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REPERTOIRE OF ANTIPREDATOR MECHANISMS IN *Proceratophrys avelinoi* (MERCADAL DE BARRIO & BARRIO, 1993) (ANURA: ODONTOPHRYNIDAE)

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Abstract: Predation plays a crucial role in the evolution of several amphibian antipredator mechanisms to avoid their predators. Knowing the antipredator mechanisms used by a species can reveal information about its ecology and natural history. *Proceratophrys avelinoi* is an inhabitant of the Atlantic Forest leaf-litter. Here, we describe the repertoire of antipredator mechanisms used by individuals found during field research in the northwestern Paraná state, revealing new mechanisms for the species and reporting their potential advantages, contributing therefore to enhance the natural history of *P. avelinoi*.

Keywords: Amphibia; behavior; ecology; natural history; zoology

Anurans are preyed on by a wide variety of animals, including vertebrates such as snakes, birds, mammals, other amphibians (Toledo *et al.* 2007), and invertebrates such as centipedes, spiders, and scorpions (Wells 2007). Predation has been an important factor in the evolution of many unique characteristics of amphibians used as a defense strategy against attacks by predators, such as toxic and unpleasant skin secretions, cryptic and aposematic coloration, and a wide variety of postures and defensive behaviors (Wells 2007, Ferreira *et al.* 2019). These mechanisms can be used in escalated phases (*e.g.* Lourenço-de-Moraes *et al.* 2016), and there can also be variations within each mechanism, as well as combinations of different mechanisms. For instance, camouflage and interrupt calling comprise primary defenses used to avoid detection by the predator (Ferreira *et al.* 2019). Then, mechanisms are used to discourage the predator from continuing the attack, such as body inflation, mouth gaping, and death feigning. Mechanisms such as distress call, secretions, and aggression are used during the attack in an attempt to hinder and prevent ingestion by the predator (Ferreira *et al.* 2019). In this sense, anurans become one of the vertebrate groups with the greatest variety of recorded antipredator mechanisms (Ferreira *et al.* 2019).

The genus *Proceratophrys* Miranda-Ribeiro 1920 is represented by 43 species distributed throughout Brazil, northeastern Argentina, and Paraguay, with 40 of these species occurring in Brazil (Frost 2023). The *Proceratophrys bigibbosa* group is composed of the species *P. bigibbosa*, *P. avelinoi*, *P. brauni*, and *P. palustris* (Kwet & Faivovich 2001), and are cryptic

species characterized by short and abrupt snout, post-ocular swellings, and the absence of eyelid appendages (Eterovick & Sazima 1998).

Proceratophrys avelinoi (Anura: Odontophrynidae) is a species that can be identified by being the smallest species in the group (Kwet & Faivovich 2001). Inhabiting the Atlantic Forest leaf-litter, this species present nocturnal habits and is specialized in the use of water bodies for reproduction, choosing shallow waters with an average depth of 10 cm and swamps with slow water flow and covered by vegetation (Caldart *et al.* 2010). However, notes on the antipredator mechanisms used by this species are still scarce (Zocca *et al.* 2022).

Here, we present the repertoire of antipredator mechanisms used by *P. avelinoi*. Among the new antipredator mechanisms registered to *P. avelinoi*, we highlight the defensive behavior called stiff-legged posture, during which the frogs flatten their bodies and remain immobile with their legs extended. This mechanism has already been recorded in anuran species such as the congener *Proceratophrys appendiculata* and also recorded for species of the *Stereocyclops parkeri* (Microhylidae), and *Zachaenus parvulus* (Cycloramphidae) (Sazima 1978, Rocha *et al.* 1998). Although the behavior of stiff-legged posture has already been observed in the genus *Proceratophrys*, this is the first report of this behavior for *P. avelinoi*.

On 21 January 2021, at 20:15 h, during a field expedition, individuals of *P. avelinoi* were located vocalizing in a fragment of the Atlantic Forest located in the municipality of Iporã, northwestern Paraná state, southern Brazil (23º59'34" S, 53º44'55" W datum WGS84). This is a 42 hectares fragment comprising by different stages of vegetation regeneration and surrounded by pastures. The individuals were located in the water next to leaves and branches on the banks of a small stream, with about 5 cm of water column. Thus, we conducted focal animal sampling to evaluate their antipredator mechanisms in the field (Altmann 1974), and used the finger-only stimulate method to simulate a predator attack: the researcher's fingers were used to stimulate the frog, with humans as the possible predator (see Lourenço-de-Moraes *et al.* 2016). A total of seven individuals was tested.

