

Reproductive Behaviour of *Raorchestes shillongensis* (Pillai and Chanda, 1973) from Meghalaya, Northeastern India

Abstract

We studied reproductive behavior of *Raorchestes shillongensis*, a poorly known endemic bush frog of Northeast India. During the study period, behavioural repertoire previously unreported from other bush frog species of India were observed. We also describe amplexus, spawning and male advertisement call of the species and provide a comparative account of reproductive mode of other bush frogs from South Asian region. Our study fills the gap of knowledge on reproductive biology of bush frogs of Northeast India.

Introduction

Among the tetrapod vertebrates, amphibians exhibit highest diversity of reproductive modes (Haddad and Prado, 2005; Wells, 2007; Crump, 2015; Gaitonde et al. 2016). There are over forty recognized reproductive modes in amphibians (Gururaja et al. 2014; Iskandar et al. 2014; Seshadri et al. 2014; Willaert et al. 2016). Among anurans, the Rhacophoridae group exhibit diverse reproductive modes, ranging from foam nests and free-feeding tadpoles to direct development (Brown & Alcalá 1982, 1994; Grosjean et al. 2008). Terrestrial direct development in Bush frogs (*Philautus*, *Pseudophilautus* and *Raorchestes*) involves eggs hatching into tiny froglets avoiding the free swimming tadpole stage (Bossuyt & Dubois, 2001; Bahir et al. 2005; Gururaja and Ramachandra, 2006; Grosjean et al. 2008; Li et al. 2009; Biju et al. 2010). Studies on the reproductive behavior of amphibian species in India has been scanty and data on breeding behavior are available for roughly 7-8 percent of the total amphibian species of the country. Studies on the breeding biology of amphibians is of crucial importance for the successful conservation the species along with their habitats (Gaitonde et al. 2016), and more so for endemic and poorly known species like *Raorchestes shillongensis*.

Meghalaya state in Northeastern India is a part of Indo-Burma global biodiversity hotspot (Mittermeier et al. 2004). The vegetation of the area ranges from tropical evergreen, tropical semi evergreen, tropical moist, riverine grassland, subtropical pine forest and temperate forest (Haridasan and Rao, 1985 & 1987).

At least 70 species of amphibians are recorded from Meghalaya state of which about 30% are endemic to the state (Ahmed et al. 2009; Mahony et al. 2013). 10 species of Bush frogs of genera *Raorchestes* and *Philautus* are known from Northeastern India. Meghalaya is home to 6 species (Frost, 2017).

In this communication, we described reproductive behaviour of critically endangered X-mas Bush frog, *Raorchestes shillongensis* which is endemic to Northeastern India.

Key words:
Raorchestes shillongensis,
endemic,
reproductive
behaviour,
Northeast India.

