Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 532314, 5 pages
doi:10.1155/2012/532314

Research Article

Eggs of the Blind Snake, *Liotyphlops albirostris*, Are Incubated in a Nest of the Lower Fungus-Growing Ant, *Apterostigma* cf. *goniodes*

Gaspar Bruner, Hermógenes Fernández-Marín, Justin C. Touchon, and William T. Wcislo

Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Panama, Panama

Correspondence should be addressed to Hermógenes Fernández-Marín, fernandezh@si.edu
and William T. Wcislo, wcislow@si.edu

Received 15 September 2011; Accepted 25 November 2011

Academic Editor: Diana E. Wheeler

Parental care is rare in most lower vertebrates. By selecting optimal oviposition sites, however, mothers can realize some benefits often associated with parental care. We found three ovoid reptilian eggs within a mature nest of a relatively basal fungus-growing ant, *Apterostigma* cf. *goniodes* (Attini), in central Panama. In laboratory colonies, *A.* cf. *goniodes* workers attended and cared for the eggs. Two blind snakes, *Liotyphlops albirostris* (Anomalepididae), successfully hatched, which is the first rearing record for this species. The ants did not disturb the snakes, and the snakes did not eat the ants; we found no ants in the dissected stomachs of the snakes. We review other associations between nesting fungus-growing ants and egg-laying vertebrates, which together suggest that attine nests may provide a safe, environmentally buffered location for oviposition, even in basal attine taxa with relatively small colony sizes.

1. Introduction

The degree to which organisms are buffered from environmental fluctuations is often reflected in basic life history strategies; at the extremes, some produce large numbers of offspring that suffer extremely high mortality rates, whereas others produce relatively few offspring but are cared for by their parents, thereby decreasing juvenile mortality [1]. For oviparous animals, a mother can realize some benefits normally associated with parental care (e.g., decreased rates of egg mortality), yet not bear the associated costs, by placing her eggs in a nest where heterospecifics tend the eggs, such as brood parasitic bees [2] or birds [3] that lay eggs in nests of other bees or birds, respectively. In some cases, the associations involve distantly related taxa. Among oviparous snakes, parental care is rare, beyond providing the eggs a relatively secure nesting site [4]. There are scattered reports of nesting associations between both amphibians and reptiles with a variety of ant species [5–9], including fungus-growing ants (Attini, Formicidae) [5, 10, 11]. All attines maintain a homeostatic environment (including humidity and temperature) in order to cultivate their fungal symbiont [12, 13], which is a stable environment that snakes exploit [5, 6]. A stable incubation temperature is important for eggs of many species of reptiles, including snakes, and can determine sex and behavior after hatching [14, 15]. Little is known about the nature of potential snake-ant associations, and whether the snakes provide any benefit to the ants or are harmless commensals. Reports thus far indicate that the ants, including soldiers with greatly enlarged mandibles, do not harm the snake eggs [5]. To date all the reported associations between fungus-growing ants and snake eggs are limited to *Atta* and *Acromyrmex* colonies, which contain thousands or millions of workers with large numbers of nest chambers [16], raising the question of whether only large colonies provide suitable abiotic conditions that are sufficiently stable for the development of snake eggs (e.g., [17, 18]). Here, we report finding three snake embryos in a nest of a phylogenetically basal attine ant with relatively small nests, *Apterostigma* cf. *goniodes*. We also provide a review of the associations among attines with both squamates and anurans and briefly sketch possible hypotheses for the origins of these associations.