During the meeting, it was possible to observe the display of a series of defensive behaviors that had not yet been recorded for *P. avelinoi* (Table 1). When they were found during vocalization, with the approach of the researcher, the individuals interrupted calling and remained immobile. Thus, this tactic associated with their cryptic coloration with the substrate (Figure 1A) contributed to not being visually recognized (Ferreira *et al.* 2019).

Table 1. Antipredator mechanisms recorded for *Proceratophrys avelinoi*.

Antipredator mechanisms	Reference
Camouflage (background matching)	Lourenço-de-Moraes and Lourenço-de-Moraes, 2012; Present study.
Immobility	Lourenço-de-Moraes and Lourenço-de-Moraes, 2012; Present study.
Interrupt calling	Present study.
Aposematism (hidden)	Lourenço-de-Moraes and Lourenço-de-Moraes, 2012; Present study.
Body inflation	Present study.
Contraction	Lourenço-de-Moraes and Lourenço-de-Moraes, 2012; Present study.
Stretching limbs	Present study.
Death feigning	Present study.
Unken reflex	Lourenço-de-Moraes and Lourenço-de-Moraes, 2012; Present study.

Figure 1. Antipredator mechanisms displayed by *Proceratophrys avelinoi*. A) Immobility and Camouflage. B) Body inflation. C) Death feigning. D) Contracting. E) Contraction flat against the ground. F) Stiff-legged keeping only one leg and Stiff-legged using two legs.

After being captured, all the individuals exhibited new antipredator behaviors. For instance, in the hands of the researcher, they inflated the body (Figure 1B) to make ingestion difficult, in addition to scare a potential predator (Toledo *et al.* 2011, Ferreira *et al.* 2019). Then, when released on the substrate, they performed death feigning and unken reflex, displaying an aposematic belly with red spots on a black background (Figure 1C, D). These behaviors are usually used after being handled by a potential predator in an attempt to discourage ingestion in animals that consume live prey (Toledo *et al.* 2010).

In addition, the contraction together with the use of aposematic coloration may indicate that this species is toxic at a certain level to its predators (Ferreira *et al.* 2019). The objective of aposematic

coloration is to warn a visually oriented predator of the potential risk of ingesting a certain prey (Toledo *et al.* 2010; Ferreira *et al.* 2019). However, sometimes this mechanism may have the opposite effect, exposing the animal that uses it in its environment (Hall *et al.* 2013). To compensate for this disadvantage, many species have aposematic coloration in less visible parts of the body, such as the belly. Combined with cryptic coloration, this tactic allows the animal to choose which strategy to use depending on the situation in which it finds itself and the position of the body that is exposed (Barnett *et al.* 2017). We observed this strategy being used by *P. avelinoi*, with the individuals after being grabbed by the researcher's hand and released on the ground, remaining with their belly facing upwards, exhibiting aposematic coloration and contracting their limbs close to the body, thus protecting vital organs, exposing its toxicity and making it difficult to ingest (Ferreira *et al.* 2019).

Also using immobility in synergy with contraction of the body under the substrate and when disturbed, the individuals jumped, flattened the body, and remained immobile with their legs extended, keeping only one or both legs stretched out (Figure 1 E, F). *P. avelinoi* exhibits skin appendices and cryptic coloration giving them the appearance of dead leaves. By assuming this posture with the legs distended, the animal takes a more elongated form, improving its appearance of a dead leaf (Figure 1 F). Such behavior probably increases the animal camouflage protection against visually oriented predators (Sazima 1978).

This series of mechanisms used by *P. avelinoi*, as well as other antipredator mechanisms already recorded for the genus *Proceratophrys*, has its evolutionary phylogenetic relationship presented by Zocca *et al.* (2022). Cryptic coloration in synergy with the use of postures such as immobility, contraction, and stretching limbs is an effective antipredator mechanism for *Proceratophys* species against predators in leaf-litter with a phylogenetic structure in the genus (Zocca *et al.* 2022). Although most records of antipredator mechanisms are recorded for Atlantic Forest species (Haddad *et al.* 2013), several aspects of the natural history of several species remain unknown, including repertoires of antipredator mechanisms. Therefore, new studies are required to enhance our understanding on the diversity of antipredator mechanisms used by species of the genus *Proceratophrys*.

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