

## Correspondence

### Oviposition of the snake *Thelotornis kirtlandii* in a parabiotic ant nest

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Snakes are ectothermic organisms, hence thermoregulation plays a pivotal role in their activity. Behavioural components of thermoregulation – e.g., actively looking for a basking site – enable snakes to maintain sufficiently high and balanced body temperatures throughout their active periods. Unfavourable weather conditions are responded to with inactivity, e.g., hibernation or aestivation. However, such behavioural thermoregulation is certainly not possible at early ontogenetic stages. Snake embryos (either eggs of oviparous species or developing embryos within the female's body in viviparous species) are exposed to ambient temperatures to a greater extent than the fully formed snake in most species. Nevertheless, females can actively choose appropriate incubation sites to lay their eggs. An optimal site is characterized by sufficiently high temperatures and humidity and ambient moisture levels without sudden and extreme changes, and is well protected or hidden from potential predators (PACKARD & PACKARD 1988, RESETARITS 1996). Consequently, many snakes use holes in the ground, former rodent burrows, or decaying substrates that will produce heat for the incubation of the developing eggs. Finding an appropriate site may have two consequences: 1) the recurrent use of an oviposition site by the same female or conspecific females over multiple reproduction seasons (e.g., BROWN & SHINE 2005), and 2) a communal use of an oviposition site by conspecific (e.g., COOK 1964, COVACEVICH & LIMPUS 1972) or allo-specific females (e.g. BRODIE et al. 1969). For example, both strategies are applied by the European whip snake, *Hierophis viridiflavus* (FILIPPI et al. 2007).

A particular case is when snakes lay their eggs in nests of termites or ants. These nests are known as constructions

that apparently offer the insects optimal microclimatic conditions and these are likewise conducive to incubating snake eggs. Myrmecophilic and termitophilic associations of reptiles including snakes have repeatedly been reported, especially from Central and South America (e.g., SCHERBA 1965, BRANDÃO & VANZOLINI 1985, BRUNER et al. 2012). Interestingly, lizards were more often associated with nests of termites (THEOBALD 1868, KNAPP & OWENS 2008), whereas snakes apparently used those of ants more frequently (RILEY et al. 1985). RILEY et al. (1985) listed 17 snake species laying their eggs in nests of termites and ants; mostly, these were tropical, ground-dwelling and arboreal ones; e.g., species of *Boaedon*, *Boiga*, *Chironius*, *Micrurus*, and some scolecophidians. Ant nests or termitaria may also be used as hibernacula by snakes (e.g., PISANI 2009). Here, we report the discovery of a new association between a snake and ants from western Africa.

The site of our study was in the Lamto Scientific Reserve in Côte d'Ivoire ( $6^{\circ}12.834' N$ ,  $5^{\circ}1.485' W$ , altitude between 75 and 175 m a.s.l.). Annual rainfall averages 1,210 mm here. A long rainy season extends from February through November, usually interrupted by a short dry season in August. The temperature is fairly constant throughout the year, with an annual mean temperature of around  $27^{\circ}C$  (ABBADIE et al. 2006).

The habitat of our observation was a gallery forest bordering the Bandama River, the main watercourse of the reserve. The local tree flora is dominated by *Croton scariosus*, *Pterocarpus santalinoides*, *Cola laurifolia*, *Cynometra megalophylla*, *Manilkara obovata*, and *Parinari congensis* (DEVINEAU 1975).

*Platythyrea conradti* (Formicidae: Ponerinae) and *Strumigenys maynei* (Formicidae: Myrmicinae) are two ant species that live in parabiosis (i.e., they nest together; also depicted in YEO et al. 2006) and are commonly found in this habitat of Lamto. The tiny *S. maynei* scavenges on prey remains of the large and aggressive *P. conradti*, which may in this manner benefit from improved nest hygiene (YEO et al. 2006). The nest is also inhabited by numerous other inquines, such as springtails, spiders, mites, silverfish, collembolans, and rove beetles (see Supplementary video). The nest of these ants is arboreal, typically found 0.5–2 m above the ground in large hollow branches of live trees (Fig. 1A). The diameter of the nest opening is variable depending on the host tree size (usually *Pancovia bijuga*, Sapindaceae), but may be enormous proportionally to the ants, ranging from 5 to ca 20 cm. In the dry season, the entrance will usually be entirely plugged with soil and organic debris deposited by the ants to protect their nest. However, the nest entrance will be conspicuous in the rainy season.

