REPTILES & AMPHIBIANS

Use of Leaf-cutter Ant Fungal Gardens as Nurseries for Eggs of Ashmead's Banded Cat-eyed Snake, *Leptodeira ashmeadii* (Hallowell 1845)

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Leaf-cutter ant nests have a complex and specialized internal structure that allows them not only to maintain stable microenvironmental conditions where they can shelter their colonies, but also to protect themselves from various predators and intruders (Bollazzi and Roces 2002; Diehl-Fleig and Diehl 2007; Velásquez-Múnera et al. 2008). These characteristics allow colonies to have multiple types of interactions with other species, varying from those that can use colony debris to others that present coevolutionary relationships (Mehdiabadi et al. 2006; Mueller et al. 2008; Lugo et al. 2013; Jesovnik et al. 2013). However, information on associations with reptiles is limited and whether any of these cases represent true coevolutionary relationships is not clear. Snakes are known to lay their eggs in nests of ants but whether these are merely cases of females opportunistically exploiting optimal microenvironmental conditions or if they represent a true coevolutionary relationship is unknown (Brandão and Vanzolini 1985; Velásquez-Múnera et al. 2008; Baer et al. 2009).

Ashmead's Banded Cat-eyed Snake (*Leptodeira ashmeadii*) is a nocturnal endemic species in northern South America (Venezuela, northern Colombia, Trinidad, and northern Brazil). This species is found on the ground or in trees near water and feeds mainly on small lizards, frogs, and their eggs (Barrio-Amorós 2019). Few studies address the natural history of the genus *Leptodeira* (Avila and Morais 2007;

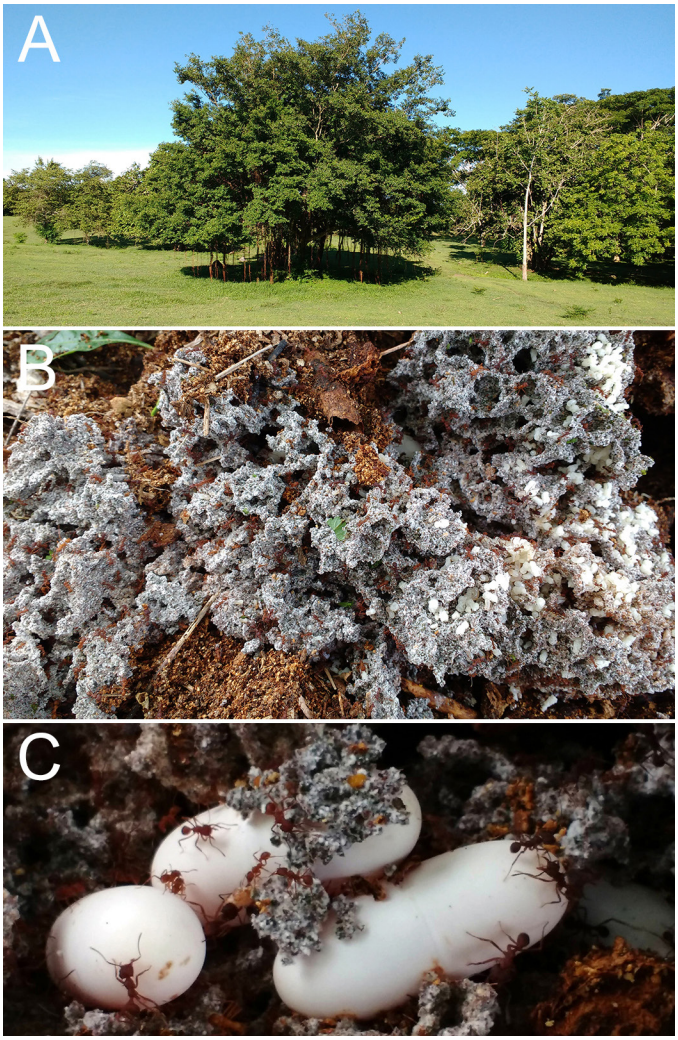

Fig. 1. General view of the habitat of *Leptodeira ashmeadii* from Las Llanadas, Corozal Municipality, Department of Sucre, Colombia (A); fungal garden cultivated by a colony of Leaf-cutter Ants (*Acromyrmex santschii*) (B), and eggs of *L. ashmeaddi* in the fungal garden (C). Photographs by Oscar Sierra-Serrano.

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Mendoza 2008), and much information about species such as *L. ashmeadii* remains unknown. Herein we report the use of leaf-cutter ant fungal gardens as nurseries for eggs of *L. ashmeadii* in the Caribbean region of Colombia and provide some new data on reproduction of this species.