Raorchestes shillongensis male calling.
Photo Credit: Abhijit Das

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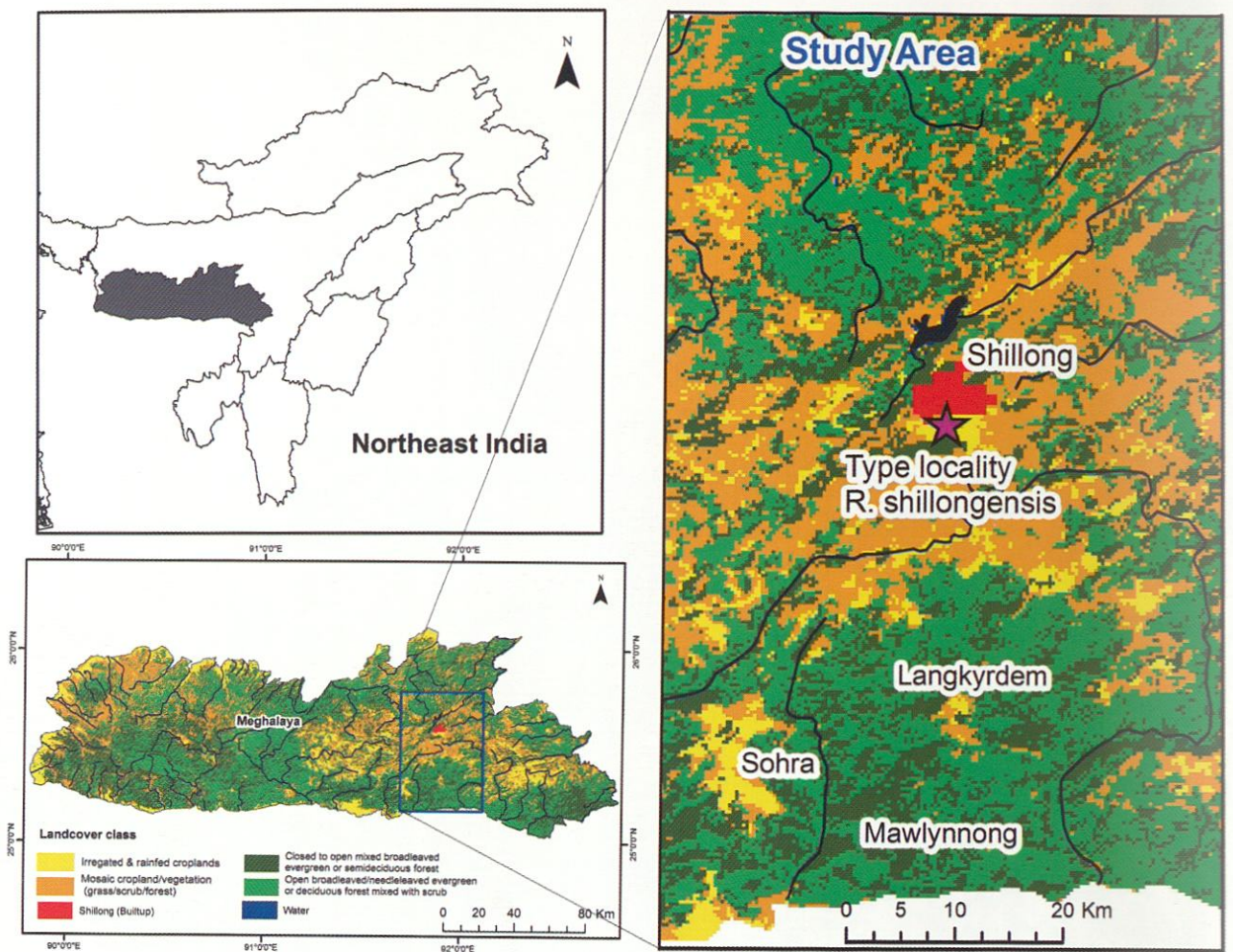
Materials and Methods

Study Area

The Xmas Bush frog *Raorchestes shillongensis* was described from Malki forest (25°33'45'' N and 91°53'19'' E) within Shillong city limit (Figure 1) in Meghalaya (Pillai and Chanda, 1973). Malki forest is a strip of subtropical wet hill forest (Champion & Seth 1968), dominated by *Pinus kesiya* and broadleaf trees (Mahony et al. 2013). Moss, ferns, tall grasses and other

shrubs cover ground floor of the forest. Forest edges mostly covered by *Eupatorium*, *Melastoma* etc. Malki forest is surrounded by thickly populated human dominated landscape. Some protected and reserve forests viz. Upper Shillong, Riat-Laban, Shyrwart, Laitkor, Mawpat, Mawlai and Riat Khawan are located at the fringe areas of the Malki forest.

Figure 1: Map showing the study area.



Field survey

Field study was conducted between May and August 2016 in the East Khasi Hills district of Meghalaya, Northeast India. During this period, selected forest areas across the district were actively surveyed to record the presence of the species. Night surveys were primarily aided by its distinctive call. The frogs begin to call with the first showers during April end. Day time

survey was also conducted to locate and determine the egg clutch size. Individuals were captured and snout-vent length (SVL) and weight were taken to the nearest 0.1 mm using a digital slide caliper and Pesola Spring balance to the nearest 0.1 g respectively before being released. Individuals were photographed and various environmental parameters of the microhabitat (e.g. ambient temperature, humidity etc.) were recorded. Male and

female were identified in the field based on presence or absence of vocal sac and externally visible ripe ova.

