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BREEDING BIOLOGY OF MALABAR TREE TOAD *PEDOSTIBES TUBERCULOSUS* (ANURA: BUFONIDAE) FROM CASTLE ROCK, KARNATAKA, INDIA

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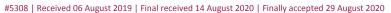
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Breeding biology of Malabar Tree Toad *Pedostibes tuberculosus* (Anura: Bufonidae) from Castle Rock, Karnataka, India

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The Western Ghats biodiversity hotspot harbors a wide variety of herpetofauna, especially anurans, with many endemic and endangered species (Roelants et al. 2004; Naniwadekar & Vasudevan 2006; Chandramouli & Ganesh 2010; Van Bocxlaer et al. 2012; Dahanukar et al. 2016). Anurans in the Western Ghats face numerous threats such as habitat loss, pollution and changing climate, and are endangered due to their small populations, specialized adaptations, and narrow distributions (Naniwadekar & Vasudevan 2006; Van Bocxlaer et al. 2012; Dahanukar et al. 2013; Gaitonde et al. 2016; Thorpe et al. 2018). Despite the endangered status of many Western Ghats endemic anurans, their natural history is largely unknown, hampering conservation efforts. We, therefore, report the breeding behavior of the Western Ghats endemic toad, Pedostibes tuberculosus from the northern Western Ghats and highlight the variation in their reproductive traits from populations distributed along the length of the Western Ghats. This will not only help in conservation prioritization, but also aid in the systematics of this group (Das et al. 2006).

The genus *Pedostibes* Günther, 1876 (Anura: Bufonidae) along with other endemic genera such as *Xanthophryene* and *Ghatophryne*, is a part of the Bufonidae lineage that dispersed into the Western

Ghats and diversified during the Miocence (Van Bocxlaer et al. 2009; Chan et al. 2016). Many species of these endemic genera are patchily distributed, and are highly specialized to their habitats with strict micro-habitat requirements, making them vulnerable to climate change (Biju et al. 2009; Gaitonde et al. 2016; Thorpe et al. 2018). For example, the northern Western Ghats endemic Xanthophryne species, exclusively use lateritic rocky outcrops as breeding habitats and lay eggs only in shallow ephemeral rocky pools/ semi-aquatic rocky habitats which are vulnerable to desiccation, and their embryos undergo largescale mortality due to stochastic fluctuations in humidity and temperature (Biju et al. 2009; Gaitonde et al. 2016; Thorpe et al. 2018). Pedostibes also exhibits several specialized adaptations such as, an arboreal habit, a short breeding period, and rapidly developing exposed embryos in aquatic/ semi-aquatic conditions and may be vulnerable to environmental fluctuations during their breeding period (Dinesh & Rahakrishnan 2013; Chan et al. 2016; Sayyed & Nale 2017). There is limited information on the reproductive biology of P. tuberculosus and its mating sequence and spawning behavior has not been documented in details.

Here, we describe the breeding behavior of *P. tuberculosus* with a pictorial sequence of their mating and spawning behavior, opportunistically encountered

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during field-work at Castle Rock, Karnataka, India.

Castle Rock (Latitude 15.41 & Longitude 74.33) is situated at an altitude of 620m, on the Goa-Karnataka border and has tropical semi-evergreen forests with numerous mountain streams. *Pedostibes tuberculosus* is locally abundant at Castle Rock and is found near perennial streams and agricultural canals. Observations for the current study were taken in an agricultural waterchannel situated in a patch of evergreen forest. The channel was mostly dry and had a single 0.3m wide and 10m long pool of water fed by a small water cascade at one end. The water column was ~2–3 inches deep and channel was filled with leaf litter and surrounded by thick undergrowth.

During light pre-monsoon rainfall, we а opportunistically encountered an aggregation of P. tuberculosus males at around 23.00h near an agricultural canal fed by a perennial stream. Males were observed calling repeatedly while perched on vegetation at a height of about ~1m from the ground (Image 1, A). Approximately, 15 males were observed in a 10m² area. The males were calling repeatedly, and constantly shifted their positions on nearby vegetation. At times, they wrestled with each other in short duels lasting 10-30 seconds. At 01.00h, the first pair in an axillary amplexus was observed on the ground, initially motionless, but soon started moving around presumably searching for a suitable oviposition site (Image 1B). At 03.00h the pair entered shallow water but were chased away by a Duttaphrynus sp. males in full breeding-color, who had also aggregated to breed in the same pool, slightly further away. Eventually, at 03.45h the P. tuberculosus pair found shallow water where they were not disturbed and started oviposition (Image 1C). The female was submerged in water up to her throat whereas; only a small part of the male's hindquarters was submerged (Image 1D). The male's hind limbs were positioned between the hind limbs of the female and the male's feet were spread beneath the female's abdomen (Image 1C). During oviposition, the female arched her back downwards and extended her hind limbs and deposited 10-20 eggs at a time. The male simultaneously performed 2-3 pelvic thrusts presumably to release sperms over the freshly released eggs. After depositing the eggs, the pair moved 10-20 cm away from the previous oviposition site and sat motionless until the female was ready to lay the next set of eggs. The female laid eggs in several bouts and the initial clutches contained 20-60 eggs (first two bouts) while subsequent clutches had fewer eggs (next four to five bouts). The female laid approximately 150 eggs by the time the mating concluded. The fresh eggs were brownish in color and a clump of eggs laid was later extended as a loose string with few detached single eggs. Subsequently, a few more pairs started oviposition at the same site. After about an hour, the focal pair had finished laying all the eggs and the male released the female from a tight axillary amplexus and crawled into the bushes. We observed the female arch her back after being released from amplexus, and soon left the oviposition site, completing the mating sequence. The other pairs formed later continued oviposition in the same manner described above until early morning (05.00h). Sympatric anurans at the breeding site of P. tuberculosus included Raorchestes sp., and Duttaphrynus sp. We also observed two species of snakes, Macropisthodon plumbicolor Green Keelback and Xenochrophis piscator Checkered Keelback, near the breeding site and may be potential predators of P. tuberculosus. The stagnant water pool where eggs were deposited and tadpoles developed, contained Chironomus larvae and plenty of leaf litter.

