The ovipositional behaviour of the endemic whistling lizard *Calotes liolepis* Boulenger, 1885 (Reptilia: Agamidae) in the Knuckles forest region of Sri Lanka

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Abstract. A mature female *Calotes liolepis* was observed laying eggs on the ground in Manigala in the Knuckles Forest Region of Sri Lanka. This is the first completely described observation of the ovipositioning as well as the captive egg hatching of *Calotes liolepis*. This ovipositional behaviour consisted of the digging of the nest cavity, oviposition, scraping of the soil to bury the eggs, filling of the spaces between the eggs, compression of the soil, and camouflage of the nest. The sizes of three eggs were increased during incubation: day 1 mean = 17.5 mm × 9.2 mm (length × width), and after 70-71 days mean = 21.7 mm × 14.4 mm. Three hatchlings were emerged (mean snout-to-vent length = 29.9 mm; tail length = 58.2 mm; head length = 10.2 mm. Immediate conservation measures are needed for this endemic and threatened lizard, and the observations related to its egg-laying could be useful in planning and implementing suitable conservation methods.

Keywords. Agamidae, Draconinae, *Calotes*, Ovipositional behaviour, Knuckles, Sri Lanka.

INTRODUCTION

Sri Lanka and Western Ghats of India is a biodiversity hotspot, rich in herpetofaunal assemblages because of favorable environmental factors such as high rainfall and humidity, and high density of undergrowth found in this region (Bossuyt et al., 2004; De Silva, 1996; Gunawardene et al., 2007; Karunarathna et al., 2006; Meegaskumbura et al., 2002; Pethiyagoda et al., 2005). There are eighteen species of agamid lizards (Family: Agamidae) in Sri Lanka including three endemic genera and 15 (83%) endemic species (Bahir and Surasingha, 2005; Manamendra-Arachchi et al., 2006; Samarawickrama et al., 2006). The 18 native species belong to the sub-family Draconinae and consists of six genera; *Calotes*, *Ceratophora*, *Cophotis*, *Lyriocephalus*, *Otocryptis* and *Sitana* (De Silva, 2006; Deraniyagala, 1953; Macey et al., 2000). The genus *Calotes* consists seven species and five of them are endemic to Sri Lanka, their are *C. ceylonensis*, *C. desilvai*, *C. liocephalus*, *C. liolepis*, and *C. nigrilabris* (Das and De Silva, 2005; Karunarathna and Amarasinghe, 2008; Manamendra-Arachchi, 1998), and all of them are nationally threatened (IUCN Sri Lanka and MENR 2007). Others non-endemic are the *C. calotes* and *C. versicolor* respectively.

Calotes liolepis has been recorded from only a few widely separated localities restricted to the sub-montane forests, mainly in heavily shaded areas of forest in the wet zone and plantations below 1000 m elevation (Asela et al., 2007; Bahir and Maduwage, 2005; Mana-mendra-Arachchi and Liyanage, 1994). This arboreal species is much slower than other Sri Lanka agamid lizards, even when tree climbing. Its conservation status is Vulnerable (IUCN Sri Lanka and MENR, 2007). This species is largely arboreal and unusual among agamid lizards due to its habit of uttering a high-pitched whistle when alarmed (Das and De Silva, 2005). Average adult snout-to-vent length (SVL) = 72 mm, head length (HL) = 28 mm, tail length (TL) = 190 mm, and axilla-to-groin length (AG) = 37 mm (Deraniyagala, 1953). Recently, the egg laying behaviour of *C. liolepis* has been documented by Asela et al. (2007), but it lacks many details. In this paper we highlight further details of the ovipositioning behaviour in the wild, as well as the captive incubation and hatching of *C. liolepis* eggs.

MATERIALS AND METHODS

The length of the enclosure is 300 mm, width 15 mm and height 100 mm. A thermometer and a hygrometer are used to monitor temperature and relative humidity fluctuations. The base of the unit was filled with soil mixed with sand to a depth of nearly 50 mm. Pieces of stones, sticks and leaf litters were also provided as hides for hatchlings. The relative humidity varied between about 65% and 75% during incubation. These values may be higher than those found in the open in the wild. The surface soil was generally kept dry but occasionally about 100 ml of tap water was sprayed in to the hatching device to maintain a more natural state. The daily temperature varied between about 1 °C and 2 °C. The above 3 eggs were buried half in the soil and covered with leaf litter.

