

**An unusual defensive strategy and distress call in
Lithobates palmipes (Spix, 1824) (Anura: Ranidae)**

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An unusual defensive strategy and distress call in *Lithobates palmipes* (Spix, 1824) (Anura: Ranidae)

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ABSTRACT

Anurans exhibit a wide range of defensive strategies that have evolved under selective pressures from predators throughout their evolutionary history. This study reports, for the first time within the family Ranidae, the occurrence of tongue protrusion as a defensive behaviour in *Lithobates palmipes* (Spix, 1824), and describes its distress call associated with stress situations. Observations were conducted at the Estação Ecológica de Caetés, Pernambuco, Brazil, during field expeditions in August 2025. The recorded behaviours included thanatosis, cloacal discharge, tongue protrusion, and distress call emission, revealing a diverse defensive repertoire for the species. The distress call exhibited a short duration (0.05 ± 0.01 s) and a dominant frequency of 1406.25 ± 187.93 Hz, consistent with short-range defensive vocalisations. Tongue protrusion, a behaviour rarely documented in anurans, appears to reinforce thanatosis by confusing potential predators. These findings expand current knowledge of the antipredator strategies of *L. palmipes* and contribute to a broader understanding of behavioural diversity within Ranidae. Furthermore, they highlight the importance of ethological and bioacoustic studies for understanding the evolution and conservation of Neotropical amphibians.

Keywords: Anura; Ranidae; defensive behaviour; thanatosis; tongue protrusion; defensive call.

1 Predation is a natural ecological phenomenon that underpins trophic dynamics within
2 ecosystems and exerts strong selective pressure on species (Ferreira et al., 2019). Throughout
3 the evolutionary history of anuran amphibians, a wide range of defensive strategies has been
4 selected to minimize predation risk, including chemical, acoustic, visual, and behavioral
5 mechanisms (Toledo et al., 2011; Ferreira et al., 2019). Depending on predator–prey
6 interactions, these strategies may operate either independently or in combination, resulting in
7 complex responses (Duellman & Trueb, 1994; Toledo et al., 2011). Understanding these
8 antipredator mechanisms provides valuable insights into intra and interspecific interactions, as
9 well as contributing to the comprehension of the adaptive value of these strategies for species
10 survival (Zug et al., 2001).

11 Among the defensive mechanisms recorded in anurans, aposematism, camouflage, thanatosis,
12 body contraction, poisonous skin secretions, and foul-smelling cloacal discharge are
13 particularly noteworthy (Toledo et al., 2011; Ferreira et al., 2019). In Atlantic Forest species,
14 anurans defensive postures such as thanatosis and changes in body shape are commonly
15 observed and may function to discourage predators, these behaviours are often associated with
16 the exposure of aposematic colouration on the ventral surfaces and limbs (Toledo & Haddad,
17 2009; Ferreira et al., 2019; Souza et al., 2021). Such postures may also be accompanied by
18 distress or alarm vocalisations, which vary according to the intensity and context of the
19 perceived threat (Toledo et al., 2015; Köhler et al., 2017; Siqueira et al., 2026).

20 Within the anuran vocal repertoire, distress calls are frequently associated with defensive
21 contexts, especially encounters with potential predators (Toledo et al., 2011; Köhler et al., 2017;
22 Siqueira et al., 2026). These vocalisations are highly variable, differing from advertisement
23 calls in both structure and function. Their acoustic features lack clear standardisation, and calls

may or may not present harmonic components, being generally associated with defensive responses (Toledo et al., 2015; Siqueira et al., 2026).

Understanding antipredator strategies provides valuable insights into species ecology and behaviour, serving as a foundation for hypothesis testing, phylogenetically informative studies, and conservation planning (Toledo et al., 2011). Predator–prey interactions are central to ecosystem dynamics and represent a major driving force of natural selection (Duellman & Pianka, 1990; Roslin et al., 2017). Among these mechanisms, tongue protrusion is a behavior rarely documented in anurans, characterized by the exposure of the tongue outside the mouth while the individual remains motionless (Borteiro et al., 2018). This tactic is thought to reinforce thanatosis by enhancing the simulation of death to potentially confuse or discourage predators (Toledo et al., 2011; Borteiro et al., 2018). Despite its relevance, this behavior has been previously reported in only a few restricted taxa, illustrating its scarcity in the current literature (Perret, 1961; Borteiro et al., 2018). Thus, the present study aims to provide the first record of tongue protrusion for the family Ranidae and to analyse and describe the distress call of *Lithobates palmipes*, contributing to a deeper understanding of the defensive behaviours associated with this species. Beyond expanding current knowledge of anuran antipredator strategies, these findings offer relevant information for future studies on the ecology and evolutionary history of this family.