2. Materials and Methods

A nest of a fungus-growing ant, *A.* cf. *goniodes,* was excavated in central Panamá (Plantation Road, Soberanía National Park, Colon Province, Panamá) in August 2009. *A.* cf. *goniodes* is a small ant (0.4 mm head width, 0.6 mm head length) with mature colonies having more than 1,000 workers (HFM, personal observation). They cultivate a G4 fungus (Family: Pterulaceae), which is phylogenetically distant from lepiotaceous fungus cultivated by *Atta* and *Acromyrmex* (G1 fungus) [17]. The nest contained four chambers with fungus gardens. In one of the four chambers, we found three ovoid snake eggs (3.0 × 0.75 cm, measured along the widest and longest axes) embedded in the fungal garden. The nest contents, including the fungus garden, workers, ant brood, and the snake eggs were collected and placed in a small plastic container with wet cotton inside to maintain humidity. In the laboratory, we maintained the embryos, along with the ant colony, in a Petri dish (6 cm × 2.5 cm), which was placed inside a larger plastic container (19 cm × 16 cm × 7 cm) at a temperature of 25°C, on a light: dark cycle of 12 : 12 hr. The ant nest was fed with corn meal and cleaned twice per week.

To determine if the ants could distinguish snake eggs from a snake-like egg made of a different material, we observed the behavioral responses of ants to natural and artificial snake eggs. We transferred the eggs from the nest to a sterile Petri dish and gently removed the fungal mycelium around the eggs using sterile forceps. We shaped plasticine into a form that mimics a snake egg and sterilized it (115°C for 20 min). Then, we placed one egg, either plasticine or natural, on the top of the fungal garden. Using a stereomicroscope (0.7x), we observed the ants' behavior toward the egg in 10-minute periods for a total of 120-minute observation for each egg. We recorded the frequency at which ants placed a piece of fungal mycelium on the egg (a known hygienic behavior [16]); made antennal contact with the egg; groomed the surface of the egg using the mouth.

We monitored the nest until the snakes hatched. Once hatched, the snakes were maintained for 2 weeks in the ant nest to observe snake-ant interactions with special emphasis on possible agonistic interactions (e.g., did the newly hatched snakes eat the ant workers?). After 18 days, two snakes were found dead in the nest and we stored them in alcohol (95%). These snakes were dissected using a surgical scalpel, and the stomach contents were analyzed for any evidence that they had fed on *A.* cf. *goniodes* workers, brood, or eggs.

Voucher specimens of the ant species is deposited in the Dry Reference Collection of the Smithsonian Tropical Research Institute and the Museo de los Invertebrados, Universidad de Panamá.

3. Results and Discussion

Two of the three embryos hatched nine and 12 days after the nest was collected, and both were identified as *Liotyphlops albirostris* (Figure 1), the white-nosed blind snake (Anomalepididae), which is a relatively basal, Central American endemic snake [22]. These small snakes (adult total length, ~ 223 mm) are fossorial, and little is known about their biology. Furthermore, to our knowledge, there are no documented accounts of their oviposition behavior.

Figure 1: *L. albirostris* egg on a colony of *A.* cf. *goniodes*. Note that the workers have planted pieces of fungal garden on the egg's shell.

Table 1: Behavioral responses (behavior/10 min ±; means ± SD) by *Apterostigma cf. goniodes* workers to natural and artificial eggs. P^* refers to the P value from Wilcoxon signed-rank tests; $n = 12$ observation periods.

	Natural egg	Artificial egg	P^*
Planting cover	2.41 ± 1.37	0 ± 0	$P = 0.019$
Antennal contact	1.66 ± 1.55	0.5 ± 1.16	$P = 0.003$
Grooming	0.66 ± 0.65	0 ± 0	$P = 0.011$

Approximately three days prior to hatching, the first embryo had longitudinal grooves on the egg and amniotic fluid was leaking from the egg. This embryo was removed from the nest and placed in a sterile Petri dish. We cut open the egg shell, and a live, well-developed neonate emerged with the yolk sac still connected. After 48 hours, the connection to the yolk sac was lost. The snake appeared healthy and was placed in the ant nest with the remaining embryos. At a similar age prior to hatching, a second egg appeared to have the same grooves, and, two days, later the snake naturally hatched and was unconnected with its yolk sac.

