trueb, 1994). These strategies could be morphological, physiological, and behavioural or combine more than one feature (see review in Toledo et al. 2011; Mailho-Fontana et al., 2013). The understanding of these strategies is crucial in unraveling aspects of their natural history and of trophic interactions.

The subfamily Phyllomedusinae Günther, 1858 (Amphibia, Anura, Hylidae) currently consists of five genera (Agalychnis Cope, 1864, Cruziohyla Faivovich et al., 2005, Phasmahyla Cruz, 1990, Phrynomedusa Miranda-Ribeiro, 1923 and Phyllomedusa Wagler, 1830) and all these genera have peculiar characteristics, including a vertical pupil, green dorsal colouration and usually hidden patterns of red, blue and yellow. Furthermore, they exhibit highly specialized reproductive strategies by laying their eggs outside the water in nests built from rolled-up leaves (Caramaschi, 2006). The genus Phasmahyla, in which seven species are recognized, is found in the Atlantic Rainforest from the Brazilian states of Bahia to southwards Paraná (Cruz, Napoli and Fonseca, 2008). *Phasmahyla guttata* (A. Lutz, 1924), occurs from Paraná to Espírito Santo (Cruz, 1990; Oliveira et al., 2009).

We found an individual of *P. guttata* (SVL 34,7 mm) at night on the edge of a creek on shrub \pm 50cm soil; humidity 83%; temperature 21,3°C; 38 m above sea level; on June 04, 2015 in Dacnis Project, município de Ubatuba (23° 27.525S; 45° 08.973W; WGS 84), São Paulo state, Brazil. We handled the animal to take a photograph at 19:41 h. At this time the dorsum had a red/orange-brown colour (Figure 1.1). After 10 minutes (19:53 h), the specimen had turned brown-green (figure 1.2) and at 20:07 h (26 minutes later) it was light green with black spots (figure 1.3 and 1.4). The specimen secreted an extremely sticky substance similar to glue.

The use of colours as a defensive strategy by anurans may be widespread and its protective effect may vary depending on the species (Toledo and Haddad, 2009). Phasmahyla guttata displays a change of colour from brown to green. These colours could be described as camouflage, where an animal blends in with a part of its environment, such as branches and leaves. Although this change in colour pattern is known for phyllomedusines as polyphenism (sensu Hanlon et al. 1999), it was described as change from purplish during night activity to greenish during daytime resting (Toledo and Haddad, 2009) for Phasmahyla cochranae (Bokermann, 1966), P. guttata, Phasmahyla jandaia (Bokermann and Sazima, 1978), and in Phyllomedusa azurea Cope (1862), Phyllomedusa megacephala (Miranda-Ribeiro, 1926), and Phyllomedusa rohdei Mertens (1926). Although Toledo and Haddad (2009) have recorded this behaviour for P. guttata, our report is the first comprehensive record on dynamic polyphenism (i.e. color change in a short period of time) in the genus Phasmahyla.

Apart from the change between two camouflage colours of the dorsum (polyphenism), *P. guttata* features striking orange and purple colouration on its sides. Such

Ibere Farina Machado^{1,*}, Rafael Costabile Mengucci², Humberto Fonseca Mendes², Matheus Toledo Moroti³

¹ Instituto Boitatá, Goiânia, Goiás, Brazil.

² Universidade Federal de Alfenas, Alfenas, Minas Gerais, Brazil;

³ Universidade do Vale do Paraíba, São José dos Campos, São Paulo, Brazil;

^{*} Corresponding author e-mail: iberemachado@institutoboitata.org



Figure 1. Adult *Phasmahyla guttata* displaying a polyphenism defensive behaviour at Dacnis Project, Ubatuba municipality (23° 27.525S; 45° 08.973W; WGS 84), São Paulo state, Brazil. 1) dorsum was red/orange brown at 19:41 h; 2) brown green at 19:53 h; 3) and 4) light green with black spots at 20:07 h. Photos by Rafael Menegucci.

colouring can be classified as aposematic. Aposematic colour is associated with poisons or unpalatability and acts as a warning to potential predators (Gamberale-Stille and Guilford, 2003). We believe that the colour change during handling might increase the contrast between the aposematic colouration of the flanks and that of the back (Figure 1.1-4).