Field expeditions were carried out during three consecutive nights in August 2025 at the Estação Ecológica de Caetés (ESEC Caetés; Fig. 1), located in the municipality of Abreu e Lima, Pernambuco, northeastern Brazil (7° 55' 26.34" S, 34° 56' 1.06" W; datum WGS84). The area is located within the Atlantic Forest domain, characterized by lowland seasonal forest with predominantly secondary vegetation (Veloso, Rangel-Filho and Lima, 1991; CPRH, 2012). The regional climate is classified as As according to the Köppen–Geiger system, corresponding to a tropical climate with a marked dry season (Peel, Finlayson and McMahon, 2007). Field

activities were conducted daily between 17:00 and 21:00 h, with researchers actively surveying the area throughout this period (Crump and Scott Jr, 1994).

Vocalizations were recorded using a Tascam DR-40 digital recorder coupled with a Yoga HT-81 directional microphone. Field procedures were conducted under permits issued by the Sistema de Autorização e Informação em Biodiversidade – SISBIO (license number 103341-1). Genetic heritage activities were registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado – SisGen under registration number A30F839).

Acoustic parameters were analyzed using Raven Pro version 1.6.5. for Windows (Cornell Lab of Ornithology – Bioacoustics Research Program, 2024). The following acoustic parameters were considered, according to the terminology established by Köhler et al. (2017): call duration (s), interval between calls (s), minimum frequency (Hz), maximum frequency (Hz), dominant frequency (Hz), and frequency bandwidth (Hz).

We generated oscillograms and spectrograms in R (R Core Team, 2016) using a window size of 512 samples, 90% overlap, and a 512-point FFT, with the packages tuneR version 1.0 (Ligges et al., 2013) and seewave version 1.7.3 (Sueur et al., 2008). No filters were necessary.

Among the four examined males of *Lithobates palmipes*, one individual exhibited tongue protrusion (Fig. 2A), whereas three individuals exhibited thanatosis (Fig. 2B), cloacal discharge, and distress calls during handling.

We recorded the distress calls of four males of *L. palmipes*, with an average of 16 calls per individual. These calls were characterized by a single multipulsed note containing 26.5 ± 9.88 pulses per call. The vocalization pattern showed a mean duration of 0.05 ± 0.01 s, indicating a short and rapid call. The minimum ($1127,12 \pm 229,23$ Hz) and maximum ($2577,14 \pm 527,17$

Hz) frequencies revealed a broad spectral range, with a dominant frequency peak at $1407,29 \pm 187,93$ Hz. The frequency bandwidth was $1512,10 \pm 507,67$ Hz, suggesting considerable variation within the frequency range produced (Fig. 3).

A previously undocumented defensive behaviour for the family Ranidae was recorded: tongue protrusion, characterised by the exposure of the tongue outside the mouth while the animal remains motionless (Borteiro et al., 2018). This strategy appears to reinforce thanatosis by potentially discouraging the predator by reinforcing the simulation of death (Toledo et al., 2011; Borteiro et al., 2018). Such behaviour is rarely described in the literature, having been previously reported only in two species of the genus *Odontophrynus* (*O. lavillai* and *O. americanus*) (Borteiro et al., 2018), and in *Acanthixalus spinosus* (Perret, 1961). Unlike our observation in *L. palmipes*, the latter possesses an aposematic-coloured tongue, which adds a visual warning component to the display (Toledo et al., 2011). This represents the first record of tongue protrusion for Ranidae, making it a valuable contribution to the understanding of defensive behaviours in anurans. In *L. palmipes*, the combination of tongue protrusion, thanatosis, cloacal discharge and distress calls reveals a complex defensive repertoire, similar to multi-component patterns described for other anuran taxa (Toledo et al., 2011). Across the genus *Lithobates* (Table 1), records show notable interspecific variation, while North American species like *L. palustris*, *L. pipiens* and *L. areolatus* tend to inflate and raise their bodies as a way to appear larger to predators (Marchisin & Anderson, 1978; Ferreira et al., 2019), *L. palmipes* relies on a different direction, prioritizing immobility and short-range acoustic signaling. This divergence supports the premise that even closely related taxa may evolve distinct behavioural solutions driven by specific predation pressures of their environments (Hillis & Wilcox, 2005; Duellman & Trueb, 1994). Furthermore, the cloacal discharge in *L. palmipes* may act as an additional deterrent, this can facilitate escape by reducing body mass or

