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Bite, shriek, or intimidate: defensive behaviours of the Apatani Horned Toad, *Xenophrys apatani* Saikia et al., 2024, and the Bicoloured Frog, *Clinotarsus curtipes* (Jerdon, 1853), from India

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Anurans exhibit a diverse array of defensive mechanisms that largely serves to mitigate predation risk by aiding to avoid detection, prevent attack by predators, or counterattack. Depending upon the criteria used for interpretation and classification, between 12 and 30 distinct anti-predator mechanisms have been documented in the literature to date (e.g., Dodd, 1976; Duellman and Trueb, 1994; Toledo et al., 2011; Lourenço-de-Moraes et al., 2016; Ferreira et al., 2019). Defensive mechanisms employed by different species are influenced by environmental conditions, the types of threats encountered, and their evolutionary histories. These mechanisms could range from simple, singular strategies to more complex ones that comprise two or more mechanisms to reduce the chances of predation and increase the probability of survivability (Wells, 2007; Toledo et al., 2011).

Various defensive or anti-predator mechanisms have been previously documented in Asian Horned Frogs (Family Megophryidae), including camouflage, aposematism, body inflation, aggressive biting, and warning sounds (e.g., Zainudin et al., 2018; Ferreira et al., 2019; Pradhan and Pradhan, 2021). Similarly, among members of the True Frogs (Family Ranidae) a diverse array of anti-predator mechanisms has been reported previously, including camouflage, aposematism, posture, escape, warning sounds, cloacal discharge, secretions, aggression, and distress calls (e.g., Choi et al., 1999; Toledo and Haddad, 2009; Toledo et al., 2011, 2015; Ferreira et al., 2019). Even though India harbours one of the highest levels of frog diversity and has seen a growing interest in amphibian research over the past two decades (Biju and Bossuyt, 2003; Vijaykumar et al., 2014; Garg and Biju, 2019), studies on defensive behaviour are relatively scarce (e.g., Kanagavel and Tapley, 2013; Jena and Palita, 2020; Khate et al., 2021). This highlights a significant gap in the current understanding of the range of natural behaviours in Indian frog species and underscores the need for further research. Here, we report defensive behaviours of the megophryid *Xenophrys apatani* Saikia et al., 2024 and the ranid *Clinotarsus curtipes* (Jerdon, 1853).

The observations were made during field studies conducted in two biodiversity hotspots of India. The study site for *Xenophrys apatani* was Pange, Tale Valley Wildlife Sanctuary, Ziro, Lower Subansiri District, Arunachal Pradesh State (27.5466°N, 93.8949°E; elevation 1907 m), which lies in the Himalaya biodiversity hotspot. The observations of *Clinotarsus curtipes* were made at Singappara, Siruvani, Palakkad District, Kerala State (10.9794°N, 76.615°E; elevation 856 m), situated in the Western Ghats biodiversity hotspot. The defensive behaviours were recorded fortuitously during both diurnal and nocturnal searches. To assess the consistency of these behaviours, we simulated a threat multiple times using a small twig or a stick. All observations and photographs were captured in the wild.

Defensive vocalisations were recorded using a Tascam DR-40X portable digital audio recorder (44.1 kHz sampling rate, 16-bit resolution) connected to a Sennheiser MKE 600 unidirectional microphone that was positioned towards the calling animal at a distance of 10 cm. Input levels were pre-adjusted to prevent amplitude clipping, ensuring a consistent signal-to-noise ratio across all recordings. Five temporal properties (call duration, call rise time, call fall time, pulses per call, pulse rate) and one spectral property (overall dominant frequency) were measured using

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Raven Pro v1.6.5 software (K. Lisa Yang Center for Conservation Bioacoustics, 2024) following Charif et al. (2010). Measurements were carried out following the definitions, terminologies, and methods of Garg et al. (2021). Oscillograms showing the amplitude versus time waveform were prepared for visual representation of the calls. The overall dominant frequency was obtained using Raven's spectrogram function (1024-point fast Fourier transformation, Hann window, 50% overlap, 43.1 Hz resolution). Spectrograms for the calls were prepared to match the time frame of the oscillograms.