Observations of the lizard were made between 1430 and 1730 h from a distance of 2 m. The animal was disturbed two times to take photographs. When disturbed, it expanded its gular sac and looked around for about 5 min (Fig. 1). The specimen was examined closely and notes on key characteristics were recorded. All measurements were taken to the nearest 0.1 mm using dial calipers. Diagnostic keys in Bahir and Maduwage (2005), Deraniyagala (1953), Manamendra-Arachchi (1990, 1998), Smith (1935) and Taylor (1953) were used for species identification. Plant nomenclature was based on Senaratna (2001) and identified using Ashton et al. (1997).

In vitro incubation

All eggs were removed from the original nest and buried in soil in a glass enclosure that allowed the penetration of light. The incubation container was placed outside the laboratory in a shady environment similar to the original habitat. Humidity inside the tank was kept near 70%. Eggs were not disturbed during incubation and all were visible to the outside so that all observations during hatching could be made without disturbance.



Fig. 1. During the disturbances it expanded its gular sac and looked around.

RESULTS

Study area and habitat

Observations were made approximately 1 km from Ilukkumbura in Manigala in the Knuckles Forest Region (altitude: 800 m) in Matale District, Central Province (7°33'46" N, 80°46'12" E). The ground was covered with 5 cm thick wet leaf litter. The soil was dark and soft with tightly bound particles, and lacked air cavities. There was approximately 75% canopy cover and the undergrowth consisted of shrubs and herbs. The average temperature and humidity were 27 °C and 70%, respectively. On the day of our observations of nesting, the weather was sunny, cloud cover was 6/8, and there was rain prior to the nesting event. The semi-evergreen forests represent the major natural vegetation type in Manigala area. The forest consists of three strata; the canopy (20-25 m), sub-canopy (5-10 m) and ground herbaceous vegetation (Bambaradeniya and Ekanayake, 2003).

These forests harbour many large canopy trees such as *Creteava religiosa*, *Phyllanthus indika*, *Sterculia foetida*, *Bombax ceiba*, *Dimocarpus longan*, *Palaquium hinmolpedda* and *Vitex altissima* species and the sub-canopy level consists of *Breynia vitisidea*, *Miliusa indica*, *Pavetta indica* and *Streblus asper* species; ground cover consists of *Begonia hirtella*, *Carex filicina*, *Carex jakiana*, *Curculio orchioides* and *Procris crenata* species (Ekanayake and Bambaradeniya, 2001). The highland areas of the Knuckles forest range is extremely wet throughout the year, with an average annual rainfall > 4000 mm, though the lower eastern slopes are much drier. Previous surveys have documented eight species of Agamid lizards inhabiting the Knuckles forest reserve (Bambaradeniya and Ekanayake, 2003; Samarawickrema et al., 2006; De Silva et al., 2005; Rajapaksha et al., 2006).

Observations on digging the nest hole

A mature female *Calotes liolepis* (SVL = 79 mm, HL = 25.1 mm, HW = 14 mm, TL = 177 mm, AGL = 51 mm) lying on the ground in the Manigala, about 1 km away from the Ilukkumbura-Manigala Nature Trail, was observed on 5th April 2008 at about 1430 h. First, the lizard looked around for about 30 min. During this time it repeatedly turned its head approximately 180° fifteen times with moving its body. The lizard then changed its body color to approximate the ground color, probably for camouflage. The female then started digging and scraping the soil with its forelimbs, in the process turning its body clockwise and counterclockwise 24 times. It took ~30 min to make the body pit prior to digging the nest hole.