Behavioural observations

Two sites (Malki forest and Risa colony) were selected for observations of breeding behavior in natural conditions. Behavioural observations were carried out from 18.00 hours onwards and sometimes continuing throughout the night. During the study period, sequences of breeding activities were observed on five occasions within these two study sites (29.05.2016/ 02.06.2016/ 06.06.2016/ 25.06.2016/ 07.07.2016). Observations were made from approximately 1m distance using a neutral white light source and sometime redlight. Time period of each event was recorded in minutes and hours using a stopwatch. Within 10 minutes after egg laying, clutch size, egg diameter, size (SVL) of the amplexant pair was recorded and evaluated the association between female's body size and clutch size and egg size. Development of the eggs was continuously observed for two clutches in natural habitat. Besides, two egg clutches were carried to the field station and kept in nature like condition (with a maximum 1°C temperature difference from the outside) to evaluate the percentage of successful hatchlings. Froglets after hatching were released back to their natural habitat. Basic statistical analysis was performed in MS excel spreadsheet.

Call record and analysis

A digital recorder (Sony IC recorder 7.4.0) was used to record call on the 30th August at 19.41 hrs. Recorded calls were visualized and call characters were obtained using Raven Pro Ver. 1.5 (Charif, Waack & Strickman, 2010). We measured a total of five temporal properties that included call group duration, inter call group interval, intra call group interval, call duration and call rate of a call bout comprising of five call groups. One spectral property, i.e., peak frequency, was also measured over the entire series of calls. Terminologies and graphical representation of the call properties follow Bee et al. (2013a) and Bee et al. (2013b).

Results

Male vocalization: Calling males were recorded from the onset of raining in April end and the call activities decreases towards August. Calling males come out at just beginning of dusk (18:30 hr) and calling is more frequent up to 24:00 hr. Calling of males also can be heard from the bushy thickets on a rainy day. Advertisement calls delivered in call groups (Figure 2) consists of two to multiple (Tick tick tick ...) calls. Duration of the call bout analysed was 22.62s and number of call group was five with call numbers varying 3-5. Duration of call group was $0.72 \pm 0.21s$ ($n=5$). Duration of call group varies with the number of calls. Inter call group interval was $4.76 \pm 0.76s$ ($n=4$). Intra call group interval was 0.19

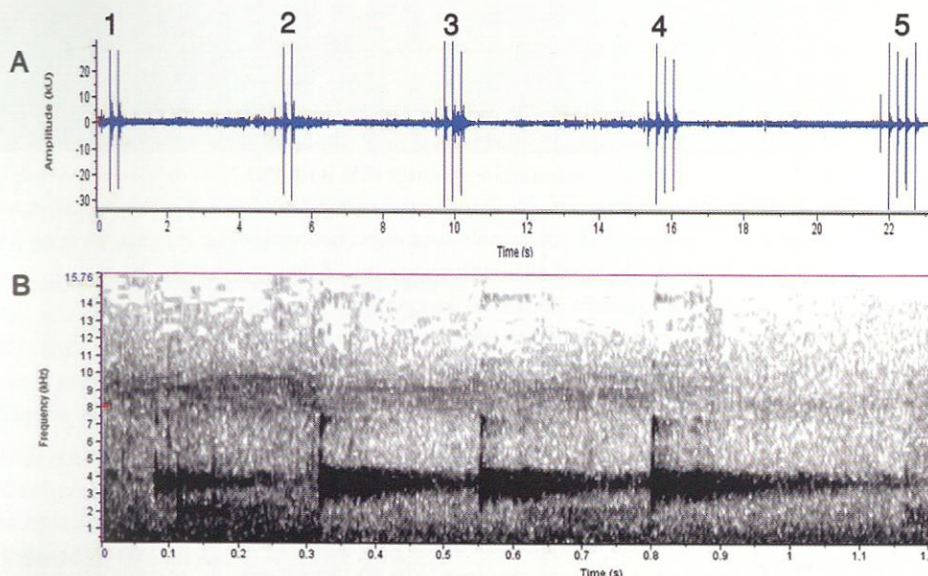


Figure 2: Advertisement call of *R. shillongensis* at ambient air temperature 24.1°C. A. A call bout comprising of five call groups (digits indicated number of calls in the respective call groups). B. Spectrogram of 4th call group with four calls depicted in A.

is 30.5 ± 0.71 days ($n=2$) (details of egg development is provided in **Table 1**). Rate of successful hatchlings is 100% ($n=2$). Body colour of the froglet is brown and slightly blackish towards lateral side of the belly or brown with dark - (shape mark on back as in adult.