Anurans have evolved a high diversity of reproductive behaviors in response to the varied habitats they occupy, and exhibit distinct reproductive strategies to overcome numerous biotic and abiotic challenges (Haddad & Prado 2005; Wells 2007; Crump 2015). The Western Ghats endemic toad lineages have locally adapted to specific microhabitats (Van Bocxlaer et al. 2010; Thorpe et al. 2018), and understanding the reproductive biology of anurans of the Western Ghats will not only help conservation prioritization by identifying breeding sites and specific micro-habitat requirements, but also help understand the role of ecological factors in shaping reproductive behaviors and mating systems. Pedostibes tuberculosus shares several traits with other Bufonid lineages, such as, a semi-terrestrial adult niche, parotid glands, ability to use a wide variety of oviposition sites, and, rapidly developing exotrophous tadpoles, that aided the dispersal of the Bufonid lineage helping it attain a global distribution (Pramuk et al. 2007; Van Bocxlaer et al. 2010). On the other hand, reproductive traits such as, a small clutch size and loose single eggs often exposed to semi-aquatic conditions, seem to have evolved in response to local conditions in Western Ghats endemic toads Pedostibes and Xanthophryene, and highlight the adaptive nature of reproductive traits in the Bufonid lineage (Gaitonde et al. 2016). A novel case of pelvic thrusts during amplexus was first reported in Xanthophryne toads (Gaitonde et al. 2016), but we observed similar behavior in P. tuberculosus from the northern Western Ghats, and the pelvic thrusts seems to be widespread among bufonid species, where they possibly function to increase fertilization success,



Image 1. Mating sequence of *Pedostibes tuberculosus* from Castle Rock, Karnataka, India: A—males usually perch on vegetation at about ~1m above ground and have a single gular vocal sac | B—a mating pair in a tight axillary amplexus | C—the female carries the male in amplexus in search of suitable oviposition sites, and the female extends her hind limbs and arches her back to release a clump of eggs. As soon as the female spawns, the male simultaneously thrusts his pelvis probably to deposit sperms on the eggs while holding the egg clump with its hind limbs | D—the oviposition site is a shallow stagnant pool of water filled with leaf litter | E—as the pair moves forward before depositing more eggs, the deposited egg clump is later extended in loosely attached eggs which float on the shallow water column. © Deepak Deshpande.

Breeding biology of Malabar Tree Toad

especially in species breeding in semi-aquatic habitats. Precise estimates of fertilization and hatching success, however, need to be measured in *P. tuberculosus*. We observed tadpoles of *P. tuberculosus* to develop rapidly and metamorphose in approximately a month, which is consistent with earlier study (Dinesh & Rahakrishnan 2013).

Pedostibes tuberculosus populations in the Western Ghats exhibit substantial variation in crucial life history traits such as clutch size and choice of the oviposition site. For example, P. tuberculosus populations in southern India are reported to oviposit approximately 1,100 eggs after a single mating (Chan et al. 2016), whereas, at Castle Rock we observed females to lay only 150 eggs. A clutch size of 250 eggs is also reported for P. tuberculosus from an unknown location as a personal observation (Van Bocxlaer et al. 2009). Such magnitude differences in crucial life history traits such as clutch size are unlikely to be observed within a species; and life history traits of P. tuberculosus need to be estimated in other populations along with evaluating species diversity. Apart from clutch size, P. tuberculosus seems to exhibit substantial variation in oviposition sites, and populations from southern Western Ghats are reported to lay eggs in hollow cavities on tree barks, or, in clumps of Ochlandrae (Dinesh & Rahakrishnan 2013; Chan et al. 2016); whereas, populations in the northern Western Ghats lay eggs in shallow ephemeral pools of water or on wet ground at the edge of streams without considerable standing water (Van Bocxlaer et al. 2009; Sayyed & Nale 2017). The variation in oviposition sites indicates the utilization of suitable sites in local habitats and suggests reproductive behavior of P. tuberculosus to be influenced by local availability of resources.

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