discourage predators via chemical aversion, much like other Neotropical ranids (Toledo & Haddad, 2009).

The distress call of *L. palmipes* exhibited a mean duration of 0.05 ± 0.01 s, indicating a short and rapid vocalisation (Köhler et al., 2017). The call was composed of a single multipulsed note, and these acoustic parameters are consistent with those described for distress calls, typically characterised by short duration and broadband frequency structure, and generally associated with acute stress situations (Toledo et al., 2015; Köhler et al., 2017). The distress call also differs markedly from the advertisement call previously reported for the species, which consists of two pulsed notes with dense harmonic structure and frequency modulation, a longer call duration (0.898 ± 0.026 s), lower dominant frequency (996.429 ± 234.861 Hz), and more pulses per note (10.104 ± 1.469) (Arrieta-García et al., 2025).

These findings highlight the importance of continued research into anuran defensive mechanisms, as the analyses of antipredator strategies provides essential insights into species ecology and behaviour (Toledo et al., 2011). Such ethological information is relevant for phylogenetic and conservation studies, potentially contributing to new hypotheses on the behavioural evolution of the genus *Lithobates*. Continued investigation into the acoustic repertoire of *L. palmipes* is crucial, as defensive behaviours and vocalisations can serve as phylogenetic characters (Silva et al., 2013; Guerra et al., 2016), aiding in the identification of cryptic species. Therefore, broader sampling efforts in different social and ecological contexts are essential for a more comprehensive understanding of behavioural plasticity and vocal diversity within Neotropical ranids.

DISCLOSURE STATEMENT

The authors report there are no competing interests to declare.

DATA AVAILABILITY STATEMENT

The audio recordings of the distress calls were deposited in the Coaxar sound library, which is part of the herpetological collection of the Universidade Federal Rural de Pernambuco, under voucher numbers SCLEHP0123, SCLEHP0124, SCLEHP0125, and SCLEHP0126.