On 21 December 2014, two pairs of snake eggs of unknown identity were found in two parabiotic nests of *P. conradti* and *S. maynei*. The eggs of both clutches were whitish, elongate, and loosely attached to each other. They had been deposited within an inner crevice of the branch, directly

onto the wood (Figs 1B and 1C), 10–15 cm deep in the nest. Eggs of the first clutch were accidentally destroyed during our ant sampling, but the second pair was carefully recovered from the nest and transported to our vivarium facility.

For incubating the snake eggs (40 × 14 and 43 × 15 mm in size, respectively), we used an organic substrate. This consisted of ½ in volume of the original substrate from the ant nest and ⅓ in volume of leaves and branches of oak and beech composted by sun beetle larvae (*Pachnoda* sp.). The development of the eggs was visually checked at regular intervals using a cold light source. Temperature was maintained at between 29 and 31°C, and relative humidity was kept very high and the substrate moist but not wet. Both eggs hatched on 14 January 2015, 24 days after collection, and we were able to identify the snakes as *Thelotornis kirtlandii* (Squamata: Serpentes: Colubridae, Fig. 1D). This is an arboreal and mostly diurnal, back-fanged colubrid species, mainly distributed in the lowland tropical forests of Africa (BROADLEY 2001). The hatchlings had total lengths of approximately 28–30 cm and moulted for the first time on 23 and 24 January 2015, respectively. Tissue samples were taken of the shed skin of both specimens, and the standard DNA barcoding marker was sequenced following the protocols of NAGY et al. (2012). This confirmed our