Associated to this behaviour, the specimen secreted a transparent and extremely sticky substance. We did not notice a strong smell, burning or irritation on contact with this substance. Secretions have been isolated from various genera of the Phyllomedusinae subfamily (Raja et al., 2013). In the genus *Phasmahyla*, only the skin of *P. jandaia* has been chemically analysed. Active ingredients were identified as peptides (e.g. sauvagine), deltorphins, dermorphins, dermaseptins, dermatoxins, phyllokinins, phylloseptin, plasticins and tryptophyllins (Rates et al., 2011). During the handling of *Phyllomedusa* and *Phasmahyla* the parotoid or laterodorsal glands often

turn to a darker colour compared to the rest of the skin (Délio Baêta, personal communication). Future studies will reveil the composition of the secretion in *P. guttata* and if the colour change of glands occurs because of a substance in the secretion. The effects of peptides include the protection against microbial infections and the defence against predators (Sazima, 1974; Leite et al., 2005).

According to Carvalho-e-Silva et al (2009) *Phasmahyla cruzi* is only known from its type locality in "Rio das Pedras Reserve, Municipality of Mangaratiba (09°19'08"S, 36°28'16"W), which is in the middle of the geographical distribution of *P. guttata*. Both species are virtually indistinguishable, except for the tadpoles which differ by the presence of one or more white spots on the back of the posterior third of the tail on *P. cruzi* (absence in *P. guttata*) and by a larger oral disc, representing 100% of the body width of *P. cruzi*, but only 70% in *P. guttata* (Carvalho-e-Silva et al.,

468



Figure 2. Tadpole morphs 1) and 2) *Phasmahyla guttata* and 3)-4) *Phasmahyla cruzi* at Dacnis Project, Ubatuba municipality (23° 27.525S; 45° 08.973W; WGS 84), São Paulo state, Brazil. Photos by Rafael Menegucci (1 and 3), Edélcio Muscat (2) and Matheus Moroti (4).

2009). Based on this similarity, Faivovich et al (2010) considered their sample *P. guttata* in Ubatuba to be *P. cruzi* (Faivovich et al., 2005, had named this eventually as *P. guttata*). In the area, we found tadpoles resembling the *P. guttata* morph and the *P. cruzi* morph (Figure 2.1-4). A recently launched study will determine the identity of these tadpoles based on molecular data. As a result we hope to answer whether this is the first record of sympatry between two species of *Phasmahyla* or if *P. guttata* and *P. cruzi* are synonymous.

Acknowledgements. We are grateful to Edélcio Muscat (for his kind permission to use the picture in figure 2.2) and Elsie Rotenberg for the study permit in the areas of Projeto Dacnis. We thank Unifal and Instituto Boitata for the support of our research, João Pedro Costa Elias and Karime Araújo Paina for their assistance in the field and Dr. Délio Baêta and anonymous reviewer for critically reading and improving our manuscript.

References

- Caramaschi, U. (2006): Redefinição do grupo de *Phyllomedusa hypochondrialis*, com redescrição de *P. megacephala* (Miranda-Ribeiro, 1926), revalidação de *P. azurea* Cope, 1862 e descrição de uma nova espécie (Amphibia, Anura, Hylidae). Arquivos Do Museu Nacional, Rio de Janeiro, **64**(2): 159–179.
- Carvalho-e-Silva, A.M.P.T. de, Silva, G.R. da, & Carvalho-e-Silva, S.P. de (2009): A new species of *Phasmahyla* Cruz, 1990 from the Atlantic Forest in the state of Rio de Janeiro, Brazil (Amphibia, Hylidae, Phyllomedusinae). Zootaxa, **2120**(2009): 15–26.
- Cruz, C.A.G. da (1990): Relações intergenéricas de Phyllomedusinae. Revista Brasileira de Biologia, 50(3): 709– 726.
- Cruz, C.A.G. da, Napoli, M.F., Fonseca, P.M. (2008): A new species of *Phasmahyla* Cruz, 1990 (Anura: Hylidae) from the state of Bahia, Brazil. South American Journal of Herpetology, 3(3): 187–195.
- Duellman, W.E., Trueb, L. (1994): Biology of Amphibians, McGrawHill Inc New York, The Johns Hopkins University Press.

