

The Amazonian toad *Rhaebo guttatus* is able to voluntarily squirt poison from the paratoid macroglands

Carlos Jared^{1,*}, Marta Maria Antoniazzi¹, Vanessa Kruth Verdade², Luís Felipe Toledo³,
Miguel Trefaut Rodrigues⁴

Abstract. Amphibian defence mechanisms commonly rely on cutaneous toxins produced in either isolated or clustered glands, such as toad paratoid macroglands. In contrast to the passive mechanism of poison liberation in other amphibians, we discovered that the Amazonian toad *Rhaebo guttatus* is unique because it can voluntarily squirt jets of poison from its paratoids.

Keywords: Amphibia, Anura, Bufonidae, defence, granular glands, poison glands.

Amphibians commonly use toxic, cutaneous secretions produced by glands spread over the body as a defence against predators (Duellman and Trueb, 1994). In bufonids, these glands are grouped behind the eyes, forming a pair of post-orbital structures, known as paratoid macroglands (Toledo and Jared, 1995; Tyler, Burton and Bauer, 2001). Amongst amphibians, toads provide a classic example of passive defence, based on contact between a predator's oral mucosa and cutaneous poisonous secretion. When threatened, they react by inflating their bodies in such a way that the paratoids become turgid and primed. When an aggressor bites a toad, it becomes the agent of its own poisoning by triggering the macroglands and, consequently, receiving jets of poison in its mouth (Jared et al., 2009).

The activation of the amphibian's chemical defence usually seems to be dependent on predator's bites (Jared et al., 2005, 2009; Toledo and Haddad, 2009; Heiss et al., 2010). Here we

present an unknown, and presumably unique, defence system found in the toad *Rhaebo guttatus* Schneider, 1799. In contrast to the passive defence mechanisms of other amphibians, we have discovered that this species is capable of voluntarily ejecting poison from its paratoids.

Until recently, all common toads were included in the genus *Bufo*. However, this genus is now restricted to species found in Europe, Asia and Africa; most Latin American species are now in the genus *Rhinella* (Frost, 2011). Basal species, such as the species formerly known as *Bufo guttatus*, are currently assigned to the genus *Rhaebo* (Pramuk, 2006; Pramuk et al., 2007). *Rhaebo* is comprised of nine species distributed across Central and South Americas (Frost, 2011). *R. guttatus* (fig. 1a) is widespread throughout the north of Brazil, in the Amazonian region (Frost, 2011).

Specimens of adult *R. guttatus* from two populations that are separated by a straight-line distance of 650 km (Jacareacanga – 06°13'20''S, 57°45'10''W, Pará state, and Borba – 04°35'49''S, 58°13'14''W, Amazonas state, Brazil) were observed in their natural habitat ($n = 21$, undefined sex, mean SVL 97.65 ± 9.09 mm). Staged encounters in the field were made by approaching an individual and registering its reaction to close approach, and gently hitting it with a stick or a finger on the head or dorsum (Toledo et al., 2011). The observations were registered by photographs and video recording.

Ten adult specimens from each population were collected for morphological studies. The voucher numbers of some sampled individuals are ZUEC 14982-85; 16040; 17150. For histological analysis, the paratoids were fixed

1 - Instituto Butantan, Av. Vital Brasil 1500, CEP 05504-900, São Paulo, Brazil

2 - Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Santo André, Brazil

3 - Museu de Zoologia, Universidade Estadual de Campinas, Campinas, Brazil

4 - Departamento de Zoologia, Universidade de São Paulo, São Paulo, Brazil

*Corresponding author; e-mail: jared@butantan.gov.br

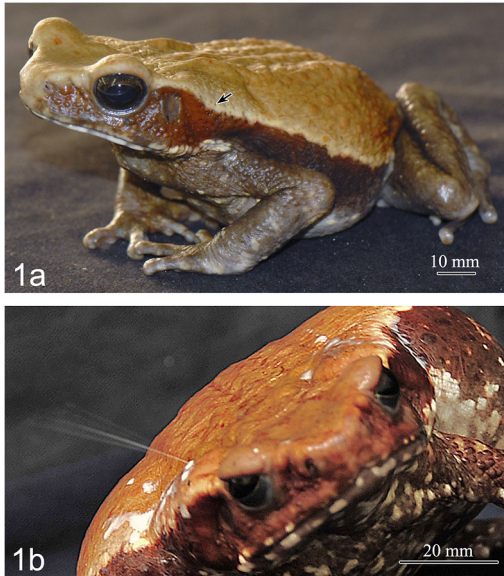


Figure 1. *R. guttatus*. (a) Note the parotoid macrogland, which is full of glandular pores (arrow). (b) The typical defensive posture the toad assumes when expelling poison jets from the parotoid. This figure is published in colour in the online version.

in Bouin's solution, dehydrated in ethanol and embedded in paraffin. Sections were stained by Haematoxylin-eosin and von Kossa methods.

