Green Anole (*Anolis carolinensis*) Eggs Associated with Nest Chambers of the Trap-Jaw Ant *Odontomachus brunneus*

Christina L. Kwapich*

Abstract - Vertebrates occasionally deposit eggs in ant nests, but the subterranean chambers of ponerine ants have not previously been reported as nesting sites for squamates. The current study reports the occurrence of *Anolis carolinensis* (Green Anole) eggs and hatchlings in a nest of the ponerine trap-jaw ant *Odontomachus brunneus*. Hatching rates suggest that *O. brunneus* nests may be used communally by multiple females, which share spatial resources with another recently introduced *Anolis* species in their native range. This opportunistic nesting behavior is placed in the context of known associations between ants and frogs, snakes, and legless worm lizards.

Introduction. Subterranean ant nests are an attractive resource for vertebrates seeking well-defended cavities for their eggs. To access an ant nest, trespassers must work quickly or rely on adaptations that allow them to overcome the strict odor-recognition systems of ants. For example, the ant-associated frog *Lithodytes lineatus* (Schneider) bears a chemical disguise that permits it to mate and deposit eggs deep inside the nests of the leafcutter ant *Atta cephalotes* (L.) without being bitten or harassed. Inside nests, tadpoles enjoy the same physical and behavioral protection as the ants' own brood, in a carefully controlled microclimate (de Lima Barros et al. 2016; Schlüter and Regös 1981, 2005; Schlüter et al. 2009).

Several reptiles in the order Squamata (scaled reptiles) are also reported to deposit eggs in the nests of attine ants. The eggs of *Leptodeira annulate* (L.) (Banded Cat-Eyed Snake) can be found embedded in the subterranean fungus gardens of *Acromyrmex octospinosus* (Reich) and *Atta colombica* Guérin-Méneville (Baer et al. 2009, Brandão et al. 1985), and the eggs of *Liotyphlops albirostris* (Peters) (Whitenose Blind Snake) can be found in the fungus gardens of the ant *Apterostigma goniodes* Lattke (Bruner et al. 2012). Like some other fungus-farming ants, *A. goniodes* workers cover their own eggs, larvae, and pupae with pieces of fungus garden, presumably to protect these brood from microbial infections. The ants also apply fungal coverings to the comparatively large eggs of the Whitenose Blind Snake, which may confer protection from pathogenic microbes (Bruner et al. 2012).

Among vertebrates, the degree to which this nesting behavior is opportunistic or obligate is not well understood. For instance, scattered encounters suggest that the legless worm lizards (Amphisbaenia) *Amphisbaena alba* L. (Red Worm Lizard), *A. mertensi* Strauch (Merten's Worm Lizard), *A. fuliginosa* L. (Speckled Worm Lizard), and *A. kingii* (Bell) (King's Worm Lizard) facultatively deposit their eggs in or near the nests of *Atta* spp. (reviewed by Andrade et al. 2006). *Leposternon microcephalum* Wagler (Smallhead Worm Lizard) eggs have been found in *Camponotus* sp. nests, though fewer associations between lizards and non-fungus-farming ant genera have been reported (Andrade et al. 2006). The current study addresses the relationship between eggs of *Anolis carolinensis* Voigt (Green Anole) (Squamata: Dactyloidae) and the trap-jaw ant *Odontomachus brunneus* (Patton) (Hymentoptera: Formicidae: Ponerinae). Though incidental, this account describes a previously undocumented nesting behavior in Green Anoles of Florida, which must partition

*Department of Biological Sciences, University of Massachusetts Lowell, One University Avenue, Lowell, MA 01854; Christina_Kwapich@uml.edu.

Manuscript Editor: Robert Jetton

spatial resources in the presence of introduced *A. sagrei* Cocteau (Brown Anole; Losos and Spiller 1999).

Methods and Results. I made these observations from May to November of 2012 in the Apalachicola National Forest, 16 km southwest of Tallahassee, FL (30°21'41"N 84°25'08"W). The study site was a mesic hammock that includes large *Quercus virginiana* Mill (Southern Live Oak) and a bed of *Cladonia* sp. lichen on top of a layer of fallen oak leaves, adjacent to a sandhill dominated by *Pinus palustris* Mill (Longleaf Pine) and *Quercus laevis* Walter (Turkey Oak).