Discussion

The present study is a first ever documentation of breeding behavior of a bush frog from Northeast India. Breeding mode of *R. shillongensis* belongs to Type 17 as delineated in Duellman and Trueb (1994), i.e. direct development on ground. Clutch size of *R. shillongensis* is smaller (except *R. chalazodes* and *R. ochlandrae*) but developmental duration is longer than other known Indian bush frogs (**Table 3**). This may be attributed to the colder and more moist conditions the region. The Amplexus mode, egg colouration and size is comparable to other Bush frogs. Reproductive mode *R. shillongensis* is similar (mode 17) with *R. tinniens*, *R. graminirupes* and *R. resplendens*. But, *R. resplendens* lays eggs under moss covered forest floor, deep inside the base of bamboo clumps (Biju et al. 2010), *R. graminirupes* lays on ground, grass clump, rocky crevices, *R. tinniens* lays in deep hole on ground (Gururaja and Ramachandra, 2006), while *R. shillongensis* lays eggs on moist soil under leaf litters. However, mixing of eggs with moist soil has not been previously observed in any bush frogs of India. In *P. variabilis*, (confined to Sri Lanka) the female guarded the eggs and chase the males (Kanamadi et al. 1996). However, we did not observe such parental care in *R. shillongensis* like in *R. chalazodes* and *R. ochlandrae* as they stay with the eggs until hatched. Other bush frogs in India like *Raorchestes gladulosus*, *R. bombayensis*, *R. nerostagona* and *R. bobingeri* deposits eggs above the ground on arboreal habitats (mode 20). Breeding behaviour of *R. shillongensis* shows some similarity with ground nesting *Pseudophilautus* spp. of Sri Lanka as they mix the eggs with soil, probably for better distribution of sperms (Bahir et al. 2005). However, *R. shillongensis* do not excavate soil to egg lay and egg separation does not occur during mixing with soil as in Sri Lankan *Pseudophilautus* (Bahir et al. 2005).

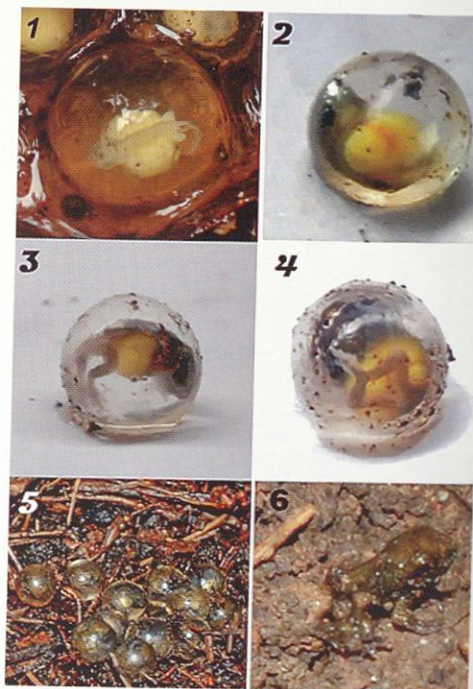
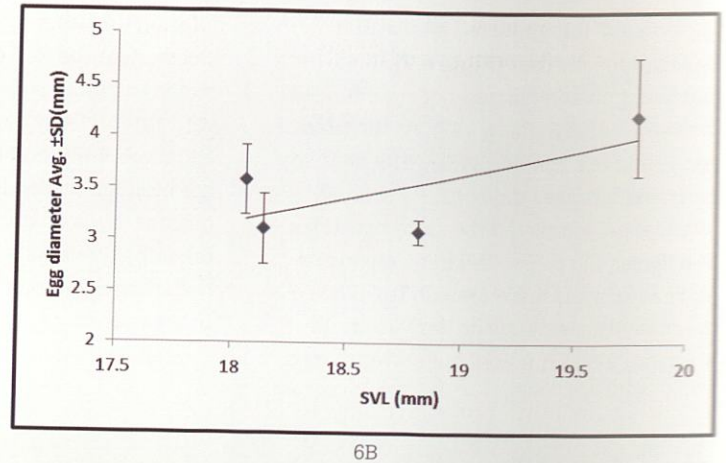


Figure 7: Developing embryo of *R. shillongensis*.

