

Defensive behaviors in *Pleurodema borellii* (Anura: Leptodactylidae) from northwest Argentina

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ABSTRACT

Anurans are common prey for several vertebrates and also invertebrates. In response to this predatory pressure, they exhibit various defensive behaviors. In this work we report liquid cloacal discharge, body inflation, stiff-legged posture and body vibration performed by *Pleurodema borellii* from San Miguel de Tucumán, northwest of Argentina. These multiple defensive behaviors performed together can hinder or disrupt the predator's action more efficiently.

Key Words: Anti-Predation Strategy; Body Inflation; Body Vibration; Cloacal Discharge; Stiff-Legged Posture.

RESUMEN

Los anuros son presa común de varios vertebrados y también de invertebrados. En respuesta a esta presión depredadora, exhiben diversos comportamientos defensivos. En este trabajo reportamos descarga cloacal líquida, inflación corporal, postura de piernas rígidas y vibración corporal realizadas por *Pleurodema borellii* de San Miguel de Tucumán, noroeste de Argentina. Estos múltiples comportamientos defensivos realizados juntos pueden obstaculizar o interrumpir la acción del depredador de manera más eficiente.

Palabras claves: Descarga cloacal; Estrategia Anti Depredación; Inflación Corporal; Postura de piernas rígidas; Vibración Corporal.

Anurans are preyed on multiple life stages by different predators like mammals, birds, reptiles, and many invertebrates (Toledo *et al.*, 2007; Fadel *et al.*, 2019). This predatory pressure has been important to the evolution of the large repertoire of defensive behaviors that anurans exhibit, including sound emission, toxic and distasteful skin secretions, cryptic and aposematic coloration, and a variety of visual displays (Martins *et al.*, 1993; Abrahams, 1995; Toledo and Haddad, 2009; Toledo *et al.*, 2011; Mailho-Fontana *et al.*, 2014; Dreher *et al.*, 2015). These behaviors can be performed isolatedly or

combinedly, such as escape followed by cloacal discharge, or immobility accompanied by body inflation (Williams *et al.*, 2000; Toledo *et al.*, 2011; Ferreira *et al.*, 2019).

Currently, more than 30 categories of defensive behaviors in response to predator attacks have been described in anurans, distributed across avoiding detection, preventing attack and counterattacking (Toledo *et al.*, 2011; Ferreira *et al.*, 2019). A reconstruction of ancestral states for the antipredator mechanisms of anurans conducted by Ferreira *et al.* (2019), showed that most of these mechanisms

are plesiomorphic and arose independently across families suggesting extensive homoplasy. However, the same authors highlight that some of the apparently homoplastic antipredator mechanisms may be a consequence of the lack of observations on species displaying them. In fact, records of these defensive behaviors are made through fortuitous observations, which generally occur when individuals are being manipulated in the field or laboratory (e.g., Wells, 2007; Pedroso-Santos *et al.*, 2022).

The genus *Pleurodema* Tschudi, 1838 comprises 15 species and is widely distributed from Panama to southern Argentina and Chile (Frost, 2023). They inhabit an extensive altitudinal range, with species that live at sea level and others in mountains with almost 5.000 m a.s.l. (Seimon *et al.*, 2007; Ferraro and Casagrande, 2009). Among these, *P. borellii* (Peracca, 1895) is a semi-aquatic species with a large size compared to other species in the genus (SVL 40.56 mm \pm 3.73; Ferraro and Barrionuevo, 2022). The species occurs in preserved or disturbed habitats in northwestern Argentina and southern Bolivia, in the Chaco-Yungas transition on the eastern slopes of the Andes and in montane grasslands, at 400 to nearly 3000 m a.s.l. (Cortez *et al.*, 2004; Frost, 2023). For species of the genus *Pleurodema*, defensive behaviors including active escape, body inflation, death feigning with limbs outstretched (later identified as stiff-legged posture), deimatic behaviors such as exposition of lumbar glands and flash coloration, elevation of the pelvic region, emerging from the ground covered with sand, body tilting towards a disturbing stimulus, secretion of the prominent lumbar glands and presence of bright color in the lumbar region have been cited (Martins, 1989; Kolenc *et al.*, 2009; Ferraro *et al.*, 2021; Velasco *et al.*, 2023). For *P. borellii*, the mentioned behaviors include tilting the body laterally in the same direction as a tactile stimulus, exhibiting the prominent lumbar glands, deimatic behavior, body inflation, presence of macrogland in the lumbar position, and bright color (yellow) in the lumbar region and thighs (Kolenc *et al.*, 2009; Ferraro *et al.*, 2021). In this work, we report the occurrence of four defensive behaviors in *P. borellii*, three of which are reported for the first time for this species.