The body pit was 150 mm in diameter. While turning around, the soil was thrown backward using forelimbs and hind limbs. The bottom of the body pit was flat and large enough for the female to lie in while egglaying. After completing the initial pit the lizard started digging the ground with its forelimbs. While digging, it made the margin and inner wall of the nest hole by compressing the soil with the supra-ocular region, snout and anterior half of its lower jaw to avoid collapse. Then it stopped digging and looked around for approximately 5 min while repeatedly turning its head 90° six times without moving its body. It continued to dig the hole for another hour, stopping three more times for 5 min per interval. The hole was vertical, 50 mm deep and 35 mm in diameter. During the rest intervals the tail was coiled inside the hole with the head bent at an angle of 90° to look around.

Laying the eggs

After 30 min of digging, the female turned its body 180° clockwise, placing the posterior part of its body at the opening of the hole and the tail coiled at the outer margin of the hole. It then looked around again. The female laid eggs in the hole without lifting its limbs where as limbs placed at the top-opposite side of the body pit by thrusting them to the front (Fig. 2). The head and breast were lying very closer to the ground during the whole period. Three eggs were laid at a rate of approximately one per three min (Fig. 3). The eggs were pure white and elliptical with a thin pliable shell. Mean length = 17.5 mm and mean width = 9.2 mm (pooling these data with previous observations of 11 eggs, mean = 17.1 mm \times 9.2 mm). After the eggs were laid the female remained motionless for 20 min.

Burying the eggs and camouflaging the nest

Following a period of inactivity, the lizard turned 180° clockwise and moved back into the hole for 10 min to pack and place the eggs below ground level using the anterior part of its lower jaw. It then remained motionless for approximately 15 min. It then began dragging soil towards the hole using its forelimbs. The excavated soil was pulled poste-



Fig. 2. The lizard laying eggs and see tail is curved.



Fig. 3. The three eggs layed by *Calotes liolepis* in Manigala.

riorly using its hind limbs. After filling approximately 1/3 of the hole in about 5 min, it turned 90° counterclockwise and started pressing the soil with the supra-ocular region, tip of the snout and anterior half of its lower jaw for 30 min. A knocking noise was produced as the lizard hit the substrate 28 times with its lower jaw to compress the soil in the hole.

It then filled another 1/3 of the hole in about 5 min, turned 90° counterclockwise again, and started compressing the soil with the anterior half of its lower jaw for 20 min.

Finally, the lizard filled last 1/3 of the hole in about 5 min. Afterward, it turned 90° counterclockwise again and started pressing the soil with the anterior half of its lower jaw for 10 min, after which the hole was filled to ground level. After looking around, it dragged nearby fallen leaves of *Dimocarpus longan* and *Palaquium hinmolpedda* (Family: Sapindaceae and Sapotaceae) over the nest for camouflage. It remained motionless for 5 min and during this time changed its body color to light greenish brown before running towards the forest. At this time it was caught for measurement and then released.

Captive eggs hatching notes

Eggs were removed from the nest cavity and the cavity was examined after the female was released. The bottom of the cavity was conical and the soil soft, dark and wet. The eggs were buried in soil in a screen-topped glass enclosure to incubate. We deposited the eggs in an artificial nest cavity similar in structure to that constructed by the female. The lid of the container was close-fitting to deter predators. After about two days the hatchlings were released to the original habitat.

DISCUSSION

The ovipositional behaviour of this species varies from the ovipositional behaviour of *Calotes versicolor* and *C. liocephalus*. According to Amarasinghe and Karunarathna (2007, 2008), *C. versicolor* places its cloacal aperture over the opening of the hole and lifts its hind limbs while laying its eggs (Fig. 4a), *C. liocephalus* places the posterior part of the body inside the hole while laying eggs (Fig. 4b), and *C. liolepis* places the posterior part of its body at the opening of the hole and the tail was coiled at the outer margin of the hole and then lay eggs to the hole without lifting its hind limbs (Fig. 4c). The egg laying of *C. ceylonensis* is similar to the egg laying of *C. liolepis* but *C. ceylonensis* lifts hind limbs then *C. liolepis* (Pradeep and Amarasinghe, 2009). The *C. versicolor* lifts the anterior part of the body with its forelimbs while turning its head to look around and *C. liocephalus* coils its entire body inside the hole while bending the anterior part of its body to look around but *C. liolepis* coiled the tail inside the hole with the head bent at an angle of 90° to looking around similar to *C. ceylonensis* (Pradeep and Amarasinghe, 2009).