With respect to the interactions between ants and snake eggs, *A.* cf. *goniodes* workers repeatedly antennated and groomed snake eggs, but were never observed to bite them (Figure 1, Table 1). Moreover, ant workers took fungus garden pieces, with and without substrate, and planted them on the eggs. This behavior is very similar to what the workers do with ant eggs, larvae, and pupae within their fungus garden, as a method for putative control of point

Table 2: General summary of Squamata associated with fungus-growing ants.

Ant species	Vertebrates	Nature of association†	Region
Apterostigma Cf. goniodes	*Liotyphlops albirostris* (A)	This study	Panama
Acromyrmex ambiguous	*Philodryas patagoniensis* (C), *Liophis obtusus* (C)	Oviposition	Uruguay [5]
Acromyrmex echinatior	Unidentified	Oviposition	Panama (H.F.M. pers. comm)
Acromyrmex heyeri	*Philodryas patagoniensis* (C), *Liophis obtusus* (C)	Oviposition	Uruguay [5]
Acromyrmex heyeri	*Pseudoblabes agassizii* (C), *Liophis obtusus* (C),	Oviposition	Uruguay [6]
Acromyrmex hispidus	*Philodryas patagoniensis* (C), *Clelia rustica* (C)	Oviposition	Uruguay [5]
Acromyrmex hispidus	*Philodryas aestivus manegarzoni* (C)	Oviposition	Uruguay [6]
Acromyrmex lobicornis	*Philodryas patagoniensis* (C), *Liophis obtusus* (C), *Micrurus frontalis altirostris* (E), *Leptotyphlops munoai* (L), *Liophis jaegeri* (C), *Pseudoblabes agassizii* (C), *Elapomorphus bilineatus* (C)	Oviposition	Uruguay [5]
Acromyrmex lobicornis	*Liophis obtusus* (C), *Clelia rustica* (C), *Philodryas patagoniensis* (C), *Liophis obtusus* (C), *Pseudoblabes agassizii* (C)	Oviposition	Uruguay [6]
Acromyrmex lundi	*Amphisbaena darwini* (Am)	Oviposition	Uruguay [6]
Acromyrmex octospinosus	*Leptodeira annulata* (C)		Venezuela [19]
Acromyrmex octospinosus	*Stenorrhina degenhardti* (C)	Oviposition	Colombia [10]
Acromyrmex octospinosus	*Tripanurgos compressus* (C)	Burrow	The island of Trinidad [7]
Acromyrmex striatus	*Philodryas patagoniensis* (C), *Teius teyou* (T)	Oviposition	Uruguay [5]
Atta cephalotes	*Amphisbaena alba* (Am)	Burrow and Predator	The island of Trinidad [20]
Atta colombica	*Leptodeira annulata* (C)	Oviposition	Panama [5]
Atta mexicana	*Sympholis* sp. (C)	Burrow	Southern Mexico [20]
Atta sexdens	*Leptodeira* sp. (C)	Oviposition	The island of Trinidad [7]
Atta sexdens	*Amphisbaena alba* (Am) and *Amphisbaena mitchelli* (Am)	Burrow	Brazil [21]
Atta sp. and *Acromyrmex* sp.	*Psuedoboa neuwiedii* (C)	Oviposition	South America [7]

Family (A: Anomalepididae, Am: Amphisbaenidae, C: Colubridae, E: Elapidae, L: Leptodactylidae, T: Teiidae).

†The associations are defined as follows: oviposition: an egg was found inside a colony; burrow: an adult or a young snake was found inside the colony; predator: analyses of intestinal or fecal contents show evidence of prey.

sources of infection [16]. When the mycelial cover of an egg was removed, the ants completely recovered the eggs with fungal garden material (as the snake embryos originally were found) but did not do so with the artificial egg. Ants spent substantially more time physically examining the snake egg than the artificial egg (Table 1), suggesting that the ants were not simply responding to natural eggs as a foreign object.