Results and Discussion

***Xenophrys apatani*.** The species is endemic to Arunachal Pradesh and currently known only from the vicinity of its type locality (Saikia et al., 2024). Our observations were made of individuals encountered between 2022 and 2024 near the forest camp in Pange, Tale Valley Wildlife Sanctuary, at the type locality of this species.

When approached initially, the individual remained immobile and relied on its cryptic body colouration that resembles the dry leaf litter of its natural habitat. Camouflage and immobility are the first and most common forms of defence among anurans (e.g., Toledo et al., 2011; Lourenço-de-Moraes et al., 2016; Ferreira et al., 2019; Barnett et al., 2021). Both of these strategies combined enable these animals to escape detection by predators that rely on the movement of prey to find them (Bertoluci et al., 2007).

To observe any additional defensive behaviours, we exposed the animal to an artificial threat by using a twig brought into close proximity of the animal. In response, the animal raised itself and inflated its body (Fig. 1D). This type of response was previously reported for *Bufo crucifer, Leptodactylus stenoderma* and *Rhinella major* (Toledo, 2004; Well, 2007; Pedroso-Santos and Costa-Campos, 2021). This was followed by mouth gaping, similar to the response observed by Guerra et al. (2018) in *Boana raniceps* (Cope, 1862). The animal maintained this position for about 10 s while the twig was kept motionless.

On touching the animal's snout with the twig, the animal initially produced a warning call (as defined by Toledo et al., 2015) and it subsequently showed aggression by biting the twig while maintaining its inflated posture (Fig. 1E–F). The bite force was of considerable strength as evident from our effort to tug the twig from the mouth of the animal. The animal returned to its pre-encounter posture after it was left undisturbed for about 20 s. This sequence of behaviour was observed consistently during four additional trials with the same sequence and stimulus, after which the frog seemed to habituate and did not respond.

While we attempted to collect the frog for photography it exhibited the same body inflation and mouth-gaping behaviour while simultaneously emitting a piercing distress call (as defined by Toledo et al., 2015). It further exhibited aggression by biting the finger of the handler and kept the mouth closed until the finger was pried out of its mouth (Fig. 1A). The distress call was emitted each time the animal was picked up but there was no consistency in the timing of the calls. Initially, the frog emitted the call as soon as it was picked up but subsequently it shrieked intermittently even when it was gently steered or handled for photography. The same defensive behavioural sequence was also observed in a female of the species during collection and photography (Fig. 1B).

For anurans encountering danger, escape is the most helpful defence but biting and distress calling behaviour are generally displayed when a frog comes in close contact with an aggressor or a predator (Lourenço-de-Moraes et al., 2016; Guerra et al., 2018). In close contact encounters, biting is one of the most aggressive behaviours observed in frogs, which has been reported in at least 30 species (e.g., Toledo et al., 2011; Lourenço-de-Moraes et al., 2016; Ferreira et al., 2019; Pradhan and Pradhan, 2021). In the Family Megophryidae, biting behaviour has previously been reported only in *Brachytarsophrys carinense* (Boulenger, 1889) and one species in the genus *Megophrys* (Toledo et al., 2011). Gaping of the mouth and body inflation during distress calling or biting appear to be synergistic behaviours in many anuran species (Lourenco-de-Moraes et al., 2016).

The intended function of distress calls is to scare away predators (Toledo et al., 2015). Even though distress calls have been reported in several frog species (e.g., Lourenco-de-Moraes et al., 2016; Guerra et al., 2018), they have previously not been reported in megophryid species. The male *Xenophrys apatani* produced a loud distress call (Fig. 1G–J) not unlike the screech of a cat. The vocalisations had a pulsatile temporal structure with a call duration of 1031.8 ms, rise time of 49.3 ms, and fall time of 982.4 ms. The calls had 301–805 pulses delivered at a pulse rate of 495.7 ± 37 pulses/s. The calls had a mean dominant frequency of 3.78 ± 0.17 kHz (Table 1). As these vocalisations were produced by

