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Report of Polyandry and Necrophilia in *Duttaphrynus himalayanus* (Günther, 1864) (Anura: Bufonidae) from Sikkim, India.

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Many species of anurans have what is called "explosive breeding," which is a short, intense male-male scramble competition to mate, where males only spend a few days at breeding sites (Wells 1977; Pough et al. 2015). Explosive breeding has been documented in anuran families such as Bufonidae, Hylidae, Microhylidae, Bombinatoridae, Dicroglossidae, Pyxicephalidae, Leptodactylidae, Rhacophoridae and Ranidae (Wells 1979; Zimmerman & Bogart 1988; Seidel 1999; Prado et al. 2005, Rodrigues et al. 2007; Izzo et al. 2012; Vivek et al. 2014; Valetti et al. 2014; Khongwir et al. 2016; Abraham et al. 2013 &2018; Cai et al. 2019). The time window for such explosive breeding is relatively short and ranges from a few days to weeks (Ayres 2008). In explosive breeding, the Operational Sex Ratio (OSR) is strongly male biased as male frogs congregate at the breeding site and stay there after the mating is over in an attempt to mate with females, whereas females usually leave the breeding site after laying eggs. This results in higher male density (Menin et al. 2006, Hartel et al. 2007, Ayres 2008, Kovács & Sas 2010). Some males use non-agonistic strategies to acquire access to females associated with the most attractive males or a most attractive breeding site occupied by dominant male. Such males are called "satellite males" (Waltz 1982). These satellite males are often attracted to the amplecting pair and attempt to displace the amplecting male resulting into a large mating ball of multiple male frogs (Wells 2010). In *Isthmohyla pseudopuma* (Günther, 1901), 16 males have been recorded in a single multiple-male amplexus (Crump & Townsend 1990). Such interactions often lead to the death of the female due to drowning. (Sztatecsny et al. 2006; Zamudio & Chan 2008). Female fatality by drowning after they were clasped by scrambling males is reported in *Lithobates sylvaticus* (LeConte, 1825) and *Bufo bufo* (Linnaeus, 1758) (Howard 1980; Verrell & McCabe 1986). An observation of amplexus with a roadkill female was recorded on *Duttaphrynus melanostictus* (Schneider, 1799) from western India (Patel et al. 2016) and one record of *Duttaphrynus himalayanus* from Nepal (Rai 2022). So far, 47 cases of necrophilia have been reported worldwide, including 14 in North America,14 in South America, 10 in Asia, eight in Europe and one in Africa (Rai 2022; Serrano et al. 2022). Bufonidae have the most records of necrophilia, followed by Ranidae and Hylidae (Bufonidae: 18, Ranidae: 16, Hylidae: eight, Ascaphidae: two, Bombinatoridae: one, Leptodactylidae: one, and Pyxicephalidae: one) (Rai 2022; Serrano et al. 2022). However, possible cases of polyandry and necrophilia have hitherto been unknown from South Asia. Herein, we provide the observation of female mortality in *Duttaphrynus himalayanus* due to aggressive male scrambling competition for mating.

Duttaphrynus himalayanus is uniformly greyish brown to dark brown from dorsal region with diffuse large dark spots or without spots. The venter is yellowish to dark grey. Dark grey spots on the venter are also observed in some specimens. Spots on the venter are small in size. Dorsally, the spots are larger and diffuse. Both the sexes develop characteristic red spots which are more prominently visible in the females, Maximum SLV is 127 mm. Adult males attain only 70% of the female body size (Schleich & Kästle 2002). This species is widely distributed along the Himalayas (Sikkim, Darjeeling, Meghalaya, Arunachal Pradesh, Manipur, Assam, Himachal Pradesh, Uttrakhand, Jammu and Kashmir, (Kashmir) Pakistan, southern slopes of the Himalayas in central and eastern Xizang (China), Tibet, Nepal) between 1000 to 3500 m altitudinal gradient (Schleich & Kästle 2002; Patel pers. Obs.).

