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Notes on breeding and behaviour in the Anamalai Dot Frog *Ramanella anamalaiensis* Rao, 1937

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Ramanella Rao and Ramanna, 1925 is a genus of microhylid frogs, which are endemic to India and Sri Lanka (Bowatte and Meegaskumbura, 2011; Manamendra-Arachchi and Pethiyagoda, 2001). Of the nine species in this genus, seven are endemic to the Western Ghats-Sri Lanka biodiversity hotspot and four are threatened (IUCN, 2014). *Ramanella anamalaiensis* Rao, 1937 (Figure 1) is endemic to the Anamalai Hills of the southern Western Ghats in India and is listed as "Data Deficient" (Biju et al., 2004). It was first described (based on a single individual) in 1937 from the base of these hills at Coimbatore, Tamil Nadu. After this, it was rediscovered in 2010 at Parambikulam Tiger Reserve, Kerala. It is known to occur in moist forests at an elevation of 100-1500 m a.s.l. and can be found on the forest floor, logs and rock crevices (Gururaja, 2012). In this note, we present observations on breeding and associated behaviour and sexual dimorphism of *R. anamalaiensis* over a period of three months from September - November 2014.

The species was confirmed as *R. anamalaiensis* from its triangular snout, chocolate brown stripe from snout to groin, rudimentary webbing in feet and absence of dilations on toes (Gururaja, 2012). A total of 1 - 244 individuals (138.7 individuals ± 86.3) were observed inside a concrete tank (4.2 × 5.8 × 2.2 m) in a plantation outside the protected area at 1350 m a.s.l. in Munnar (Kerala) during 12 survey nights between 19:00 - 00:30 h. The geographical coordinates of the location are not provided here to safeguard the location from collections for research purposes that is currently rampant outside the protected area network in the Western Ghats. The air temperature during these observations ranged 17.7-20.3 ºC, substrate temperature was 20.7-22.7 ºC, water temperature inside the tank was 20.9-22.0 ºC and ambient humidity was 55-82 %. During the study period, individuals were occasionally encountered outside the tank; these specimens were excluded from further observations. This species has not been observed in such large congregations on the forest floor and was rarely encountered (relative abundance=0.02, up to elev. 1650 m) during surveys undertaken at shola-grassland forests in the region (A. Kanagavel, unpublished data). This relatively large congregation observed in the present study has not been reported in any other *Ramanella* species. The breeding in *R. nagaoi* and *R. montana* occurs in water-filled tree hollows, in groups of 9-13 individuals (Krishna et al., 2004; Krishna and Bosch, 2007; Manamendra-Arachchi and Pethiyagoda,

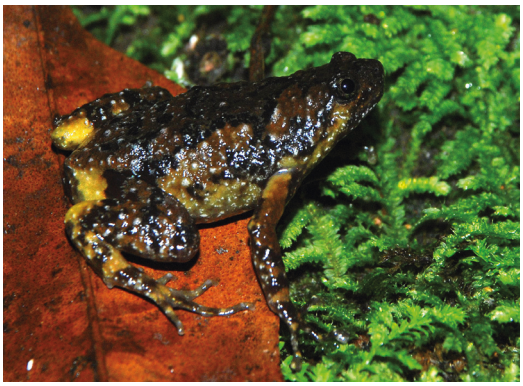

Figure 1. The Anamalai Dot Frog (*Ramanella anamalaiensis*) (Photo by Arun Kanagavel).

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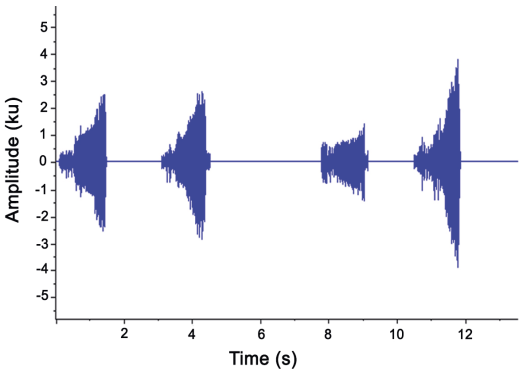

Figure 2. An oscillogram of four continuous calls of a single male *R. anamalaiensis.*

2001). The *R. anamalaiensis* congregations were also not observed during the day time surveys undertaken at the tank.