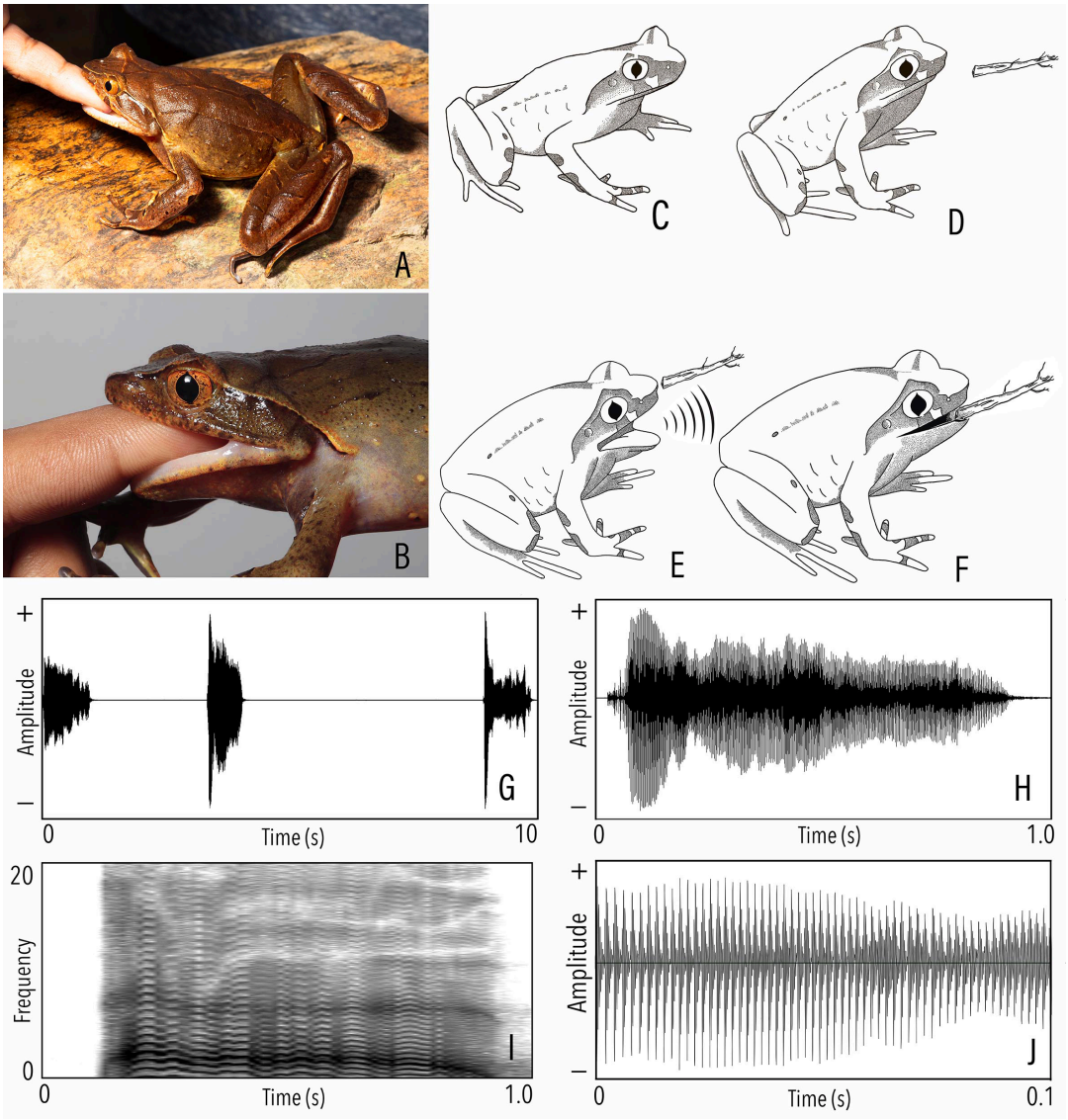

Figure 1. Defensive behaviour in *Xenophrys apatani*. (A) Biting by a female. (B) Biting by a male. (C–F) Illustrations showing the sequence of defensive behaviours when a threat stimulus is presented. (C) Normal posture. (D) Body inflation and raising of the head. (E) Mouth-gaping and production of a 'warning call'. (F) Biting. (G–J) Waveform and spectrogram of a 'distress call'. (G) Oscillogram for a 10-s call segment. (H) Oscillogram for 1.0-s call segment. (I) Spectrogram for a 1.0-s call segment. (J) Oscillogram for a 0.1-s call segment. Photos by S.D. Biju.

	Mean	SD	Minimum	Maximum
Call duration (ms)	1031.8	552.0	571.3	1821.1
Call rise time (ms)	49.3	37.1	6.6	97.2
Call fall time (ms)	982.4	0.5	0.6	1.7
Pulses per call (#)	440	322–728	301	805
Pulse rate (pulses/s)	495.7	37.0	442.0	526.8
Dominant frequency (kHz)	3.80	0.17	3.62	3.96

Table 1. Acoustic properties of distress calls emitted by *Xenophrys apatani*. For each characteristic, we report mean ± standard deviation and the range. Because pulses are indivisible units, we report medians and interquartile ranges based on individual means in place of means and standard deviations.

Figure 2. Body-raising behaviour in *Clinotarsus curtipes*. (A) Crouching down to a lower-than-normal sitting posture. (B) Head-raising with support of the forelimbs. (C) Initial raising of the hind body with support of both fore- and hind limbs. (D) Fully raised body with forelimbs vertically stretched and hind limbs nearly so. (E) Return to the normal sitting posture after about 8 s. (F) Raising of the complete body once more within 15 s. Photos by S.D. Biju.

the animal with its mouth agape while being captured, and the vocalisations exhibited dense and multiple harmonics, we classify this vocalisation as a distress call in accordance with Toledo et al. (2015) and Köhler et al. (2017).