We observed the reproductive behaviour of *Duttaphrynus himalayanus* near Rahbangla village, Sikkim, India (27.30079167 N, 88.35187778E, 1770 m a.s.l.) on 22 April 2018. The toads were amplecting in an artificial tank

(dimension: 10 m × 5 m) surrounded by agricultural fields. NP observed the amplexus event from 00:33 hrs until the female drowned in the water at 02:57 h. We attempted to photograph with a digital camera (EOS 60D; Canon Corporation), at one-minute intervals during the mating activity. From 145 minutes, a total of 117 minutes were documented.

At the beginning of our observation at 00:33 h, we recorded six males in amplexus with a single female. The males were clasping female from dorsal region in axillary position, from ventral region in axillary position, from dorsolateral region, on the hind limbs and cephalic region (Fig. 1A: Fig. 2). At a time, we recorded a maximum of eight males and a minimum of two males during the multiple amplexus (Fig. 1A &;1B). We documented a *D. melanosticus* that approached the mating event and attempted to clasp the female in the multiple amplexus (Fig. 2). The *D. melanosticus* is large sized and sturdy toad (Maximum SVL of 129 mm) with a head that is noticeably broader than long (Schleich & Kästle 2002). The snout is slightly pointed and the head and back are densely covered with warts. The warts on the sides of the body and venter are ash grey, similar to the basic body colour (Schleich & Kästle 2002). Supraorbital ridges are strongly developed, high, and black, which is absent in *D. himalayanus* (Schleich & Kästle 2002). The *D. melanostictus* individual was not able to compete with other males and left the amplexus. This *D. melanostictus* individual was there for about a minute in the amplexus. Males involved in mating were continuously pushing and kicking the other toads with their hind limbs. Due to this scrambling competition number of males were frequently changing from two to eight (Fig. 3). During the first 30 minutes of our observation, the amplecting male numbers ranged from two to eight. The amplecting male toad numbers ranged from six to eight from 30 to 60 minutes. During this time, the female struggled to stay afloat and remained motionless most of the time. Between the 60th minute to 144th minute, as time progressed, the