We have observed adults of *L. albirostris* in nests of other attines, including *Trachymyrmex cornetzi* (one observation in 188 nest excavations), *Trachymyrmex* sp. 10 (two observations in 35 nest excavations), and *Atta cephalotes* (one observation in 12 nests excavations), but no embryos have been observed (H.F.M. and G.B. personal observations). The reproductive biology of *L. albirostris* is unknown, and few comparative data are available for blind snakes in general, so we do not know if this species regularly oviposits in nests of ants or other social insects. Some blind snakes have a specialized diet of ants and termites and have an olfactory system that allows them to detect the pheromone trails of their prey and conspecifics [23, 24], raising the possibility that *L. albirostris* might follow the *A. cf. goniodes* workers into the nest, but again we have no data to indicate the snakes are feeding on the ants. Alternatively, the adult snakes might use the nest as a temporary refuge [25] and occasionally oviposit there.

Large colonies of leafcutter ants appear to provide a suitable environment for oviposition by small vertebrates, such as reptiles and amphibians (Table 2). The most comprehensive reports are from the subtropical temperate zone in Uruguay, where 82 of 577 nests of *Acromyrmex* spp. contained squamate eggs [5, 6] (Table 2). Squamate egg development can take up to 90 days [26], and thus a thermally stable refuge for incubation may be more valuable in temperate regions than in the tropics. Some small subterranean reptiles, including blind snakes, are known to prey on ants [23, 24, 27]. We did not observe, however, any disturbance of the fungus garden or any antagonistic interactions between worker ants and the snakes throughout the posthatching period, despite the fact that *L. albirostris* are presumably large enough to feed on *A.* cf. *goniodes* workers. Thus, the relationship between *L. albirostris* and *A.* cf. *goniodes* is unclear. To date, the benefits seem more apparent for the snake. Nothing is known about the chemical ecology of snake-ant interactions, nor how the snakes might interact with possible ant parasites or agropredators, such as *Gnamptogenys* and *Megalomyrmex* [28, 29], and any possible benefit for the ants is unknown. Another possibility is that the snake egg may provide a temporary hygienic platform on which the ants can cultivate incipient gardens, in the same manner that attines use other found objects in their environment [30]. This study also raises the question of whether some chemical components of the snakes or the egg surface have been modified to mimic the ants' cuticular hydrocarbons used for recognition, and, if so, to what extent does diversification in these chemical signals help explain the diversity of snakes reported in association with fungus-growing ants (Table 2).

Our finding is the first report of a squamate-attine interaction involving a more basal fungus-growing ant species, and the first report of the oviposition behavior for the Central America endemic species *L. albirostris*. Further, we provide the first behavioral observations of squamate-attine interactions, both before and after hatching. Our review demonstrates that at least 20 species of squamates have been reported to oviposit in nests of 13 species of attine ants. These associations are remarkable in part because they occur underground in ant nests, which makes them extremely difficult to locate and observe. Thus, attine nests as oviposition sites for squamate eggs may be substantially more common than previously believed.

Acknowledgments

The authores thank Francisco Pimentel for help in the laboratory, Ulrich Mueller for help with ant identifications, and the Autoridad Nacional del Ambiente (ANAM) for permits. G. Bruner and H. Fernández-Marín were supported by Secretaria Nacional de Ciencia, Tecnología e Innovación (SENACYT) grant, H. Fernández-Marín by a postdoctoral fellowship from STRI, and general research funds from STRI to W. T. Wcislo. J. C. Touchon was supported by the National Science Foundation (DEB-0716923).

References

[1] R. H. MacArthur and E. O. Wilson, *The Theory of Island Biogeography*, Princeton University Press, 1967.

[2] W. T. Wcislo, "The roles of seasonality, host synchrony, and behavior in the evolutions and distributions of nest parasites in Hymenoptera (Insecta), with special reference to bees (Apoidea)," *Biological Reviews of the Cambridge Philosophical Society*, vol. 62, no. 4, pp. 415–443, 1987.