On 18 July 2020, we found a snake nest with six eggs in the Las Llanadas, Corozal Municipality, Department of Sucre, Colombia (9.146583 N, 75.277944 W, 95 m asl; Fig. 1A). The nest was inside a decomposing trunk under a tree in a pasture exposed to the sun, and the eggs were in a cavity with a fungal garden of a leaf-cutter ant colony (Figs. 1B–C). The temperature was 30 °C and the relative humidity was 60%. At the same locality and during the same year, we found another snake nest with four eggs on 7 October about 1.5 m above the ground inside a trunk of a *Gliricidia sepium* used as a living fence. Additionally, on 29 September, we rescued a female snake (583 mm SVL; 801 mm total length) on 21 August and kept it in captivity due to some injuries; it laid six eggs inside the terrarium on 30 September and six more on 9 November. All snakes were identified as *L. ashmeadii* using the criteria of Barrio-Amorós (2019) and Costa et al. (2022); the leaf-cutter ants were identified as *Acromyrmex santschii*, based on Fernández et al. (2015); and the fungus was identified as *Leucoagaricus* cf. *gongylophorus*, following Espinoza et al. (2017) (Fig. 2).

The eggs were white and oval (Table 1). Temperature and humidity at the wild nest sites were consistently 32 °C and 60%, respectively, and hatching occurred five to six weeks after the initial discovery. No aggressive behavior or predation of eggs or hatchlings by ants was observed in the first nest. Eggs obtained in captivity were incubated at the same temperature and relative humidity as the wild nests, but coconut fiber was used as substrate in the container where these were kept, and hatching occurred after seven to 11 weeks of incubation. The hatchlings in nature and those obtained in captivity were measured and weighed (Table 1). The rehabilitated female snake and the juveniles that hatched in captivity were released in a safe place and close to the sites where the wild nests had been found.

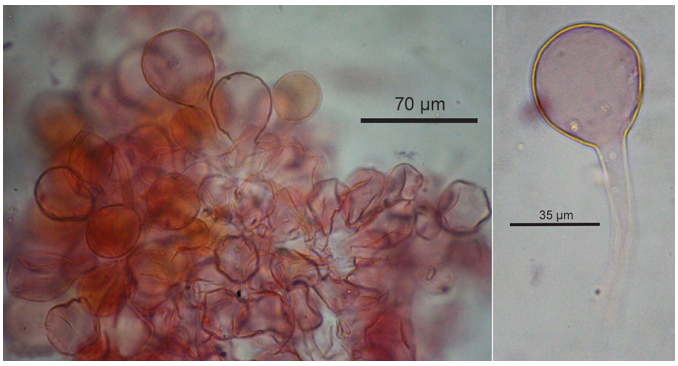

Fig. 2. Gongylidia (30–45 µm) of the fungus, *Leucoagaricus* cf. *gongylophorus*, stained with Congo red and photographed through a 40X objective (left), and an isolated gongylidium photographed through a 40X objective (right). Photographs by Anny L. Marmolejo-Vargas.

The selection of the oviposition site has important repercussions for the success of egg and offspring development (Plummer and Snell 1988; Blouin-Demers et al. 2004; Refsnider 2016). Snakes possibly could lay their eggs in ant fungal gardens to exploit stable temperatures and humidity and ants might provide some protection from predation (Brown and Shine 2004; Baer et al. 2009). Our record, with other observations about the nesting of snakes of the genus *Leptodeira*, confirms the use of ant nests of the Attini tribe as nesting sites (Brandao and Vanzolini 1985; Baer et al. 2009), and the measurements of gongylidia of *L.* cf. *gongylophorus* are similar to those reported in other ant fungal gardens of the Attini tribe (de Fine Licht et al. 2014; Masiulionis et al. 2014; Espinoza et al. 2017). However, we remain uncertain about some relevant aspects, such as the possibility of a biochemical

Table 1. Characteristics of eggs and hatchlings of *Leptodeira ashmeadii* that were monitored from nests in the wild and in captivity from the Las Llanadas village, Corozal municipality, department of Sucre, Colombia.

Characteristics		Wild		Captivity	
		Nest 1	Nest 2	Nest 1	Nest 2
Eggs (n = 22)	Mean Length (mm)	27.28 (± 1.614)	28.78 (± 0.954)	27.08 (± 1.614)	26.80 (± 1.643)
	Mean Width (mm)	12.47 (± 0.307)	12.33 (± 0.690)	14.50 (± 0.682)	16.6 (± 0.631)
Hatching date		20 August 2020	18 November 2020	19 November 2020	26 January 2021
Hatchlings (n = 20)	Mean Length (mm)	158 (± 0.9)	159 (± 1.0)	160 (± 1.0)	158 (± 1.0)
	Mean Body mass (g)	2.03 (± 0.27)	2.53 (± 0.47)	2.53 (± 0.47)	2.40 (± 0.47)

factor that prevents both snakes and eggs from being attacked by ants, whether female *L. ashmeadii* actively select a nest or merely exploit a random encounter, and what kinds of signals might lead a female to select a particular ant nest.

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