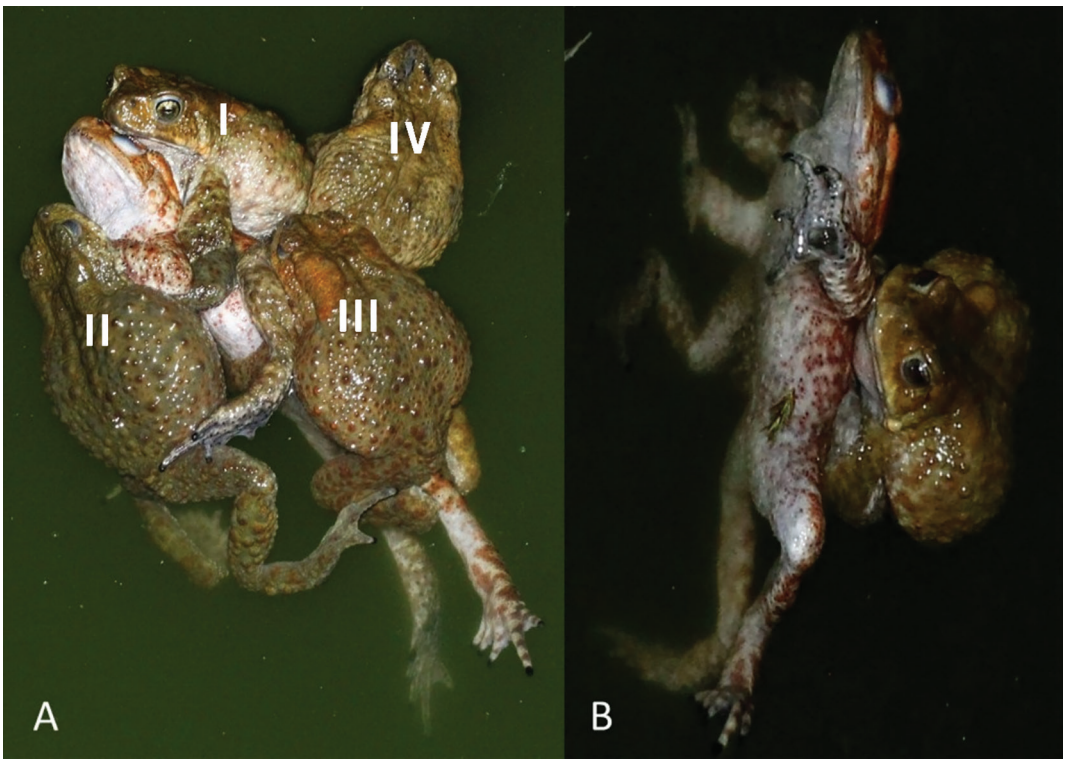

Figure 1. A) Four individual males trying to clasp the female from the dorsal axillary (I), ventral axillary (II) and dorsolateral (III) inguinal region. The fourth (IV) male clasped the first(I) male from the dorsal region B) Female was found dead at 2:24 am (112 minutes of the observation), at this moment two males were recorded clasping the female.

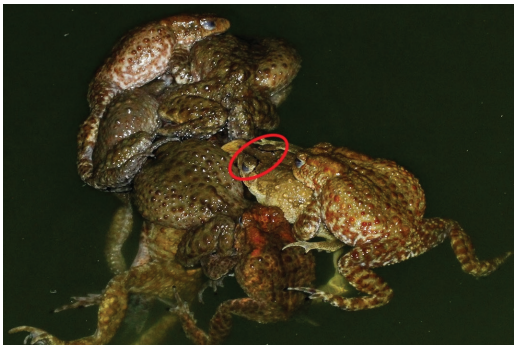

Figure 2. *Duttaphrynus melanostictus*, denoted by the red circle, was only seen once during the breeding event.

number of male toads kept changing from three to six individuals. After 112 minutes, the female died; at this moment only two toads were clasping the female (Fig. 1 B). We suspect that female died because of drowning. Males did not make any mating call or release call during our observation. We could not observe further after the female drowned. During the entire event eggs were not released by the female.

As time progressed, there was a constant change in the number of males attempting to clasp the female (Fig. 3). Most of the time smaller males were displaced by larger males (Wells 1979; Howard & Kluge 1985) due to the advantage of their size. In the present study, *D. melanostictus* got displaced by other males possibly due to bigger size of the amplecting males. The occurrence of mating events between *D. melanostictus* and *D. himalayanus* is possible as the hybrid between *D. melanostictus* and *D. himalayanus* was reported by Nanhoe & Oubotter (1987) in the Himalayan region. In the lower elevational limit of their distribution (~1000 m a.s.l.), *D. himalayanus* share their geographic range with *D. melanostictus* (Schleich & Kästle 2002). However, we encountered amplexus of *D. himalayanus* and *D. melanostictus* at the elevation of 1700 m a.s.l.