On 30 May 2012, I uncovered 8 reptile eggs in the top chambers of an active nest of an *O. brunneus* colony while conducting research on ant behavior. I confirmed ant species identity using the key to *Odontomachus* of the United States by Deyrup and Cover (2004). The nest entrance of the colony was partially occluded by a fallen oak branch and descended vertically into damp sand. Removal of the log exposed interconnected upper chambers that extended 2 to 5 cm below the ground surface before narrowing in to a downward-facing shaft, typical of this species (Cerquera and Tschinkel 2010). The reptile eggs were clean and free of debris. After inspection, I returned them to the nest and suspended further excavation. I used a mouth aspirator to clear sand that had fallen into the exposed chamber area and replaced the branch that protected it. I flagged the nest and visited it frequently over subsequent weeks to check for evidence of hatching or predation by the ants.

On 24 June, 2012, I found 1 hatchling Green Anole inside the nest's upper chambers, near its own eggshell and the remaining 7 eggs. Although the lizard was olive green in color, I determined that it was a Green Anole rather a Brown Anole by the length and shape of its snout and lack of patterning on its back (Köhler 2014). The lizard's leathery eggshell was mostly intact, but had a collapsed appearance and a single, large exit-hole. I photographed the active lizard before releasing it (Fig. 1). There was no evidence that it had been injured by the ants, and the other eggs in the chamber were not damaged, though 1 egg was noticeably darker in color. Eight days later, 2 additional eggs were absent, and 1 additional eggshell was visible, in the same condition as the first. The other eggs were unchanged. Further monitoring of the eggs was not possible due to inclement weather and other logistical constraints.

During the observation period, the ant colony foraged actively, and the ants did not abandon or move their nest's location. Though mostly nocturnal, they were easily coaxed out of the nest entrance during the day by placing shortbread cookie crumbs a short distance away. Close inspection of the nest entrance with a flashlight revealed several ants standing in the entrance chamber shared with the Green Anole eggs.

In November 2012, I excavated the *O. brunneus* nest fully, collected its inhabitants, and censused them in the laboratory. To excavate the colony, I dug a large pit adjacent to the nest, and revealed its chambers, centimeter-by-centimeter, with a brick trowel while inside the pit (per Kwapich and Tschinkel 2016). The nest was 120 cm deep and included 61 workers, 1 queen, and no brood. No additional evidence of lizards or lizard eggs were found inside, and it is likely that eggshells were discarded outside the nest along with the ants' normal food waste. In the spring, I returned the ant colony to its original territory as suggested by the accepted ASAB/ABS guidelines for the treatment of animals in behavioral research and teaching.

Discussion. Green Anoles are monoallochronic and produce eggs by alternating the use of their ovaries; consequently, mothers lay 1 egg each week during the breeding period rather than laying a large clutch all at once (Smith et al. 1972, Toda et al. 2013). The eggs I observed within the *O. brunneus* nest hatched on separate days, indicating that the nest was either revisited by the same female multiple times or was visited by several

Figure 1. Top: A Green Anole hatchling and eggs recovered from a nest of the trap-jaw ant *Odontomachus brunneus*. Bottom: The hatchling was released on a fallen branch bordering the focal ant nest.

females, as is common among other communal-nesting *Anolis* species (Rand 1967), and has been observed in Green Anoles living in cypress swamps with limited nesting sites (Godfrey et al 2019). Green Anoles do not guard their eggs, and incubation takes 29 to 38 days, depending on temperature (Tiatragul et al. 2019). Given the minimum incubation time, and based on the latency between discovery and hatching, the eggs described in this paper were at least 4 days old when they were discovered. Female Green Anoles deposit eggs in predefined cavities or dig shallow nesting holes and then use their snouts to roll the eggs into a suitable position, before pushing them deeper into cavities (Propper et al. 1991), which may explain why the observed eggs appeared along a 5-cm length of connected chambers in the ant nest.

