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SHORT NOTE

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Direct development of the Reza Khan's bush frog *Raorchestes rezakhani* in north-east India

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ABSTRACT - There are currently 76 species of frog in the genus *Raorchestes*, these undergo direct development, i.e. there is no free living tadpole stage, instead a froglet emerges from the egg. We documented the development of the recently described Reza Khan's bush frog, *Raorchestes rezakhani*. Twenty-three eggs were laid in a hole in a tree stump about 1.5 m above the ground. On average the eggs were 5.5 ± 0.26 mm in diameter and weighed 0.9 ± 0.07 g. We describe six stages of development at temperatures between 23–26° C and relative humidities of 73–86%. The froglets emerged from the egg 13 days after the embryo had developed head, tail, limb structures and body pigmentation.

INTRODUCTION

Anurans have the greatest diversity of reproductive modes among tetrapod vertebrates, with at least 42 being currently recognised (Malagoli et al., 2021). The majority of frogs lay eggs from which free living aquatic larvae (tadpoles) hatch. These tadpoles metamorphose into froglets that resemble the adult. However, 27.2% of known anuran species (1,928) have adopted 'direct development' (Liedtke et al., 2022) where there is no free living larval stage, instead the embryo develops directly into a froglet that then hatches from the egg (De Lima et al., 2016; Schweiger et al., 2017; Goldberg et al., 2020). Direct development may be favoured where environmental conditions do not provide suitable aquatic habitats for tadpoles (Liedtke et al., 2017; Salica et al., 2023).

The family Rhacophoridae contains 76 species of small-bodied, terrestrial bush frogs in the genus *Raorchestes* (Biju et al., 2010), that are found from southern India to Nepal and in mainland south-east Asia (Biju & Bossuyt, 2009; Vijayakumar et al., 2016; Frost, 2023). There is little data on clutch sizes and development times for the few species that have adopted direct development. Here we report the direct development of the little known Reza Khan's bush frog *Raorchestes rezakhani* Al-Razi, Maria & Muzaffar, 2020, a small cryptic bush frog with a mean snout-vent length (SVL) of 20.06 ± 0.87 mm, originally described from north-eastern Bangladesh based on morphological characters, genetics and bioacoustics (Al-Razi et al., 2020). This species was later discovered in various locations in Mizoram, India (Decemson, 2021; Hlondo, 2021) where it was found mainly on the edge of streams and near man-made trails in primary and secondary forests. During the monsoon season, it frequently uses hilly slopes for calling, where individuals perch on leaves and branches of small trees and on bamboo trunks (Al-Razi et al., 2020; Decemson et al., 2021; Hlondo, 2021).

Figure 1. Adult female *Raorchestes rezakhani* (MZMU 2173)

METHODS

On 25 May 2022, during a herpetofaunal survey along stretches of the Sipai stream, Lunglei, Mizoram, north-eastern India (22° 53'18.7908" N, 92° 45'37.1046" E, 817 m a.s.l.), an adult female *Raorchestes* (Fig. 1) sitting near a developing clutch of eggs was found and collected at a depth of 7 mm inside a hole in the stump of Chinese albizia *Albizia chinensis* about 1.5 m above ground (Fig. 2 A&B). The specimen was preserved and deposited in the Departmental Museum of Zoology, Mizoram University (catalogue number MZMU 2173). The site was marked and covered with nets to keep out predators. The air temperature and relative humidity were measured using KusamMeco KM-918 Thermo

Figure 2. Breeding site of *Raorchestes rezakhani* in a hole in a tree stump by the Sipai stream, Lunglei, Mizoram - **A.** Tree stump covered with net to protect the eggs from predators, **B.** Close up view showing the eggs within the hole in the tree stump

Figure 3. *Raorchestes rezakhani* eggs after transfer to a plastic terrarium under natural light with organic soil, mixed vegetation, and leaf litter to maintain humidity and temperature

Hygrometer between 25 May and 6 June 2022. Though we are not sure at what time and date these eggs were laid, it was confirmed that the microhabitat was recently utilised for breeding by this species, as calls of this species were heard frequently in this specific area during the previous survey. The study site was visited daily until 31 May 2022. Following that, eggs with developing embryos were collected and transferred to a plastic terrarium (Fig. 3) to observe and document the development of the species until froglets emerged. The plastic terrarium (23 x 20 x 12 cm) had natural lighting and was provided with organic soil, mixed vegetation and leaf litter to maintain the relative humidity at 73–86% to trigger development. Eggs were misted daily with water and humidity inside the terrarium was carefully monitored. Room temperature ranged between 23–26 °C as per ambient conditions. The stages of the eggs were classified by comparing them to images of the various stages provided by Bahir et al. (2005). The dimensions and weight of the eggs and frogs were recorded using Mitutoyo Vernier Caliper and MH Series pocket scale, respectively.

RESULTS

The adult frog that we collected was confirmed as *R. rezakhani* based on the original description provided by Al-Razi et al. (2020) which includes - relatively small size with a SVL of 21.36 mm, indistinct tympanum with supratympanic fold weakly distinct, both inner and outer metacarpal and metatarsal tubercles absent; in addition, genetic studies (Gen Bank accession no. OR482067) showed only 1.80% genetic divergence from the holotype (Gen Bank accession no. MN072375). From the date of encounter until emergence from the eggs, *R. rezakhani* developmental stages were documented. Within the vitelline membrane the egg developed, and a juvenile frog emerged. There were a total of 23 eggs, with diameters ranging from 4.82–6.24

Table 1. Observations of embryonic development in *Raorchestes rezakhani* in May/June 2022. The stages of the eggs were categorised by comparing the eggs to photographs of the various stages provided by Bahir et al. (2005).