The behaviour of venom squirting was observed in different adult individuals from both populations in the wild, just after the animals were captured, or in captivity. Out of the 21 adult *R. guttatus* encountered in the wild, 12 (about 57%) showed a defensive display that culminated in active poison squirting. Most of them (8) displayed the series of behaviours after our approaching, while the remaining 4 individuals displayed it only after finger or stick hitting. The complete defensive display consists on body rising by stretching out the front legs, inflating the lungs, opening and closing the mouth rapidly and repeatedly, tilting the body and aiming one of the parotoids towards the aggressor (fig. 1b). The toad then lowers its scapular waist and moves its head laterally towards the aggressor. With its eyes and mouth usually closed, the toad squirts poison in several yellowish jets that can extend a distance of up to two metres. Individuals that did not displayed poison squirt-

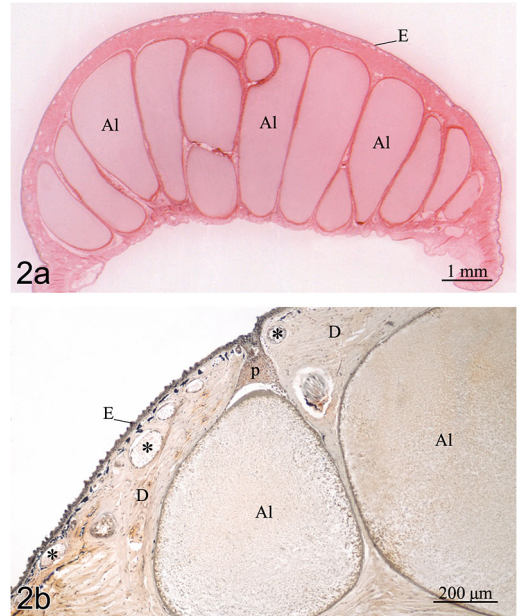


Figure 2. *R. guttatus*. (a) Transverse histological section of the parotoid, which shows the internal alveoli (Al) that lay side by side. E, epidermis. (b) Higher-magnification-histological section showing two alveoli (Al), full of poison, that are closed on the top by an epithelial plug (p). Asterisks indicate the superficial skin glands. E, epidermis; D, dermis. This figure is published in colour in the online version.

ing, did displayed other defensive behaviours, such as remaining motionless, fleeing, body rising, puffing up the body, body tilting, and opening and closing the mouth rapidly and repeatedly. Such defensive repertoire was not quantified. As in the field, in captivity poison squirting was also observed during toad manipulation, but seems to diminish with time.

The morphological study revealed that, similar to *Rhinella jimi* (Jared et al., 2009), the parotoids of *R. guttatus* present a honeycomb-like-internal structure consisting of juxtaposed alveoli. Each alveolus contains a very large, bottle-shaped gland, which is full of a poisonous secretion and is closed on top by an epithelial tissue plug (fig. 2). In both cases, poison release is not related with the presence of compressor muscles. For *R. jimi*, it was shown that it is the external pressure from the bite of a predator that abruptly increases the internal pressure, caus-

ing the plug to burst and the poison to jet forth (Jared et al., 2009).

The structural similarities between the parotoids of both toad species suggest that *R. guttatus* voluntarily poison squirt is more related to behaviour than to parotoid morphology. Like in *R. jimi* (Jared et al., 2009), the lung inflation expands *R. guttatus*' body and creates pressure at the base of the parotoids. When turning towards the side where an aggressor is located, the cranium pushes the corresponding parotoid backwards. The movement of the forelimbs changes the position of the supra-scapula, which pulls the wall of the macroglad. The effect of this movement can also influence its internal pressure. This possibly creates an analogous situation to the pressure caused by a predator's bite and triggers both the bursting of the plugs and the discharge of poison jets (fig. 1b).

The height and distance reached by the jets suggest that this defensive mechanism is used against large (and possibly visually oriented) predators, such as mammals or birds.

The fact that not all *R. guttatus* tested squirt poison can be explained by the large individual variation observed in defensive behaviour among anurans (Toledo et al., 2005, 2011). Furthermore it is important to take into consideration that poison squirting only can take place if the parotoids were not recently used, being sufficiently full for poison jets to be launched. In captivity, it was observed that poison squirting tends to decrease in frequency.

The absence of poison-squirting ability in other anurans suggests that this is an evolutionary novelty that appeared in *Rhaebo*. In this context, it would be important to analyse the behaviour and morphology of the parotoids in the other closely related species to verify whether this phenomenon represents a synapomorphy of the genus.