Sex was determined by the presence or absence of vocal sac following the methods of Manamendra-Arachchi and Pethiyagoda (2001). Male individuals had a single vocal sac and the advertisement calls consisted of a loud, high pitched note (trrrrru). The vocalizations of all the individuals overlapped temporally. The call of a single vocalising male was recorded using a mobile phone (Nokia Lumia 650) from a distance of about 10 cm. Four continuous calls from this recording were analysed using Raven Pro 1.4. A single call lasted for 1.37 s and attained peak amplitude of 127 kU at the beginning and reached 2440 kU just before the end (Figure 2). The frequency of the call ranged from 0.2-3.1 kHz while the sound power at the beginning was 55.5 dB, reduced to 16.7 dB in between and ended with 3.5 dB (Figure 3). The male individuals were partly submerged in the water and lined themselves along the inner walls of the tank. An amplectant pair that had dislodged post disturbance from other males was observed further. The male was visibly smaller than the female. Individuals that lacked vocal sacs did not vocalise and more often observed on the side or top of the tank away from the water were assumed to be females. *R. anamalaiensis* was found to exhibit sexual dimorphism with the females (SVL: 3.1 cm ± 0.29, HL: 0.9 cm ± 0.21, HW: 1.1 cm ± 0.37, n = 8) being larger than males (SVL: 2.4 cm ± 0.10, HL: 0.6 cm ± 0.08, HW: 0.8 cm ± 0.06, n = 7). This size related sexual dimorphism has been observed in other *Ramanella* species (Krishna and Bosch, 2007; Manamendra-Arachchi and Pethiyagoda, 2001). The ventral surface of the males usually had a black marbled pattern, while that of females was duller (Figure 4a, b). However, a few, dull coloured males were also observed, therefore, colouration cannot be used as a reliable characteristic to determine the sex of *R. anamalaiensis*.

A maximum of 12 amplectant pairs (2.9 pairs ± 4.1) were observed inside the tank during the survey (Table 1). One amplectant pair was also observed outside the tank but no additional search effort was targeted towards observing breeding on the ground. The male clasped the female in the pectoral region and formed an axillary amplexus (Figure 5). The amplexus was mostly formed while the frogs lay submerged in water or while the female clung onto the side of the tank above the water. During amplexus in the water, several attempts were made by multiple males to dislodge the mating pair. Such attempts at dislodging pairs have been observed in other amphibians (Chuang et al., 2013; Oliveira et al., 2014; Wogel et al., 2005). This behaviour could be the result of competition due to a skewed sex ratio, with the presence of greater number of males than females (Mollov et al., 2010). We observed on numerous occasions the male kick away other approaching males whilst they were in amplexus with a female. Amplectant pairs then often moved up the tank wall, away from the water, probably to avoid disruption. When amplectant pairs were dislodged from the wall, the amplectant pair would swim to another location and cling to the wall below the water level. A pair remained fully submerged

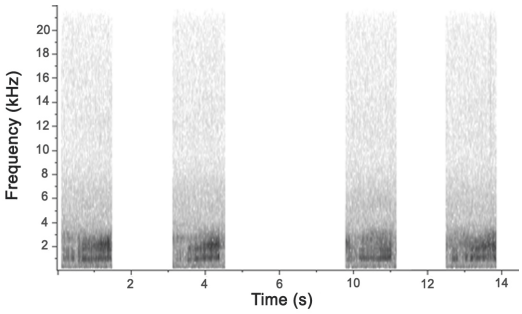

Figure 3. A spectrogram of four continuous calls of a single male *R. anamalaiensis.* Sections inbetween calls (with calls of other individual frogs) have been manually removed from the figure.

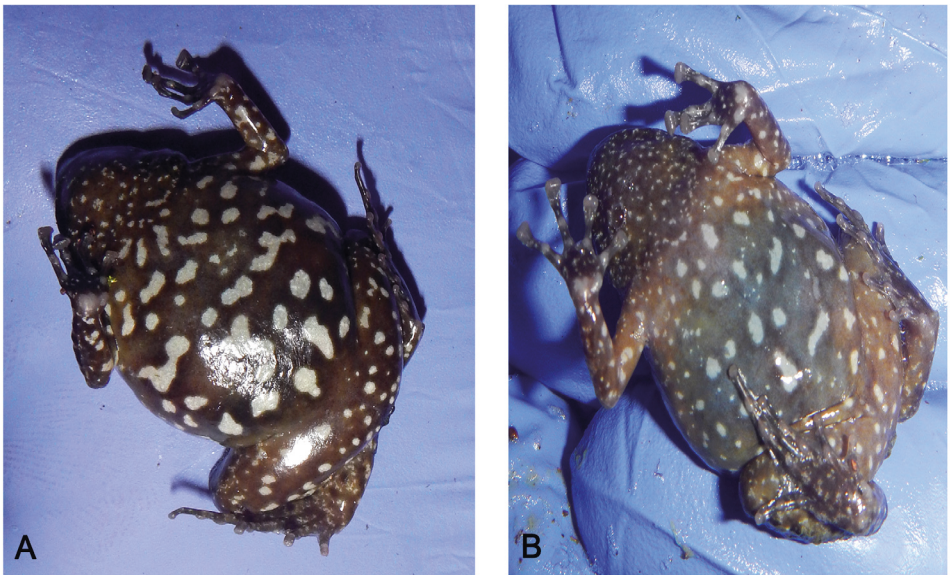

Figure 4. Ventral view of (A) male and (B) female *R. anamalaiensis* (Photo by Monica Harpalani).

for approximately eight minutes and on returning to the water surface, similar events were repeated. A pair was observed in amplexus for two and a half hours, despite four attempts by other males to dislodge the amplectant male.