1. Stage 2-3,
2. Stage 5-6,
3. & 4. Stage 10
5. Stage 14,
6. A newly hatched froglet.

Clutch size of *R. shillongensis* is relatively smaller than other bush frogs except *R. chalazodes*, *R. ochlandrae* and Sri Lankan bush frog *Pseudophilautus regius*. The change in body colour of *R. shillongensis* to camouflage with soil during egg laying is not reported in any Indian bush frogs except Sri Lankan ground nesting bush frogs (Bahir et al. 2005). The colour change of ground-nesting bush frogs (*Pseudophilautus*) during egg laying is probably to reduce the predation risk, especially these frogs often nest in daytime (Bahir et al. 2005).

Despite the smaller distribution range of *R. shillongensis* (approximately 530 sq. km) in the East Khasi Hills, it is relatively abundant in the backyards and forest edges especially around the Shillong city. During

the study period, we observed that the habitat of the Malki forest and adjacent areas are rapidly degrading due to various anthropogenic activities like fire wood collection, intentional forest fire, excessive use of detergent for cloth washing in the forest streams (Mahony et al. 2013), garbage dumping by the local people as well as by the tourist etc. Besides the above factors, use of chemicals for cultivation in the surroundings of Malki and Upper Shillong areas could be detrimental to the long term survival of this endemic bush frog.

Shillong Plateau is well known for high endemism of amphibian species, but government-protected forests of the East Khasi Hills district (2,752 km²) represent only 18.65 km² in seven locations, the largest being the Upper Shillong Protected Forest with only 7.66 km² area (Mahony et al. 2013). Deteriorating habitat quality, unregulated mining in protected and unprotected areas in the state (Gilbert, 2012), may have considerable affect in the survival of amphibian fauna of the region (Mahony et al. 2013). Therefore, conservation of the last remaining forest patches is of urgent priority, which harbor many threatened and endemic species of the region.

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Raorchestes spp. of north-east India largely represent cryptic diversity.
Photo Credit: Abhijit Das



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Table 1: Developmental stages of embryos of *R. shillongensis* (Based on clutch laid on 08. 06. 2016). Stages are in comparison to *Pseudophilautus viridis* from Sri Lanka (Bahir et al., 2005).

Date	Days from the date of laid	Stage	Characteristics
19.06.2016	12	Stage 2-3	Limb buds clearly visible, unpigmented subderal eyes, tail elongated.
22.06.2016	15	Stage 4	Eyes large and black, pigment on dorsal side of the yolk
24.06.2016	17	Stage 5-6	Toe demarcation initiated, eye pupil visible.
28.06.2016	21	Stage 10	Limbs fully developed, toes and fingers visible, pigmentation covers limbs and spread towards lateral side of the yolk.
05.07.2016	28	Stage 14	Tail almost absent, little amount of yolk present, looks like adult.

Table 2: Ecological parameters and sequences of breeding behaviour of *R. shillongensis* during the study period.

Date	Temp/ Humidity (°C/%)	Perch height (M/F) (cm)	SVL (M/F) (mm)	Perching distance between M & F (cm)	Amplexus start time
29.05.16	17.1/ 93	51/120	17.56/18.06	100	21:05 hr
02.06.16	17.8/ 92	63/40	16.37/18.81	23	21:50 hr
07.06.16	18.3/89	70 (same leaf)	18.23/21.28	very close	21:15 hr
25.06.16	20.8/92	52/78	17.73/18.14	30	22:25 hr
06.07.16	21.6/82	77/50	16.85/19.78	60	23:40 hr

Table 3: A comparative chart of different reproductive modes in Bush frogs of India and Sri Lanka.