Odontmachus brunneus are large-bodied ants that form small colonies. Nest depth is highly seasonal, such that colonies deepen their nests prior to overwintering (Hart and Tschinkel 2012). Nests may include 17 chambers spread along a vertical shaft that extends up to 184 cm below the surface, with as many as 177 workers and 340 cm^2 of chamber floor space (Cerquera and Tschinkel 2010). Nest chambers are up to 10 cm long and occasionally intersect rodent burrows and root cavities. Casts of these aberrant nests have clear lateral projections that were not observed in the current study (Cerquera and Tschinkel 2010). Future studies of egg-laying behavior in Green Anoles should consider the possibility that ant nests are suitable nesting sites for these lizards. Although subterranean ant nests may extend several meters below the ground surface, nest entrances can be cryptic. The presence of discarded insect parts, caterpillar frass, small boluses of mined soil, and other debris may indicate that a cavity housing lizard eggs is also an ant nest, even if the ants themselves are not visible or active during daylight hours.

Although *O. brunneus* workers retreat into their nests when harassed, they bear a powerful sting and are endowed with large mandibles that are held perpendicular to the head while hunting outside the nest. When triggered, the mandibles snap shut on prey, or on the ground surface as a mechanism of escape (Gronenberg et al. 1993, Larabee and Suarez 2015). These exaggerated mandibles are suited to prey capture, nest excavation, and brood care (Cerquera and Tschinkel 2010). It is unknown whether they possess utility for cutting open leathery reptile eggs, but the apparent lack of disturbance of by *O. brunneus* in this study suggests either they are unable or uninterested, or perhaps that the 25-mm eggs are too large and bulbous for the 7.5-mm ants to manipulate. The more surprising observation is the ants' apparent tolerance for a soft-bodied anole hatchling, though further observations are needed

Odontomachus brunneus use a specific blend of cuticular hydrocarbons to discern nestmates from non-nestmates. The degree of dissimilarity from the colony odor corresponds with worker aggression towards non-nestmates and nestmates with artificially manipulated odor profiles within and between populations (Smith et al. 2012, 2013). Newly hatched anoles inside *O. brunneus* nests may avoid conflict with workers during the daytime, as *O. brunneus* are largely nocturnal.

The use of *O. brunneus* nests by female Green Anoles is likely opportunistic or highly seasonal, as I have not encountered nor have others reported accounts of anole eggs during extensive nest excavations of this or other ant species in the same forest and habitat. On an evolutionary scale, opportunistic use of ant nest architecture likely precedes the preference for ant nests exhibited by more regular vertebrate associates of ants. The behavioral transition to nest parasitism by myrmecophilous aleochorine beetles is thought to have followed an analogous stepwise progression, beginning with exploitation of external ant-foraging trails and trash dumps in superficial nest chambers (Hölldobler and Kwapich 2019, Hölldobler et al. 2018).

Acknowledgments. This work was conducted under US Forest Service permit number APA583. C.L. Kwapich was supported through a research assistantship from National Science Foundation grant number IOS-1021632, awarded to Walter R. Tschinkel.

Literature Cited

Andrade, D., L. Nascimento, and A. Abe. 2006. Habits hidden underground: A review on the reproduction of the Amphisbaenia with notes on four neotropical species. Amphibia–Reptilia 27:207–217.

Baer, B, S.P.A. Den Boer, D. Kronauer, D.R. Nash, and J.J. Boomsma. 2009. Fungus gardens of the leafcutter ant *Atta colombica* function as egg nurseries for the snake *Leptodeira annulata*. Insectes Sociaux 56:289–291.

Brandão, C.R.F., P.E. Vanzolini, and P. Vanzolini. 1985. Notes on incubatory inquilinism between Squamata (Reptilia) and the neotropical fungus-growing ant genus *Acromyrmex* (Hymenoptera: Formicidae). Papéis Avulsos de Zoologia 36:31–36.

Bruner, G., H. Fernández-Marín, J.C. Touchon, and W.T. Wcislo. 2012. Eggs of the Blind Snake, *Liotyphlops albirostris*, are incubated in a nest of the Lower Fungus-growing Ant, *Apterostigma* cf. *goniodes*. Psyche 2012:532314.