On 6 March 2023 at 7 pm, we collected a juvenile of *Pleurodema borellii* (voucher: FML 31440; SVL: 31 mm) in an urban area of the Capital department, in the main city of San Miguel de Tucumán, province of Tucumán, northwest of

Argentina (26°49'08.50"S 65°14'30.8"W; datum: WGS84; 451 m a.s.l.). The specimen was accommodated in a plastic bag containing humid leaves and posteriorly was taken to the laboratory and transferred to a glass terrarium (30cm x 30cm) containing substrate (sphagnum) and water. The next day, at 9 am, we removed the individual from the terrarium to take photographs and it performed a sequence of four defensive behaviors (Fig. 1; Video 1). When handled, the specimen performed: (1) liquid cloacal discharge, (2) simultaneously body inflation, (3) stiff-legged posture and (4) body vibration. This latter is similar to muscle spasms, visibly occurring in some muscles of the trunk (longissimus dorsi, latissimus dorsi, ilio-lumbaris, coccygeo-iliacus) and the legs (triceps femoris, semimembranosus, gastrocnemius, peroneus), with intense movement of the feet. When placed on a substrate (burlap), it kept the body inflated and stiff-legged vibrating the body for approximately 15s and then returned to normal posture. On the same day, at 5 pm, the individual was handled again and performed the same four behaviors, however, he stretched the legs partially and remained there for 10s before returning to his normal posture. The behavior of stiff-legged while vibrating the body was performed by the same individual in another situation, without being handled. When we removed the pot of water from the terrarium for cleaning, it collided with the side of the terrarium and made a noise/vibration, triggering this behavior in the individual, who remained for 7s with stiff-legged and vibrating before returning to normal posture. After behavioral observations, the individual was killed using 2% lidocaine, fixed in 10% formalin, and transferred to permanent storage in 70% ethanol. We classified the individual as juvenile considering its body size, absence of male sex characters (vocal slits and nuptial pad), and undeveloped gonads. Voucher is housed in the herpetology collection of the Fundación Miguel Lillo (FML), San Miguel de Tucumán municipality, Tucumán province, Argentina. The video of defensive behaviors (ZUEC-VID 1323) is deposited in the audiovisual collection of the Museu de Diversidade Biológica da UNICAMP, Campinas municipality, São Paulo state, Brazil.

Our observations show that *Pleurodema borellii* may exhibit multiple defensive behaviors. When handled, the individual simultaneously displayed cloacal discharge, an inflated body, completely rigid legs with exposed hidden parts, and body vibration.