Calotes versicolor makes a knocking noise while packing and placing the eggs in the hole using its lower jaw (Amarasinghe and Karunarathna, 2007) while *C. liocephalus* and *C. ceylonensis* places them softly without making any noise (Amarasinghe and Karunarathna, 2008; Pradeep and Amarasinghe, 2009) and *C. liolepis* did not show any packing behaviour after laying eggs. However it made a knocking noise while compressing soil in the hole during egg laying. Neither *C. versicolor* nor *C. liocephalus* marked the body pit to dig the nest hole as *C. liolepis* and *C. ceylonensis* (Pradeep and Amarasinghe, 2009) and also they did not use hind limbs to pull the soil to the hole. *Calotes versicolor, C. ceylonensis* and *C. liocephalus* threw the soil backward under the body through raised hind limbs whereas *C. liolepis* threw the

soil backwards alongside of its body. *C. liolepis* dug the hole straight inward to the ground while other two species dug the hole into the ground at a 45° angle. We have observed Gray hornbills feed on *C. liolepis* several times. During this ovipositioning we heard a call of gray hornbill but the lizard continued its digging without any change in behaviour.

Asela et al. (2007) observed three egg laying episodes of *C. liolepis* from three different conditions between 1300 h and 1540 h in the month of February. We also made the observation during 1430 h and 1730 h in April. These observations suggest that this species prefers to lay eggs at the afternoon during February-April. Here we compared egg measurements with the earlier observations by Asela et al. (2007). We can conclude that



Fig. 4. a: *Calotes versicolor* places the cloacal aperture over the opening of the hole while laying eggs; **b**: *Calotes liocephalus* places the posterior part of the body inside the hole while laying eggs; **c**: *Calotes liolepis* places the posterior part of its body at the opening of the hole and the tail was coiled at the outer margin while laying eggs (drawings made by A.A. Thasun Amarasinghe).

Ecological Data	Delwala	Rojersongama - 1 Rojersongama - 2		Manigala
Month	February	February	February	April
Time duration	1300-1400	1400-1500	1500-1540	1430-1730
Soil	-	Humus	-	Wet, Dark, Soft
Leaf litter thickness (mm)	40	40	-	50
Canopy Cover (%)	75	-	-	75
Cloud cover	-	5/8	6/8	6/8
Temperature (°C)	27.1	-	-	27.0
Diameter of the body pit (mm)	140	-	-	150
Depth of the hole (mm)	40	48	38	50
Diameter of the hole (mm)	45	33	30	35
No. of eggs laid	2	4	2	3

Table 1. Ecological factors and egg hole description of *Calotes liolepis* at four sites. Delwala, Rojersongama1 and 2, based on Asela et al. (2007).

C. liolepis lays 2-4 eggs during 1300-1730 h in February-April, constructs a hole 30-45 mm diameter (average is 35.75 mm) and hole depth = 38-50 mm (average = 44 mm). The body pit is 140-150 mm diameter (average = 145 mm; Table 1). We conclude that there is substantial behavioural consistency in nesting among female *C. liolepis*.

The *C. liolepis* specimen was observed after egg laying crawling from forest edge to deep forest through dry leaves, which helped it to be camouflaged. The agamid took about 10 min to complete its journey climb the ten-meter *Sterculia foetida* tree. The specimen of *C. liolepis* was very active when captured, and a bright orange skin tone could be seen between gular scales of throat. Additionally, shook its tail slowly and rhythmically. A few diagrams, brief descriptions and notes of *C. liolepis* are available in popular journals, books and magazines but almost nothing exists on the pre- and post-mating behaviour, egg laying, captive breeding or hatchlings. Immediate conservation measures are needed for this endemic and nationally threatened lizard, and the observations related to its egg-laying could be useful in planning and implementing suitable conservation.

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