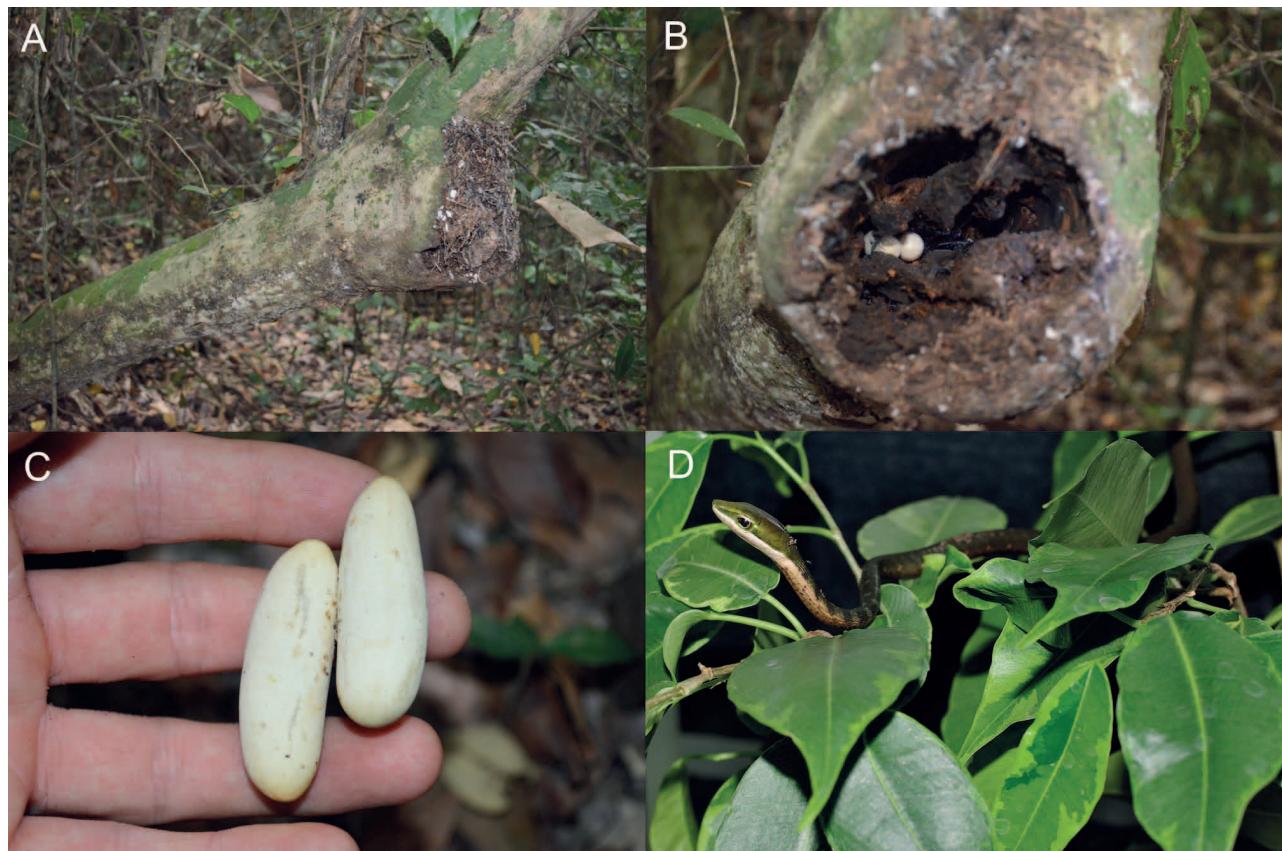


Figure 1. Oviposition of *Thelotornis kirtlandii* in a parabiotic ant nest in the Lamto Scientific Reserve, Ivory Coast. (A) Arboreal nest of the ants *Platythyrea conradti* and *Strumigenys maynei*; the entrance is plugged with soil and vegetal debris. (B) Nest opening cleared of debris; the two eggs of *T. kirtlandii* and some workers of *P. conradti* are visible. (C) *T. kirtlandii* eggs on 21 December 2014. (D) One of the two *T. kirtlandii* hatchlings on 15 January 2015. Photos: T. DELSINNE (A, B, C) and F. DE BLOCK (D).

identification based on their morphology: the COI haplotypes of the two hatchlings were identical, thus only one of them has been uploaded to GenBank (KT818832), and the sequences were closely similar to conspecific sequences of *T. kirtlandii* from western regions of the Democratic Republic of the Congo (NAGY et al. unpublished). The specimens are currently kept in captivity and will be deposited in the herpetological collections of the RBINS after their natural death.

The presence of snake eggs in parabiotic nests of *P. conradti* and *S. maynei* was previously observed on several occasions (K. YEO pers. obs.), suggesting that this association is a regular occurrence. To our knowledge, it is the first report of the use of a ponerine ant nest as an oviposition site of a snake species.

Following RILEY et al. (1985), such choice for oviposition brings a number of advantages: 1) relatively stable temperature and humidity values, and 2) protection from predators and fungal infections. The downsides of this temporal co-existence may be 1) the difficulty for the female snake to enter a nest, especially in the dry season, and 2) for the juveniles to exit it after hatching. First, they apparently will have to dig through the 1–5 cm plug of debris that blocks the nest entrance. Second, ant bites, especially those of *Platythyrea* may pose a serious risk to freshly hatched snakes, therefore a rapid escape is probably needed. To avoid the latter problem, at least by amphibians, use of an active chemical camouflage is known (RÖDEL et al. 2013; they worked in the same reserve). It was also hypothesized for snakes that some form of "chemical insignificance" helps eggs to remain ignored (BAER et al. 2009). Workers of *P. conradti* were observed walking on the eggs, apparently ignoring them, which suggests that eggs of *T. kirtlandii* may indeed be chemically camouflaged. Further studies are needed to confirm this observation and find out whether such a mechanism is present in hatchlings and/or adult snakes. Furthermore, the skin of freshly hatched snakes is moist and sticky during the first hours and therefore often covered by some substrate that may render it chemically camouflaged. Indeed, our two hatchling snakes were all but completely (except the heads) coated with substrate for about an hour after hatching. This may protect them from bites of ants.