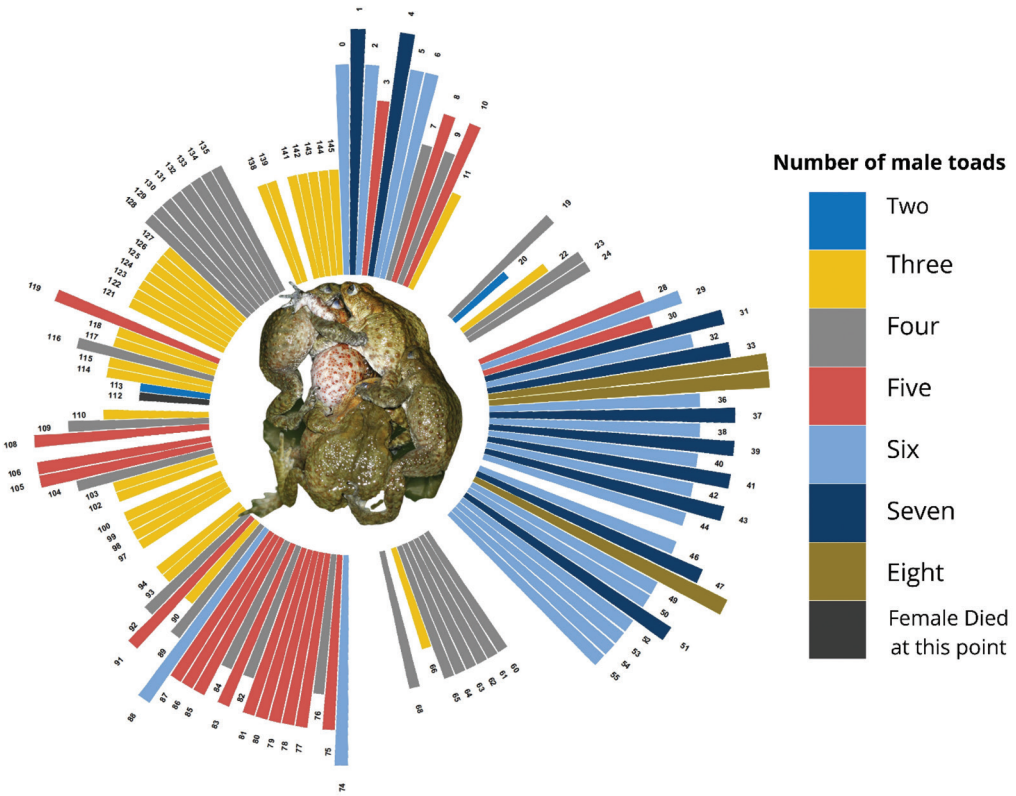

Figure 3. Number of male toads of *D. himalayanus* involved in the multiple-male amplexus as time progressed. A circular bar graph from 0 minute to 145 minutes showing number of male toads of *D. himalayanus* involved in the multiple-male amplexus.

Three males were clasped to the female most often during the breeding observation. Generally, the dorsally amplecting male tends to have an advantage in such scrambling competition over other males involved in the multiple-male amplexus (Wells 1979). Females are bigger in size compared to males (Schleich & Kästle 2002), hence the amplexus with a single individual male would not cause the female to drown. However, in the case of a mating ball where multiple males are trying to form an amplexus with the female, it becomes difficult for the female to stay afloat with other scrambling individuals. In this study, we did not observe the time at which the mating process started. We presume that this event might have started four to five hours (around 7 :30 pm or 8:30 pm) before we started recording it. Pintanel et al. (2021) suggested that necrophilia is primarily due to two causes: (1) failure in recognising live females by males, or (2) drowning to death during amplexus. (Marco & Lizana 2002; Đorđević & Simović 2014). In the case of necrophilia, the death of the female individuals in the population without successful reproduction is deemed maladaptive (Ayres 2010). Males who engage in necrophilia are deprived of time, energy, and the opportunity to mate with other potential females; they also face an increased risk of predation, roadkill, and disease transmission. (Meshaka 1996; McLister 2003; Ayres 2010; Costa et al 2010, Beebee 2012; Carmona-González et al. 2020). The possible evolutionary reason behind necrophilia could be selection for the stronger and persistent males, as the males having a morphological advantage in size or keratinized spinules will dominate the multiple-male amplexus (Izzo et al. 2012). However, there have been reports of female drowning to death because of intense multiple-male amplexus, leading to reproductive failure (Marco & Lizana 2002). As observed in this study once the female died, there was a decline in the number of male toads involved in the multiple-male amplexus, which suggest that males may need some feedback stimulation from the females in order to continue the amplexus, as observed in *Dryophytes versicolor* (LeConte, 1825) (McLister 2003). Our understanding of necrophilia is still in its infancy, and any additional research could clarify whether environmental and climatic conditions may promote these events and/or how sampling biases such as fieldwork funding and inventory completeness influence these spatial patterns (Serrano et al. 2022).

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