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Defensive behaviour of the El Rincón stream frog *Pleurodema somuncurens* in Argentina

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Anurans exhibit numerous antipredator mechanisms to avoid predation. More than 30 behavioural categories (Kowalski et al., 2018) associated with various morphological and physiological modifications in body size, colour patterns and toxin production are known (Ferreira et al., 2019; Pedroso-Santos et al., 2020) although the most commonly reported are escape behaviours (active escape or fleeing) and death feigning (Toledo et al., 2011).

Reports of these behaviours often come from casual observations of individuals being manipulated in the field or laboratory. However, for more controlled observations there are techniques that can be used to trigger defensive responses in anurans, in particular simulations of predator attack such as hitting gently with sticks or with the forefinger on the head and/or the back (Green, 1988; Chiochio et al., 2022), lightly pinching the head, arms and legs with blunt forceps (Blanchette & Saporito, 2016), by presenting a non-venomous snake to the frog (Villa, 1969; Toledo et al., 2011) or by using predator models (Bulbert et al., 2015).

Pleurodema Tschudi, 1838 is a Neotropical genus of leptodactylid frogs that currently includes 15 species. Three of them occur in southern South America (Faivovich et al., 2012; Ferraro, 2022): *P. bufoninum*, *P. somuncurens* and *P. thaul*, all within the *P. thaul* Clade. *Pleurodema bufoninum* and *P. thaul* are distributed in Argentina and Chile’s terrestrial habitats and are categorised as of Least Concern (IUCN, 2016). *Pleurodema somuncurens* is an entirely aquatic microendemic species from the Argentinean Patagonia (Velasco et al., 2016) and is categorised as Critically Endangered (IUCN, 2016). *Pleurodema bufoninum* and *P. thaul* have well-developed lumbar macroglands, while *P. somuncurens* has a slightly protuberant patch of glandular tissue in this region (Ferraro et al., 2013). *Pleurodema bufoninum* and *P. thaul* exhibit deimatic anti-predator behaviour associated with their macroglands (Cei & Espina Aguilera, 1957; Kolenc et al., 2009; Ferraro et al., 2021). Deimatic behaviour includes four movements that the frog displays synchronically (Fig. 1A) – (i) ‘crouching down’ behaviour (Marchisin & Anderson, 1978; Toledo et al., 2011); (ii) rear elevation (Ferreira et al., 2019); (iii) body inflation (Noble, 1931) and (iv) eye-protection (Toledo et

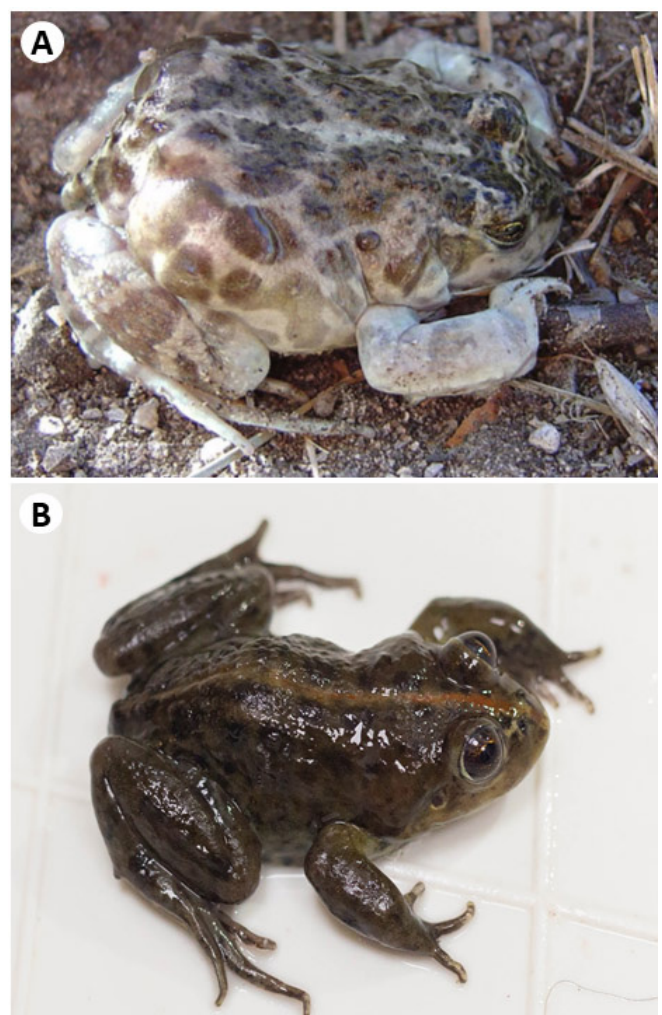


Figure 1. A. *Pleurodema bufoninum* showing deimatic behaviour, B. *Pleurodema somuncurens* not displaying deimatic behaviour following an ex situ test attempting to elicit a response

al., 2011). It was not known whether *P. somuncurens* also displayed deimatic behaviour, so we undertook in situ and ex situ experiments with adult and immature *P. somuncurens* to test for this.

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- The in situ experiment was conducted in January 2015 in the Valcheta stream (Río Negro province, Argentina, 40.98° S, 66.62° W) at night, between 21:00–22:00 h. We captured individuals in their aquatic habitat and placed them on the ground, blocking their path to prevent their escape. Approximately two minutes later we delivered a mechanical stimulus by gently hitting the snout of the frog with a 20 cm stick three times and pressing the stick to the snout for five seconds. We examined 34 individuals of *P. somuncurens*: 13 males, 13 females and eight juveniles (less than 28 mm and without secondary sexual characters). The ex situ experiment was undertaken in 2020 with 30 adult individuals of *P. somuncurens* (without differentiating between males and females) obtained from a survival assurance colony at the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Buenos Aires, Argentina. The study was the same as the in situ test but with the following differences: a) the individuals were removed from their aquariums and placed in a tray to prevent them from escaping and b) the testing was done during the day, between 12:00–13:00 h.
- The in situ and ex situ experiments revealed similar results. None of the frogs showed any of the four defensive postures associated with deimatic behaviour, instead the *P. somuncurens* were quiescent or remained motionless (sensu Toledo et al., 2011) (Fig. 1B). However, during the fieldwork in the Valcheta stream (2013–2021), we observed that *P. somuncurens* displayed active escape or fleeing behaviour (Toledo et al., 2011; Ferreira et al., 2019) when approached or when a flashlight was shined on them. This behaviour involved submerging quickly into the water and hiding under the aquatic vegetation, occasionally also burying themselves in the mud at the bottom of the stream.
- The absence of deimatic behaviour in *P. somuncurens* is probably related to its aquatic and nocturnal habits (Velasco et al., 2016). Deimatic behaviour is considered a plesiomorphic condition in *Pleurodema* (Faivovich et al., 2012; Ferraro et al., 2021). Consequently, the absence of deimatic behaviour in *P. somuncurens* is likely a secondary loss. This condition and the escape mechanism described herein differentiate *P. somuncurens* from the other two terrestrial species within the *P. thaul* Clade (i.e. *P. bufoninum* and *P. thaul*).

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