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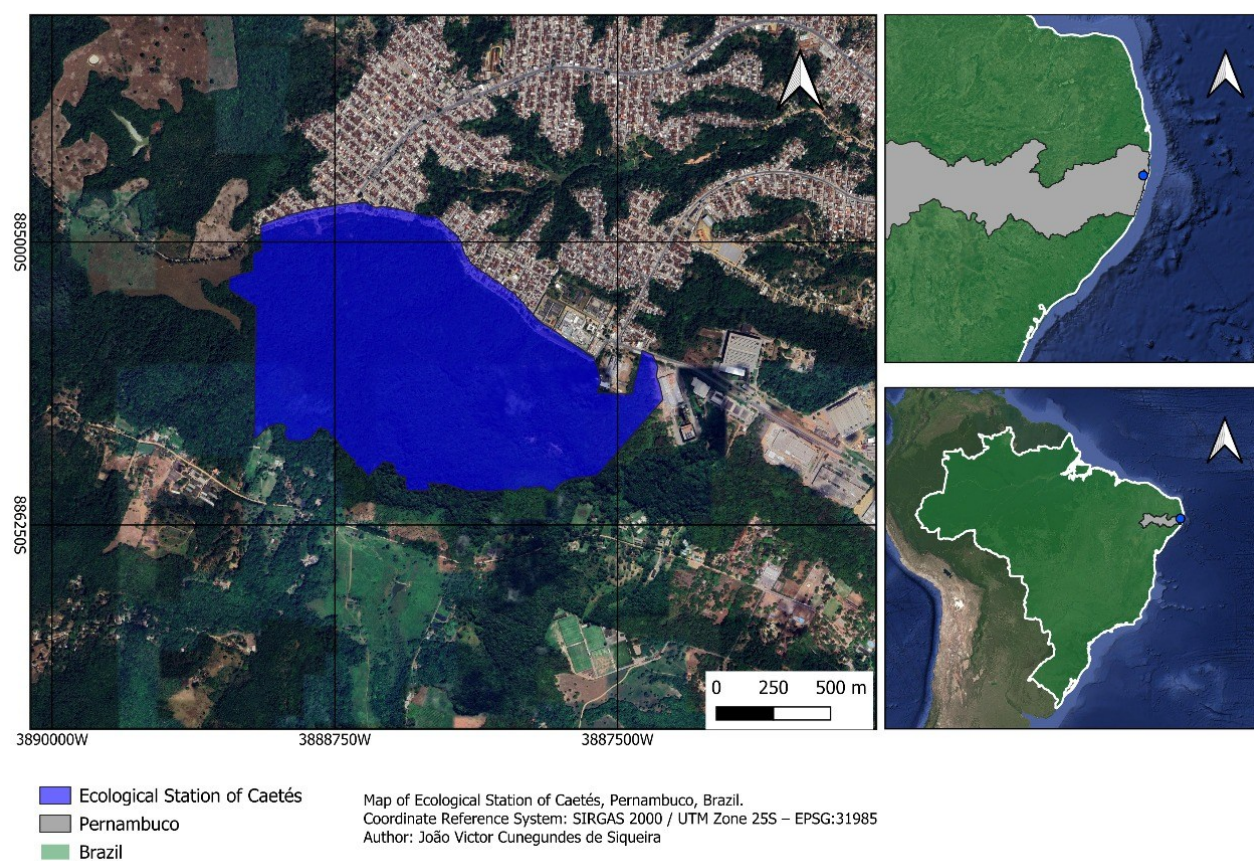
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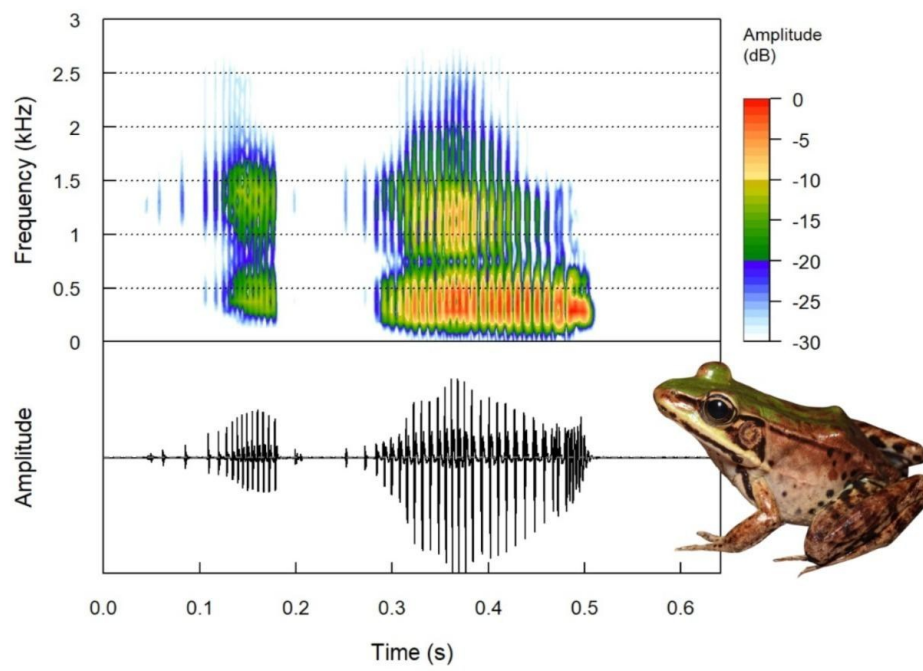
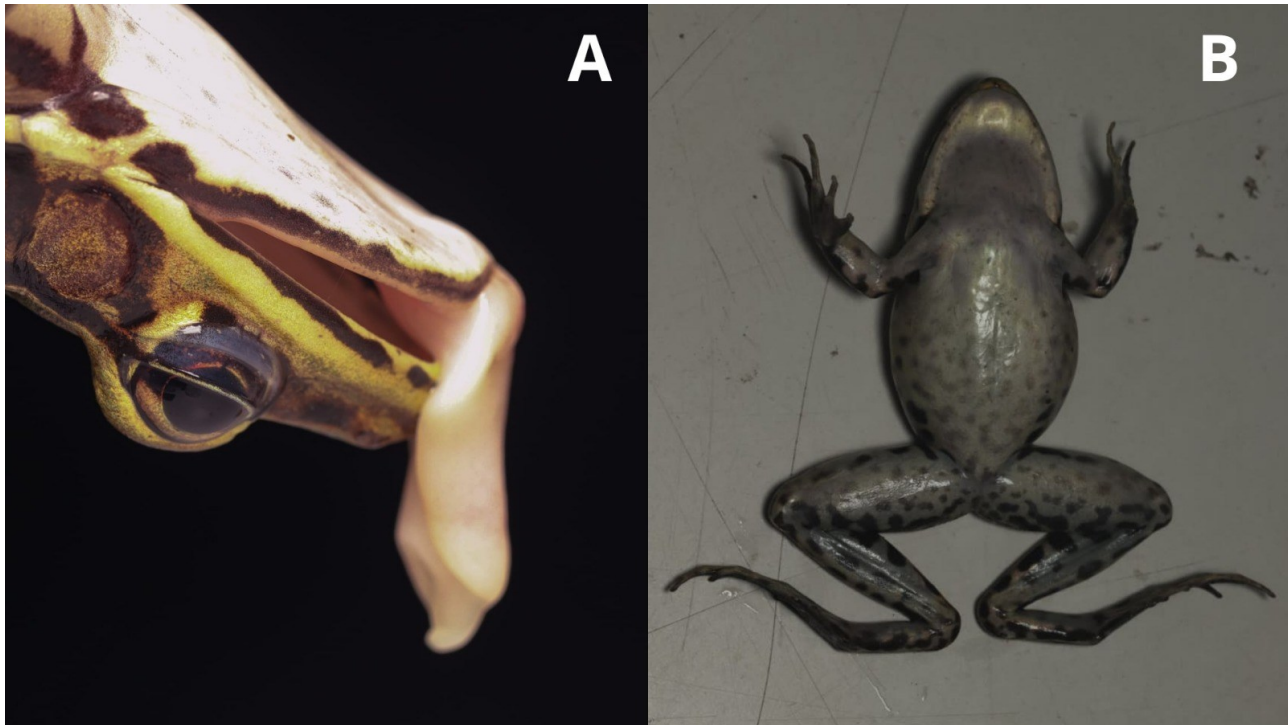
TABLES