Observation period day/date	Stage	Developmental Characteristics
Day 1 25 May	1	Unpigmented subdermal eyes visible; head become prominent; tail elongated and dorsal and ventral fin transparent; body pigmentation appears on dorsal and lateral body surface; forelimb elbows emerge.
Day 4 28 May	2	Cornea transparent; eyes pigmented; pigments denser on head, snout and dorsum; toe indentations visible.
Day 7 31 May	3	Toes separated; expanded abdominal folds on venter; pigmentation progresses from the head to the tail region of the body; eyes more defined; iris black.
Day 10 3 June	4	Pigmentation on abdomen; hands clearly distinguishable on forearms; head region longer; eyes larger; hindlimbs lengthen, tips of toes enlarged; tail fin fully developed; upper eyelid visible.
Day 12 5 June	5	Most parts of tail have resorbed; dorsal and ventral fins disappear; mouth fully developed; body greyish brown; abdominal folds disappear; head and jaw developed and fully structured; body is stouter; subarticular tubercles under fingers/toes developed.
Day 13 6 June	6	First hatchling emerged; dorsal colour greyish brown with some small dark brown/ grey spots; froglet has internal yolk sac; a small tail tip remains.

Figure 4. Various developmental stages of *Raorchestes rezakhani* - **A.** Eggs with embryos in which the head and tail area demarcated and body pigmentation developed, **B.** Subarticular patches developed on fingers and toes, **C.** Embryo fully developed, yolk visible inside abdomen, **D.** A newly hatched froglet

mm (5.5 ± 0.26 mm) and weight 0.82–1.26 g (0.9 ± 0.07 g). The six major features of development of the froglet are described in Table 1, with some stages illustrated in Figure 4. At the time of observation, the embryo was encased in a clear gelatinous capsule and had already attained a well demarcated head and tail as well as limb structures. Body pigmentation developed and the tail increased in length with a transparent blade. Subsequently, the eyes developed a black iris and translucent cornea. Toes separated and sub-articular patches became visible. Finally, the tail shrunk and then the first froglet hatched on the 13th day from the start of observation; the last hatchlings emerged three days later. The tail remnant was resorbed within the next two to three days. From 23 eggs, 17 froglets hatched successfully and 12 of these were released in the forested area in the University campus, while the remaining five were preserved as reference specimens in the Departmental Museum. The SVL of the froglets range from 5.51–5.98 mm (5.7 ± 0.17 mm, N = 5).

DISCUSSION

The embryonic development of only a few *Raorchestes* spp has been described due to the difficulty of finding their eggs in the natural environment. Like its congeners *Raorchestes huanglianshan* (Zhang et al., 2022) and *Raorchestes longchuanensis* (Yan et al., 2021), owing to direct development *R. rezakhani* embryos did not develop many of the structures associated with tadpoles, such as external gills, tadpole teeth or a coiled intestine, and the front and

hind limbs developed nearly simultaneously. Interestingly, the newly hatched froglet (Fig. 4D) still had a small tail tip.

Raorchestes spp are known to lay eggs in a variety of locations. Bush frogs such as *Raorchestes bombayensis* (Bossuyt et al., 2001), *Raorchestes glandulosus* (Krishnamurthy et al., 2002), *Raorchestes nerostagonaq* (Biju & Bossuyt, 2005a), *Raorchestes bobinger* (Biju & Bossuyt, 2005b) and *Raorchestes chalazodes* (Seshadri et al., 2015) have been observed laying eggs on the upper side of plant leaves or in deep holes in trees. *Raorchestes rezakhani* appears typical of this group by spawning in a hole in the stump of a tree. In contrast, some bush frogs have been recorded laying their eggs in grass clumps or in soil, e.g. *Raorchestes graminirupes* (Biju & Bossuyt, 2005b), *Raorchestes resplendens* (Biju et al., 2010), *Raorchestes ghatei* (Padhye et al., 2013) and *Raorchestes tinniens* (Princy & Kannan, 2018).

The clutch size of *Raorchestes* may vary between species. We observed a clutch size of 23 in *R. rezakhani* while *R. huanglianshan* has been reported laying 13 eggs (Zhang et al., 2022) and a pair of *R. longchuanensis* in amplexus laid eggs twice within one month; the first clutch included 12 eggs, and the second included ten eggs (Yan et al., 2021), *R. glandulosus* has been reported to lay a clutch of 14 eggs (Krishnamurthy et al., 2002), and *R. tinniens* a clutch of 30 eggs (Princy & Kannan, 2018).

In our present work, the developmental period of *R. rezakhani* is uncertain as we did not observe the eggs from the time of egg laying but only from a stage at which the embryos were already relatively advanced with head, limbs and tail. In other species under both natural and captive conditions the period for oviposition to hatching has been 22–28 days in *R. glandulosus* (Biju, 2003; Krishnamurthy et al., 2002), 17–19 days in *R. tinniens* (Princy & Kannan, 2018), in captivity alone 25–30 days in *R. longchuanensis* (Yan et al., 2021) and 31–35 days in *R. huanglianshan* (Zhang et al., 2022).

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