- 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
- Faivovich, J., Haddad, C.F.B., Baêta, D., Jungfer, K.-H., Álvares, G.F.R., Brandão, R.A., Sheil, C., Barrientos, L.S., Barrio-Amorós, C. L., Cruz, C.A.G., Wheeler, W.C. (2010): The phylogenetic relationships of the charismatic poster frogs, Phyllomedusinae (Anura, Hylidae). Cladist, 26(2010): 227–261.
- Gamberale-Stille, G., Guilford, T. (2003): Contrast versus colour in aposematic signals. Animal Behaviour, 65: 1021–1026.
- Hanlon, R.T., Forsythe, J. W., Joneschild, D.E. (1999). Crypsis, conspicuouness, mimicry and polyphenism as antipredator defences of foraging octopuses on Indo-Pacific coral reefs, with a method of quantifying crypsis from video tapes. Biological Journal of the Linnean Society, 66(1): 1–22.
- Leite, J.R.S.A., Silva, L.P., Rodrigues, M.I.S., Prates, M. V., Brand, G.D., Lacava, B.M., Azevedo, R.B., Bocca, A.L., Albuquerque, S., Bloch, C. (2005): Phylloseptins: a novel class of antibacterial and anti-protozoan peptides from the *Phyllomedusa* genus. Peptides, **26**(4): 565–73.
- Mailho-Fontana, P.L., Antoniazzi, M.M., Toledo, L.F., Verdade, V.K., Sciani, J.M., Barbaro, K.C., Pimenta, D.C., Rodrigues, M.T., Jared, C. (2013): Passive and active defense in toads: The parotoid macroglands in *Rhinella marina* and *Rhaebo guttatus*. Journal of Experimental Zoology, **9999**: 1–13.
- Oliveira, J.C.G., Coco, L., Deus, F.F. de, Pagotto, R.V., Silva, E.S. da, Rocha, C.F.D. da, & Vreibradic, D. (2009): Geographic distribution: *Phasmahyla guttata*. Herpetological Review, **40**(4): 446.

- Raja, Z., André, S., Piesse, C., Sereno, D., Nicolas, P., Foulon, T., ... Ladram, A. (2013): Structure, Antimicrobial Activities and Mode of Interaction with Membranes of Bovel Phylloseptins from the Painted-Belly Leaf Frog, *Phyllomedusa sauvagii*. PLoS ONE, 8(8): e70782.
- Rates, B., Silva, L.P., Ireno, I.C., Leite, F.S.F., Borges, M.H., Bloch, C., Lima, M.E., Pimenta, A.M.C. (2011): Peptidomic dissection of the skin secretion of *Phasmahyla jandaia* (Bokermann and Sazima, 1978) (Anura, Hylidae, Phyllomedusinae). Toxicon, 57(1): 35–52.
- Sazima, I. (1974): Experimental predation on the Leaf-frog *Phyllomedusa rohdei* by the water snake *Liophis miliaris*. Journal of Herpetology 8: 376-377
- Tilman, D. (1986): A consumer-resource approach to community structure. American Zoologist, 26(1): 5–22.
- Toledo, L.F., & Haddad, C.F.B. (2009): Colors and Some Morphological Traits as Defensive Mechanisms in Anurans. International Journal of Zoology, 2009: 1–12.
- Toledo L.F, Sazima I, & Haddad C.F.B. 2011. Behavioural defences of anurans: an overview. Ethology Ecology & Evolution 23: 1–25.