Behaviour	Specie	Literature
Tongue protrusion	<i>Lithobates palmipes</i> (Spix, 1824)	This study
Thanatosis	<i>Lithobates palmipes</i> (Spix, 1824)	This study
Cloacal discharge	<i>Lithobates palmipes</i> (Spix, 1824)	This study
Body inflation	<i>Lithobates palustris</i> (LeConte, 1825)	Marchisin & Anderson, 1978
	<i>Lithobates pipiens</i> (Schreber, 1782)	Marchisin & Anderson, 1978
Body elevation	<i>Lithobates areolatus</i> (Baird and Girard, 1852)	Ferreira <i>et al.</i> , 2019
Unspecified	<i>Lithobates capito</i> (LeConte, 1855)	Means, 2004

Table 1. Defensive behaviours recorded in species of the genus *Lithobates* Fitzinger, 1843, indicating behavioural categories, species, and corresponding literature sources.

FIGURES





FIGURES CAPTIONS

Figure 1. Map of Ecological Station of Caetés, Pernambuco, Brazil. Author: Siqueira J. V. C.

Figure 2. Defensive behaviours exhibited by individuals of *Lithobates palmipes*. (A) Tongue protrusion. B) Thanatosis. Author: Negromonte, I. O.

Figure 3. Oscillogram and Spectrogram of the Distress call in *Lithobates palmipes*.