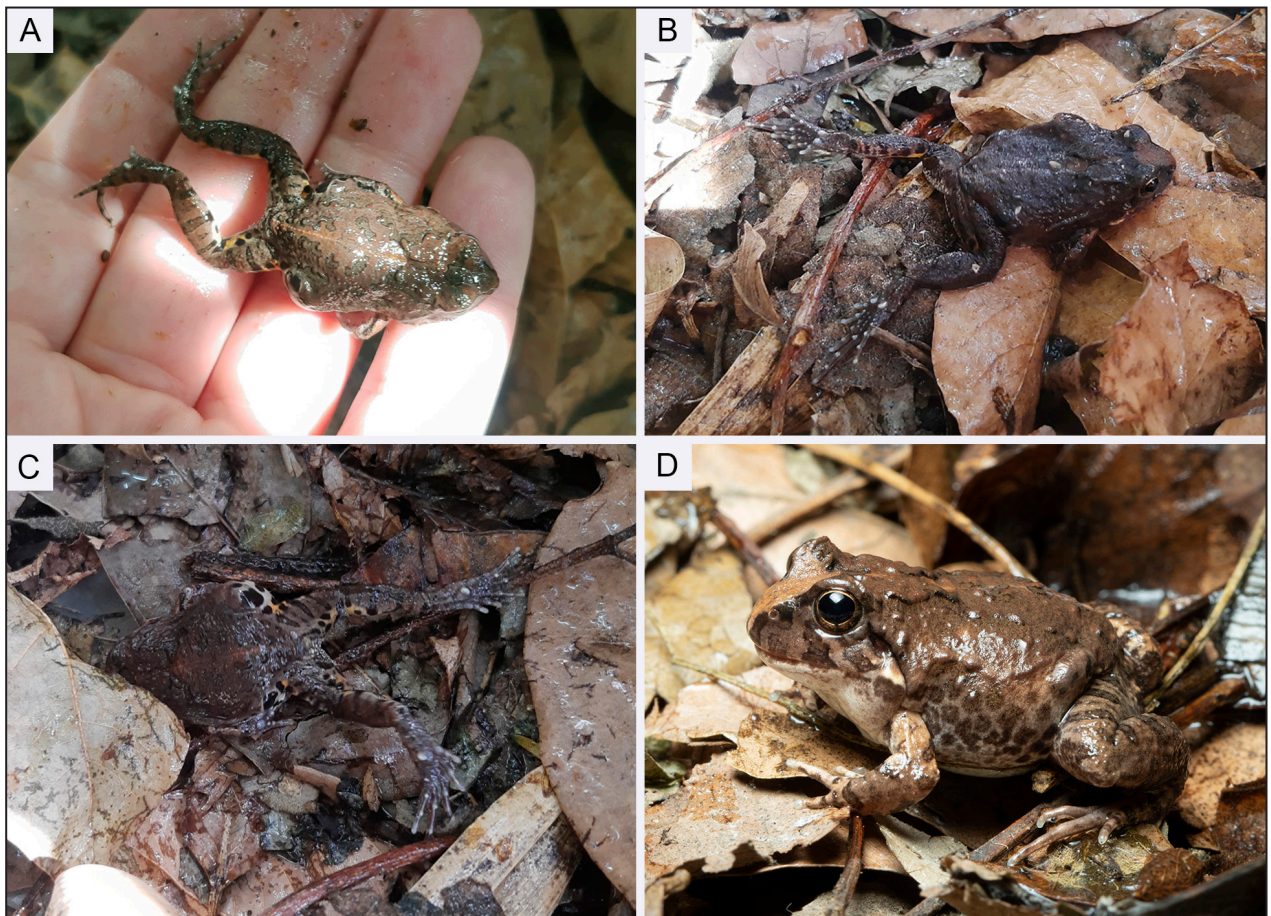


Figure 1. (A) Juvenile of *Pleurodema borellii* (FML 31440, SVL = 31 mm) performing body inflated, stiff-legged posture and vibration in the body when handled; (B) the same individual performing partial and (C) full stretching legs on the burlap substrate; (D) natural posture of the species.

The fact that *P. borellii* demonstrates this defensive repertoire during handling indicates that the trigger for these behaviors is seizure or touch by the predator. This is consistent with cloacal discharge and body inflation behavior, which are performed when an anuran is seized or touched in a predation event (Toledo *et al.*, 2011; Folly *et al.*, 2019). Liquid cloacal discharge can cause a bad taste to the predator if it reaches its mouth, or induce a recoil response in an endothermic predator with the cold liquid, in addition to making the frog lighter and facilitating its escape (Kokubum, 2002; Toledo *et al.*, 2011). Regarding body inflation (also identified as lung inflation and puffing up the body; Wells, 2007; Toledo *et al.*, 2011), it is a behavior that increases the size of the frog in the face of a predator (Stebbins and Cohen, 1995; Williams *et al.*, 2000). This posture makes the prey more difficult to grab and can deter the predator from handling, manipulating, or ingesting it (Caro, 2014).