Amongst caudates, Brodie and Smatresk (1990) show that *Salamandra salamandra* is the only species able to launch poison. In contrast to *R. guttatus*, the poison jets in this species come from rows of enlarged mid-dorsal skin glands,

which are pressed upon by surrounding epaxial musculature.

Although *R. guttatus* has been described 200 years ago, the defensive behaviour of squirting poison remained unnoticed. This fact may be explained by the little information about this species when compared to other toads in which the poison squirting behaviour has never being observed.

There is a well-disseminated mistaken belief that toads are capable of squirting their poison into people's eyes (Jared and Antoniazzi, 2009). Our discovery shows that, at least in the Amazonian region, this belief can find support in the defensive behaviour of *R. guttatus*.

Acknowledgements. We thank T.P. Vasconcellos, O.G.S. Araújo, N.R. Silva, P.L. Mailho-Fontana, J. Cassimiro, J.M. Guellere and S.M. Souza for help in the field and S.G.S. Jared and L.F. Drake for technical assistance. CNPq, INCTTOX-CNPq and FAPESP provided financial support. IBAMA provided permissions to collect animals: #058/2007 to M.T. Rodrigues, and #17242-1 to L.F. Toledo.

References

- Brodie Jr., E.D., Smatresk, N.J. (1990): The antipredator arsenal of fire salamanders: spraying of secretions from highly pressurized dorsal skin glands. *Herpetologica* **46**: 1-7.
- Duellman, W.E., Trueb, L. (1994): *Biology of Amphibians*. Baltimore, The Johns Hopkins University Press.
- Frost, D.R. (2011): *Amphibian species of the world*: an online reference. American Museum of Natural History, New York, USA. Electronic database accessible at <http://research.amnh.org/vz/herpetology/amphibia/> Version 5.5.
- Heiss, E., Natchev, N., Salaberger, D., Gumpenberger, M., Rabanser, A., Weisgram, J. (2010): Hurt yourself to hurt your enemy: new insights on the function of the bizarre antipredator mechanism in the salamandrid *Pleurodeles waltl*. *J. Zool.* **280**: 156-162.
- Jared, C., Antoniazzi, M.M. (2009): Anfíbios: Biologia e Venenos. In: *Animais peçonhentos no Brasil. Biologia, clínica e terapêutica dos acidentes*, p. 317-330. Cardoso, J.L.C., França, F.O.S., Wen, F.H., Málaque, C.M.S., Haddad Jr., V., Eds, São Paulo, Sarvier Publisher.
- Jared, C., Antoniazzi, M.M., Navas, C.A., Katchburian, E., Freymüller, E., Tambourgi, D.V., Rodrigues, M.T. (2005): Head, co-ossification, phragmosis and defence in the casque-headed tree frog *Corythomantis greeningi*. *J. Zool.* **265**: 1-8.

- Jared, C., Antoniazzi, M.M., Jordão, A.E.C., Silva, J.R.M.C., Greven, H., Rodrigues, M.T. (2009): Parotoid macroglands in toad (*Rhinella jimi*): their structure and functioning in passive defence. *Toxicon* **54**: 197-207.
- Pramuk, J.B. (2006): Phylogeny of South American *Bufo* (Anura: Bufonidae) inferred from combined evidence. *Zool. J. Linn. Soc.* **146**: 407-452.
- Pramuk, J.B., Robertson, T., Sites Jr., J.W., Noonan, B.P. (2007): Around the world in ten million years: biogeography of the nearly cosmopolitan true toads (Anura: Bufonidae). *Global Ecol. Biogeogr.* **17**: 72-83.
- Toledo, L.F., Haddad, C.F.B. (2009): Colors and some morphological traits as defensive mechanisms in anurans. *Int. J. Zool.* **2009**: 1-13.
- Toledo, L.F., Tozetti, A., Zina, J. (2005): *Leptodactylus labyrinthicus* (Pepper Frog): Defensive repertoire. *Herpetol. Bull.* **90**: 29-31.
- Toledo, L.F., Sazima, I., Haddad, C.F.B. (2011): Behavioral defenses of anurans: an overview. *Ethol. Ecol. Evol.* **23**: 1-25.
- Toledo, R.C., Jared, C. (1995): Cutaneous granular glands and amphibian venoms. *Comp. Biochem. Physiol.* **111**: 1-29.
- Tyler, M.J., Burton, T., Bauer, A.M. (2001): Parotoid or parotid: on the nomenclature of an amphibian skin gland. *Herpetol. Rev.* **32**: 79-81.

Received: May 14, 2011. Accepted: September 19, 2011.