A normal clutch of *Thelotornis kirtlandii* is likely to comprise more than two eggs; the clutch size of the congeneric *T. capensis* is 4–7 eggs (SHINE et al. 1996). This means that females of *T. kirtlandii* probably distribute their eggs over different ant nests. Consequently, they have to find nests and surmount ant defences several times. It has been demonstrated that the inquiline *Amphisbaena alba* detects and follows pheromone trails of its ant host, *Atta laevigata* (RILEY et al. 1986, CAMPOS et al. 2014). It would be interesting to investigate if *T. kirtlandii* may also identify ant pheromone of *P. conradti* and (or) *S. maynei*, or whether this snake finds nests by active visual search or by chance. Foraging activity of *P. conradti* is much reduced during the dry season (YEO et al. 2006), and it is mostly active at dawn during the rainy season (DEJEAN 2011). At present, we have

no information whether the oviposition of *T. kirtlandii* (or other snakes) is related to the seasonal activity of ants. So far, eggs were mainly found during the dry season (K. YEO pers. obs., this study), hence lower ant activity might facilitate the nest use by snakes.

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### References

- ABBADIE, L., J. GIGNOUX, X. LE ROUX & M. LEPAGE (2006): Lamto. Structure, Functioning, and Dynamics of a Savanna Ecosystem. *Ecological Studies*, **179**. – Springer, New York, NY.
- BAER, B., S. P. A. DEN BOER, D. J. C. KRONAUER, D. R. NASH & 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 (1985): Notes on incubatory inquilinism between Squamata (Reptilia) and the Neotropical fungus-growing ant genus *Acromyrmex* (Hymenoptera: Formicidae). – *Papéis Avulsos de Zoologia, Museu de Zoologia da Universidade de São Paulo*, **36**(3): 31–36.
- BRODIE, E. D. J., R. A. NUSSBAUM & R. M. STORM (1969): An egg-laying aggregation of five species of Oregon reptiles. – *Herpetologica*, **25**: 223–227.
- BROADLEY, D. G. (2001): A review of the genus *Thelotornis* A. Smith in eastern Africa, with the description of a new species from the Usambara Mountains (Serpentes: Colubridae: Diapholidini). – *African Journal of Herpetology*, **50**, 53–70.
- BROWN, G. P. & R. SHINE (2005): Nesting snakes (*Tropidonophis mairii*, Colubridae) selectively oviposit in sites that provide evidence of previous successful hatching. – *Canadian Journal of Zoology*, **83**: 1134–1137.
- BRUNER, G., H. FERNANDEZ-MARIN, J. C. TOUCHON & 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*, Article ID 532314, 5 pages. DOI:10.1155/2012/532314.
- CAMPOS, V. A., W. DÁTTILO, F. H. ODA, L. E. PIROSELI & A. DARTORA (2014): Detection and use of foraging trails of the leaf-cutting ant *Atta laevigata* (Hymenoptera: Formicidae) by *Amphisbaena alba* (Reptilia: Squamata). – *Acta Zoológica Mexicana* (n.s.), **30**: 403–407.
- COOK, F. R. (1964): Communal nesting in the smooth green snake. – *Herpetologica*, **20**: 206.
- COVACEVICH, J. & C. LIMPUS (1972): Observations on community egg-laying by the yellow-faced whip snake, *Demansia psammophis* (Schlegel) 1837 (Squamata: Elapidae). – *Herpetologica*, **28**: 208–210.
- DEJEAN, A. (2011): Prey capture behavior in an arboreal African ponerine ant. – *Public Library of Science, ONE*, **6**: e19837. DOI:10.1371/journal.pone.0019837.

DEVINEAU, J.-L. (1975): Etude quantitative des forêts-galeries de Lamto (Moyenne Côte d'Ivoire). – PhD thesis, Université Paris 6, 190 pp. – Available at [https://tel.archives-ouvertes.fr/file/index/docid/363065/filename/THESE\\_3emeCycle\\_1975.pdf](https://tel.archives-ouvertes.fr/file/index/docid/363065/filename/THESE_3emeCycle_1975.pdf).