Cerquera, L.M., and W.R. Tschinkel. 2010. The nest architecture of the ant *Odontomachus brunneus*. Journal of Insect Science 10:64.

de Lima Barros, A., J.L. López-Lozano, and A.P. Lima. 2016. The frog *Lithodytes lineatus* (Anura: Leptodactylidae) uses chemical recognition to live in colonies of leaf-cutting ants of the genus *Atta* (Hymenoptera: Formicidae). Behavioral Ecology and Sociobiology 70:2195–2201.

Deyrup, M., and S. Cover. 2004. A new species of *Odontomachus* ant (Hymenoptera: Formicidae) from inland ridges of Florida, with a key to *Odontomachus* of the United State. Florida Entomologist 87:136–144.

Godfrey S.T., J.A. Duberstein, J. Mota, and W. Moore 2019. *Anolis carolinensis* (Green Anole) nest sites and communal nesting. Herpetological Review 49:115.

Gronenberg, W., J. Tautz, and B. Hölldobler. 1993. Fast trap jaws and giant neurons in the ant *Odontomachus*. Science 5133:561–563.

Hölldobler, B., and C.L. Kwapich. 2019. Behavior and exocrine glands in the myrmecophilous beetle *Dinarda dentata* (Gravenhorst, 1806)(Coleoptera: Staphylinidae: Aleocharinae). PLoS One 14:e0210524.

Hölldobler, B., C.L. Kwapich, and K.L. Haight. 2018. Behavior and exocrine glands in the myrmecophilous beetle *Lomechusoides strumosus* (Fabricius, 1775)(formerly called *Lomechusa strumosa*)(Coleoptera: Staphylinidae: Aleocharinae). PLoS One 13:e0200309.

Köhler, G. 2014. Characters of external morphology used in *Anolis* taxonomy: Definition of terms, advice on usage, and illustrated examples. Zootaxa 3774:201–257.

Kwapich, C.L., and W.R. Tschinkel. 2016. Limited flexibility and unusual longevity shape forager allocation in the Florida Harvester Ant (*Pogonomyrmex badius*). Behavioral Ecology and Sociobiology 70:221–235.

Larabee, F.J., and A.V. Suarez. 2015. Mandible-powered escape jumps in trap-jaw ants increase survival rates during predator–prey encounters. PloS One 10:e0124871.

Losos, J.B., and D.A. Spiller. 1999. Differential colonization success and asymmetrical interactions between two lizard species. Ecology 80:252–258.

Propper, C.R., R.E. Jones, M.S. Rand, and H. Austin. 1991. Nesting behavior of the lizard *Anolis carolinensis*. Journal of Herpetology 25:484.

Rand, A. 1967. Communal egg laying in anoline lizards. Herpetologica 23:227–230.

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

Schlüter, A., and J. Regös. 2005. In der Höhle des Löwen – *Lithodytes lineatus*. Amphibia 4:23–27.

Schlüter, A., P. Löttker, and K. Mebert. 2009. 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 2:101–105.

Smith, A. A., J.G. Millar, L.M. Hanks, and A.V. Suarez. 2012. Experimenta levidence that workers recognize reproductives through cuticular hydrocarbons in the ant *Odontomachus brunneus*. Behavioral Ecology Sociobiology 66:1267–1276.

Smith, A. A., J.G. Millar, L.M. Hanks, and A.V. Suarez. 2013. A conserved fertility signal despite population variation in the cuticular chemical profile of the trap-jaw ant *Odontomachus brunneus*. Journal of Experimental Biology 216:3917–3924.

Smith, H., G. Sinelnik, J. Fawcett, and R. Jones. 1972. A survey of the chronology of ovulation in anoline lizard genera. Transactions of the Kansas Academy of Science 75:107–120.

Tiatragul, S., J.M. Hall, N.G. Pavlik, and D.A. Warner. 2019. Lizard nest environments differ between suburban and forest habitats. Biological Journal of the Linnean Society 126:92–403.

Toda, M., N. Komatsu, H.Takahashi, N. Nakagawa, and N. Sukigara 2013. Fecundity in captivity of the Green Anoles, *Anolis carolinensis*, established on the Ogasawara Islands. Current Herpetology 32:82–88.