***Clinotarsus curtipes*.** This ranid species is endemic to the Western Ghats of India and can be characterised by its highly contrasting dorsal and ventral body colouration. A pronounced 'body-elevation' or 'body-raising' behaviour was incidentally observed when a male individual was gently being picked up during daytime photography in the field. This was re-tested in the wild by using a small twig as a threat stimulus. The adult fully extended its fore- and hind limbs vertically, raising its body off the ground level while its eyes remained wide open. Three individuals of *C. curtipes* consistently exhibited this body-raising behaviour each time they received the stimulus to the back or the snout from a distance of ca. 30 cm. All individuals that exhibited this behaviour began by raising the body slowly and then repeated the posture more rapidly two to three times. This deimatic postural display exposed

the dark blackish-brown limbs and ventral body surfaces in contrast to the light-brown dorsal colouration, potentially to intimidate a perceived predator, and continued for about 10 s before the individual returned to its normal posture (Fig. 2). However, this behaviour was not exhibited by individuals in captivity.

To the best of our knowledge, this is the first report of a body-raising defensive behaviour for a ranid frog in India. This display could be assigned to the 'body elevation' with 'legs vertically stretched' category described by Toledo et al. (2011), which may function as a deceptive anti-predator mechanism by increasing the perceived body size of the individual in an attempt to avoid subjugation (Toledo et al., 2011; Ferreira et al., 2019). Within Ranidae, a similar defensive behaviour has been recorded for *Lithobates areolatus* (Baird & Girard, 1852), a species that fully extends all four limbs (Ferreira et al., 2019). This behaviour was also observed, albeit rarely, in *Rana temporaria* Linnaeus, 1758 and *Pelophylax* kl. *esculentus* (Kowalski et al., 2018). Such postural displays are commonly observed in many toxic frog species (Toledo et al., 2011). Although we do not have evidence for toxicity in *C. curtipes*, it was observed that individuals of this species produce a sticky secretion with foul odour when handled. Further investigations are required to understand the biochemical properties and function of these secretions.

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