[3] M. Andersson and M. Åhlund, "Host-parasite relatedness shown by protein fingerprinting in a brood parasitic bird," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 24, pp. 13188–13193, 2000.

[4] J. D. Reynolds, N. B. Goodwin, and R. P. Freckleton, "Evolutionary transitions in parental care and live bearing in vertebrates," *Philosophical Transactions of the Royal Society B*, vol. 357, no. 1419, pp. 269–281, 2002.

[5] R. Vaz-Ferreira, L.C. de Zolessi, and F. Achaval, "Oviposición y desarrollo de ofidios y lacertilios en hormigueros de *Acromyrmex*," *Physis*, vol. 29, pp. 431–459, 1970.

[6] R. Vaz-Ferreira, L. C. de Zolessi, and F. Achaval, "Oviposición y desarrollo de ofidios y lacertilios en hormigueros de *Acromyrmex*," *Trabajos del V Congreso Latinoamericano de Zoologia*, vol. 1, pp. 232–244, 1973.

[7] J. Riley, A. F. Stimson, and J. M. Winch, "A review of Squamata oviposition in ant and termite nests," *Herpetological Review*, vol. 16, pp. 38–43, 1985.

[8] A. Schlüter, P. Löttker, and K. Mebert, "Use of an active nest of the leaf cutter ant *Atta cephalotes* (Hymenoptera: Formicidae) as a breeding site of *Lithodytes lineatus* (Anura: Leptodactylidae)," *Herpetology Notes*, vol. 2, no. 1, pp. 101–105, 2009.

[9] A. Schlüter and J. Regos, "Lithodytes lineatus (Schneider, 1799) (Amphibia: Leptodactylidae) as a dweller in nests of the leaf cutting ant *Atta cephalotes* (Linnaeus, 1758) (Hymenoptera: Attini)," *Amphibia-Reptilia*, vol. 2, pp. 117–121, 1981.

[10] E. Velásquez-Múnera, A. Ortiz-Reyes, and V. P. Páez, "Oviposition of *Stenorrhina degenhardti* (Serpentes: Colubridae) in nest of *Acromyrmex octospinosus* (Hymenoptera: Formicidae)," *Actualidades Biológicas*, vol. 30, no. 88, pp. 199–201, 2008.

[11] B. Baer, S. P. A. den Boer, D. J. C. Kronauer, D. R. Nash, and J. J. Boomsma, "Fungus gardens of the leafcutter ant *Atta colombica* function as egg nurseries for the snake *Leptodeira annulata*," *Insectes Sociaux*, vol. 56, no. 3, pp. 289–291, 2009.

[12] M. Bollazzi and F. Roces, "To build or not to build: circulating dry air organizes collective building for climate control in the leaf-cutting ant *Acromyrmex ambiguus*," *Animal Behaviour*, vol. 74, no. 5, pp. 1349–1355, 2007.

[13] U. G. Mueller, A. S. Mikheyev, E. Hong et al., "Evolution of cold-tolerant fungal symbionts permits winter fungiculture by leafcutter ants at the northern frontier of a tropical ant-fungus symbiosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 10, pp. 4053–4056, 2011.

[14] J. Xiang and W.-G. Du, "The effects of thermal and hydric environments on hatching success, embryonic use of energy and hatchling traits in a colubrid snake, *Elaphe carinata*," *Journal of Herpetology*, vol. 35, no. 2, pp. 186–194, 2001.

[15] R. Shine, "Life-history evolution in reptiles," *Annual Review of Ecology, Evolution, and Systematics*, vol. 36, pp. 23–46, 2005.

[16] N. A Weber, *Gardening Ants, the Attines*, American Philosophical Society, Philadelphia, Pa, USA, 1972.