Species name	SVL (mm)	Clutch size	Days to hatch	Reproductive Mode
<i>Philautus cf. leucorhinus</i>	♀: 33.7♂: 28.9	51	19	20 (Direct development and arboreal)
<i>P. glandulosus</i>	♀: 24.5-26♂: 20-22.9	41	28	20 (Direct development and arboreal)
<i>P. variabilis</i>	♀ & ♂: 30.0 ± 4.5	54-62	-	20 (Direct development and arboreal)
<i>P. nerostagona</i>	♀: no report ♂: 30.1-34	41	20	20 (Direct development and arboreal)
<i>P. tinniens</i>	♀: 25♂: no report	-	-	17 (Direct development on ground)
<i>R. bombayensis</i>	-	26-27	-	20 (Direct development and arboreal)
<i>P. bobingeri</i>	♀: 23.5-26♂: 21.3-24.8	24	18	20 (Direct development and arboreal)
<i>P. graminirupes</i>	♀: 27.3-29.4♂: 21.4-22.6	30-38	24	17 (Direct development on ground)
<i>R. resplendens</i>	♀: 25.2-28.3♂: 22.7-24.5	18-28	-	17 (Direct development on ground)
<i>R. ochlandrae</i>	♀: 23.3♂: 24.0 ± 1.38 (5)	6	-	20 (Direct development and arboreal)
<i>R. chalazodes</i>	♀: 25.2♂: 23.7 ± 2.66 (3)	5-8	-	20 (Direct development and arboreal)
16 species of <i>Pseudophilautus</i> #	-	6-155	24-68	17 (Direct development on ground)
<i>Pseudophilautus femoralis</i>	-	7-22	37-49	20 (Direct development and arboreal)
<i>P. regius</i>	-	17	-	17 (Direct development on ground)
<i>R. shillongensis</i>	♀: 18.47 ± 1.6 (12) ♂: 16.5 ± 1.31 (25)	8-17	30-31 (2)	17 (Direct development on ground)

Time of descending to ground	Time of entering under leaf litters	Time of egg laying completion	Male dislodge time	Time of completion of egg mixing process	Female Leaving time	Clutch size	L/B of clutch (mm)
05:00 hr	07:05 hr	08:00 hr	08:02 hr	08:20 hr	08:23 hr	10	12.58/10.23
05:45 hr	07:35 hr	07:55 hr	07:57 hr	08:10 hr	08:13 hr	12	19.44/16.67
05:15 hr	06:55 hr	07:30 hr	07:31 hr	07:45 hr	07:50 hr	17	19.01/11.51
05:30 hr	07:20 hr	08:10 hr	08:13 hr	08:37 hr	09:05 hr	8	14.51/9.04
04:00 hr	05:15 hr	10:10 hr	10:14 hr	10:25 hr	10:27 hr	17	15.66/13.72

Egg Laying Habitat	Parental Care	Egg diameter (mm)	Study Locality	Data source
Above ground, (10cm) on wet leaves, between rocks	Pair separates after spawning	3.5 ± 0.16 (51)	Karnataka	Gururaja & Ramachandra, 2006
Above ground (1.5-3m) on wet leaves	Pair separates after spawning	4.4 ± 0.2 (48)	Waynaad, Kerala	Biju, 2003
Above ground	Eggs beneath abdomen of female, chasing intruding males	4.1 ± 0.2 (30)	Karnataka	Kanamadi et al., 1996
Above ground (10m) in tree hole (10cm deep)	-	4.5 ± 0.3 (41)	Waynaad, Kerala	Biju & Bossuyt, 2005
On ground	-	-	Nilgiri hills, Tamil Nadu	Bossuyt & Dubois, 2001; Dubois, 1986
Above ground on wet leaves	-	-	Karnataka	Bossuyt et al., 2001
Above ground (4m) on Acacia tree	-	3.9 ± 0.4 (24)	Ponmudi hills, Kerala	Biju & Bossuyt, 2005
On ground, grass clump, rocky crevice	-	4.9 ± 0.5 (38)	Ponmudi hills, Kerala	Daniels, 2005
under moss cover forest floor	Leaves after spawning	4.1 ± 0.4 (24)	Eravikulam National Park, Western Ghats	Biju et al., 2010
Above ground, inside bamboo internode	Male attends the eggs until hatch	4.94 ± 0.06 (developing embryo)	Kerala	Gururaja et al., 2007
Above ground (25cm), inside bamboo internode	Male attends the eggs until hatch	5.73 ± 0.66 (28)	Kalakad Mundanthurai Tiger Reserve, Western Ghats	Seshadri et al., 2014
In soil cavity (1.5-50 cm ³)	Male voluntarily departures, female abandons after concealing the eggs	3.7-5.7	Sri Lanka	Bahir et al., 2005
on underside of leaf above ground (0.3-2m)	after male dislodged female sits on the eggs for 1-3 hrs	-	Sri Lanka	Bahir et al., 2005
Soil cavity	Female burrows the eggs and leaves	3.1 (17)	Sri Lanka	Karunaratna & Amarasinghe, 2007
On moist soil under leaf litters	Male leaves after fertilization and female leaves after egg mixing with soil	3.59 ± 0.63 (47)	Malki Forest Shillong, Meghalaya	Present study