FILIPPI, E., C. ANIBALDI, D. CAPIZZI, A. CECCARELLI, M. CAPU-  
LA & L. LUISELLI (2007): Long-term fidelity to communal ovi-  
position sites in *Hierophis viridiflavus*. – Herpetological Jour-  
nal, **17**: 7–13.

KNAPP, C. R. & A. K. OWENS (2008): Nesting behavior and the  
use of Termitaria by the Andros iguana (*Cyclura cychlura cy-  
chlura*). – Journal of Herpetology, **42**: 46–53.

NAGY, Z. T., G. SONET, F. GLAW & M. VENCES (2012): First large-  
scale DNA barcoding assessment of reptiles in the biodiversity  
hotspot of Madagascar, based on newly designed COI prim-  
ers. – Public Library of Science, ONE, **7**: e34506. DOI: 10.1371/jour-  
nal.pone.0034506.

PACKARD, G. C. & M. J. PACKARD (1988): The physiological ecolo-  
gy of reptilian eggs and embryos. – pp. 523–606 in: GANS, C.  
& R. B. HUEY (eds): Biology of the Reptilia, Vol. 16. Ecology  
B, Defense and Life History. – C. Alan R. Liss Inc., New York.

PISANI, G. R. (2009): Use of an active ant nest as a hibernaculum  
by small snake species. – Transactions of the Kansas Academy  
of Science, **112**: 113–118.

RESETARITS, W. J. JR (1996): Oviposition site choice and life his-  
tory evolution. – American Zoologist, **36**: 205–215.

RILEY, J., A. F. STIMSON & J. M. WINCH (1985): A review of Squa-  
mata ovipositing in ant and termite nests. – Herpetological  
Review, **16**: 38–43.

RILEY, J., J. M. WINCH, A. F. STIMSON & R. D. POPE (1986): The as-  
sociation of *Amphisbaena alba* (Reptilia: Amphisbaenia) with  
the leaf-cutting ant *Atta cephalotes* in Trinidad. – Journal of  
Natural History, **20**: 459–470.

RÖDEL, M.-O., C. BREDE, M. HIRSCHFELD, T. SCHMITT, P. FA-  
VREAU, R. STÖCKLIN, C. WUNDER & D. MEBS (2013): Chemical  
camouflage – a frog's strategy to co-exist with aggressive ants.  
– Public Library of Science, ONE, **8**: e81950. DOI:10.1371/jour-  
nal.pone.0081950.

SCHERBA, G. (1965): Observations on *Microtus* nesting in ant  
mounds. – Psyche, **72**: 127–132.

SHINE R., P. S. HARLOW, W. R. BRANCH & J. K. WEBB (1996): Life  
on the lowest branch: sexual dimorphism, diet, and repro-  
ductive biology of an African twig snake, *Thelotornis capensis*  
(Serpentes, Colubridae). – Copeia, **1996(2)**: 290–299.

THEOBALD, W. (1868): Catalogue of the reptiles of British Burma  
embracing the provinces of Pegu, Martaban, and Tenasserim;  
with descriptions of new or little-known species. – Journal of  
the Linnean Society London (Zoology), **10**: 3–67.

YEO, K., M. MOLET & C. PEETERS (2006): When David and Go-  
liath share a home: Compound nesting of *Pyramica* and  
*Platythyrea* ants. – Insectes Sociaux, **53**: 435–438.

### Supplementary material

Supplementary video. Video sequence showing the parabiotic ant  
nest of *Platythyrea conradti* and *Strumigenys maynei* with two  
eggs of *Thelotornis kirtlandii*. Available at [http://www.salamandra-journal.com/images/stories/Nagy\\_et\\_al-0879-Nest\\_4-Parabiosis.MTS](http://www.salamandra-journal.com/images/stories/Nagy_et_al-0879-Nest_4-Parabiosis.MTS)