Reproductive activity in *R. anamalaiensis* could be influenced by weather conditions. During heavy rainfall the frogs began to leave the tank and there was no competition or vocalisations. The overall number of amplectant pairs reduced during heavy rainfall (pre rainfall = 8 pairs, during heavy rainfall = 5 pairs) but pairs that remained in amplexus were not disturbed by neighbouring males. However, no significant correlation (Spearman's rank) was found between the number of individuals and amplectant pairs observed with the air temperature (R=0.12, n=15, p=0.67 and R=0.28, n=15, p=0.32), substrate temperature (R=-0.22, n=15, p=0.44 and R=-0.18, n=15, p=0.52), water temperature (R=-0.03, n=14, p=0.93 and R=-0.11, n=14, p=0.71) and humidity (R=-0.03, n=15, p=0.92 and R=0.01, n=15, p=0.99).

Multiple cases of unusual amplexus were also observed, which has not been reported in other *Ramanella* species. On several occasions, the males of *R. anamalaiensis* tried to mount one another. The male being mounted, immediately vocalised and pushed the other away with its foot. At times this resulted in a combat, which lasted to a maximum of six seconds wherein the male being mounted fanned its hind limbs to push the approaching male away. At times males attempted to mount one another in the gular region, either in the usual amplectant position or their ventral sides facing each other, while

Table 1. Temporal details of amplexus, eggs and tadpoles observed of *Ramanella anamalaiensis.*

Date	*Time* (hour)	*Amplexus/eggs/tadpoles observed*
7.10.2014	18:59	Amplexus
31.10.2014	19:22	Amplexus
3.11.2014	19:30	Amplexus, egg mass
4.11.2014	19:38	Egg mass
5.11.2014	20:11	Amplexus, egg mass
7.11.2014	19:30	Amplexus
9.11.2014	19:30	Tadpoles
10.11.2014	22:35	Amplexus, tadpoles
14.11.2014	19:29	Amplexus, tadpoles
17.11.2014	19:43	Tadpoles
19.11.2014	19:13	Amplexus, tadpoles

Figure 5. *R. anamalaiensis* in axillary amplexus (Photo by Monica Harpalani).

(D'Amore et al., 2009).

Spawning was not observed in any of the amplectant pairs. However, egg clutches were seen on the surface of the water and on the tank side above the water level, these were much like the clutches documented in *R. montana* (Krishna et al., 2004). No attempts were made to measure the eggs, which were grey in color and, the vitellus was also not observed. The larva of *R. anamalaiensis* requires formal scientific description. Black-coloured microhylid-like larvae were observed in the tank after three to five days of the eggs being first observed (Table 1) and we suspect, although cannot confirm, that these were larvae of *R. anamalaiensis*.

These observations highlight the significance of stationary pools of freshwater for the reproduction of this species. The congregation could have occurred from the tank being large enough to accommodate these population sizes. However, this is in contrast to *R. obscura*, which is thought to avoid large pools due to higher resource competition (Bowatte and Meegaskumbura, 2011). The aggregation in the tank could be a result of other factors such as a lack of natural water-filled cavities in the plantation, where only the primary canopy vegetation is intact. Furthermore, the plantation where this study was undertaken is further

kicking rapidly. Both the males called vigorously during the combat. The calls emitted by males during male – male combat sounded the same as the advertisement calls, although calls emitted during these interactions were not recorded. Furthermore, numerous instances of interspecific amplexus were observed between male *R. anamalaiensis* and, *Fejarvarya* sp. and *Indirana brachytarsus* (Figure 6). On one instance, after being dislodged by the *Fejervarya* individual, the *R. anamalaiensis* followed and re-mounted it. These unusual amplexus between conspecific males and heterospecific individuals have generally been observed in large breeding congregations (Mollov et al., 2010). It is believed that due to the lack of any specific cue for sex recognition, males would generally clasp any moving object; a male of its own kind or an individual of any other species as well (Simović et al., 2014). Another reason heterospecific amplexus could occur is the preference of males for a larger-sized mate

Figure 6. Interspecific amplexus between *R. anamalaiensis* male and *Indirana brachytarsus* (Photo by Monica Harpalani).

surrounded by other similar plantations, tea estates and tourism-related infrastructure. These observations also represent a significant range extension of *R. anamalaiensis* by 62 km north-west from Parambikulam Wildlife Sanctuary, the closest known distribution to Munnar in Kerala. The IUCN Red List status for R. anamalaiensis could be revised from "Data-deficient" to "Endangered" on the basis of its restricted range.

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