[17] J. K. Webb, G. P. Brown, and R. Shine, "Body size, locomotor speed and antipredator behaviour in a tropical snake (*Tropidonophis mairii*, colubridae): the influence of incubation environments and genetic factors," *Functional Ecology*, vol. 15, no. 5, pp. 561–568, 2001.

[18] J. J. Kolbe and F. J. Janzen, "Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats," *Ecology*, vol. 83, no. 1, pp. 269–281, 2002.

[19] C. R. F. Brandão and P. E. Vanzolini, "Notes on incubatory inquilinism between Squamata (Reptilia) and the neotropical fungus-growing ant genus *Acromyrmex*," *Papéis Avulsos de Zoologia*, vol. 36, no. 3, pp. 31–36, 1985.

[20] J. Riley, J. M. Winch, A. F. Stimson, and R. D. Pope, "The association of *Amphisbaena alba* (Reptilia: Amphisbaenia) with the leaf-cutting ant *Atta cephalotes* in Trinidad," *Journal of Natural History*, vol. 20, no. 2, pp. 459–470, 1986.

[21] C. Azevedo-Ramos and P. R. S. Moutinho, "Amphisbaenians (Reptilia: Amphisbaenidae) in nests of *Atta sexdens* (Hymenoptera: Formicidae) in eastern Amazonia, Brazil," *Entomological News*, vol. 105, no. 3, pp. 183–184, 1994.

[22] O. Rieppel, N. J. Kley, and J. A. Maisano, “Morphology of the skull of the white-nosed blindsnake, *Liotyphlops albirostris* (Scolecophidia: Anomalepididae),” *Journal of Morphology*, vol. 270, no. 5, pp. 536–557, 2009.

[23] F. R. Gehlbach, J. F. Watkins, and J. C. Kroll, “Pheromone trail-following studies of typhlopid, leptotyphlopid, and colubrid snakes,” *Behaviour*, vol. 40, no. 3, pp. 282–294, 1971.

[24] J. F. Watkins, F. R. Gehlbach, and R. S. Baldridge, “Ability of the blind snake, *Leptotyphlops dulcis*, to follow pheromone trails of army ants, *Neivamyrmex nigrescens* and *N. opacithorax*,” *The Southwestern Naturalist*, vol. 12, no. 4, pp. 455–462, 1967.

[25] F. R. Gehlbach and R. S. Baldridge, “Live blind snakes (*Leptotyphlops dulcis*) in eastern screech owl (*Otus asio*) nests: a novel commensalism,” *Oecologia*, vol. 71, no. 4, pp. 560–563, 1987.

[26] R. Shine, “Egg-laying reptiles in cold climates: determinants and consequences of nest temperatures in montane lizards,” *Journal of Evolutionary Biology*, vol. 12, no. 5, pp. 918–926, 1999.

[27] J. F. Watkins, F. R. Gehlbach, and J. C. Kroll, “Attractant-repellent secretions of blind snakes (*Leptotyphlops dulcis*) and their army ant prey (*Neivamyrmex nigrescens*),” *Ecology*, vol. 50, pp. 1098–1102, 1969.

[28] M. B. Dijkstra and J. J. Boomsma, “*Gnamptogenys hartmani* Wheeler (Ponerinae: Ectatommini): an agro-predator of *Trachymyrmex* and *Sericomyrmex* fungus-growing ants,” *Naturwissenschaften*, vol. 90, no. 12, pp. 568–571, 2003.

[29] R. M. M. Adams, U. G. Mueller, T. R. Schultz, and B. Norden, “Agro-predation: uisurpation of attine fungus gardens by *Megalomyrmex* ants,” *Naturwissenschaften*, vol. 87, no. 12, pp. 549–554, 2000.

[30] H. Fernández-Marín, J. K. Zimmerman, and W. T. Wcislo, “Fungus garden platforms improve hygiene during nest establishment in *Acromyrmex* ants (Hymenoptera, Formicidae, Attini),” *Insectes Sociaux*, vol. 54, no. 1, pp. 64–69, 2007.