The stiff-legged posture (also identified as “stretching limbs”; Ferreira *et al.*, 2019), characterized by full or partial extension of the limbs, is also a defensive behavior stimulated by predator touch (Ferreira *et al.*, 2019). This behavior is useful for camouflaging itself on the substrate and avoiding visually oriented predators, being carried out mainly by forest anurans (e.g., Sazima, 1978; Toledo *et al.*, 2011; Mângia and Santana, 2013), but also by species from open areas (e.g., Borteiro *et al.*, 2018, Ferrante *et al.*, 2020; Nehemy *et al.*, 2022). Unlike the stiff-legged posture, in hidden aposematism, there is the exposure of conspicuous parts in the axilla, underside of the body, tongue, thighs or post-femoral region, in order to discourage the predator (Ferreira *et al.*, 2019; Pedroso-Santos *et al.*, 2022). In the case of *Pleurodema borellii*, at first, we thought that the stiff-legged posture could mimic fallen leaves, due to its dorsal color, or a pretense of death. However, when performing body inflation along with rigid legs, both

the glands and the bright coloration (yellow) become evident. The exposure of this bright-coloured part, together with the vibration of the body, would nullify the effect of camouflage or the feigning of death. These sudden displays, known as ‘deimatic displays’, can cause predators to recoil reflexively (Ferraro *et al.*, 2021). In this sense, it is likely that showing the bright region is more important for the species than appearing dead or imitating leaves.

In relation to body vibrations, this behavior resembles preventive sexual vibration behavior (“warning vibration”), a release mechanism observed in some species of the genus *Pleurodema* from Chile and in other anuran genera (Noble and Aronson, 1942; Aronson, 1944; Cei and Espinosa-Aguilera, 1957; Diakow, 1977). This mechanism involves a series of abdominal and thoracic muscular contractions when the animal is stimulated in the dorsal and lateral regions and is typically observed during amplexus in both heterosexual and homosexual pairs (Cei and Espinosa-Aguilera, 1957). In some species, this signal is given simultaneously with a release call (Penna and Veloso, 1982). However, this vibratory behavior seems to be different from that observed in *P. borellii*, as it does not naturally occur in juveniles unless induced by injecting specific hormones (gonadotrophins and testosterone) (Blair, 1946, 47b). Additionally, the individual also performed body vibrations without being touched (which is not consistent with repulsion to amplexus), and moved his feet intensely, a fact not reported during warning vibration. In this case, we propose three hypotheses for this behavior. The first is that this vibration, when made on the ground, could transmit a seismic warning signal to neighboring specimens, since amphibians are exceptionally sensitive to this type of stimulus (Narins, 2019). The second is that body vibration would function as a signal to non-visually oriented predators, as the display of their aposematic coloration would have no effect. Thirdly, the anthropogenic environment from which the individual originates may have triggered a hormonal disorder or imbalance, causing it to exhibit this behavior, consistent with the “warning vibration” mentioned previously. Finally, new studies are needed to understand this behavior in *P. borellii* and to investigate whether this defensive vibration behavior is restricted to this population and/or species or whether it encompasses others of the genus, and whether it was triggered by some environmental factor. Additionally, it would be interesting to study

the presence/absence and structure of this behavior in other populations of the species that live in more preserved habitats.

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Competing interests

The authors declare that they have no conflict of interest.

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Appendix I

Video 1. Defensive behaviors of *Pleurodema borellii* (ZUEC-VID 1323), deposited in the audiovisual collection of the Museu de Diversidade Biológica da UNICAMP, Campinas municipality, São Paulo state, Brazil. Available at <https://www2.ib.unicamp.br/fnjv/>