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Oviposition of the snake *Thelotornis kirtlandii* in a parabiogenic 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 allospecific 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°12.834' N, 5°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°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 scaricius*, *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 inquilines, 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 1/3 in volume of the original substrate from the ant nest and 2/3 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

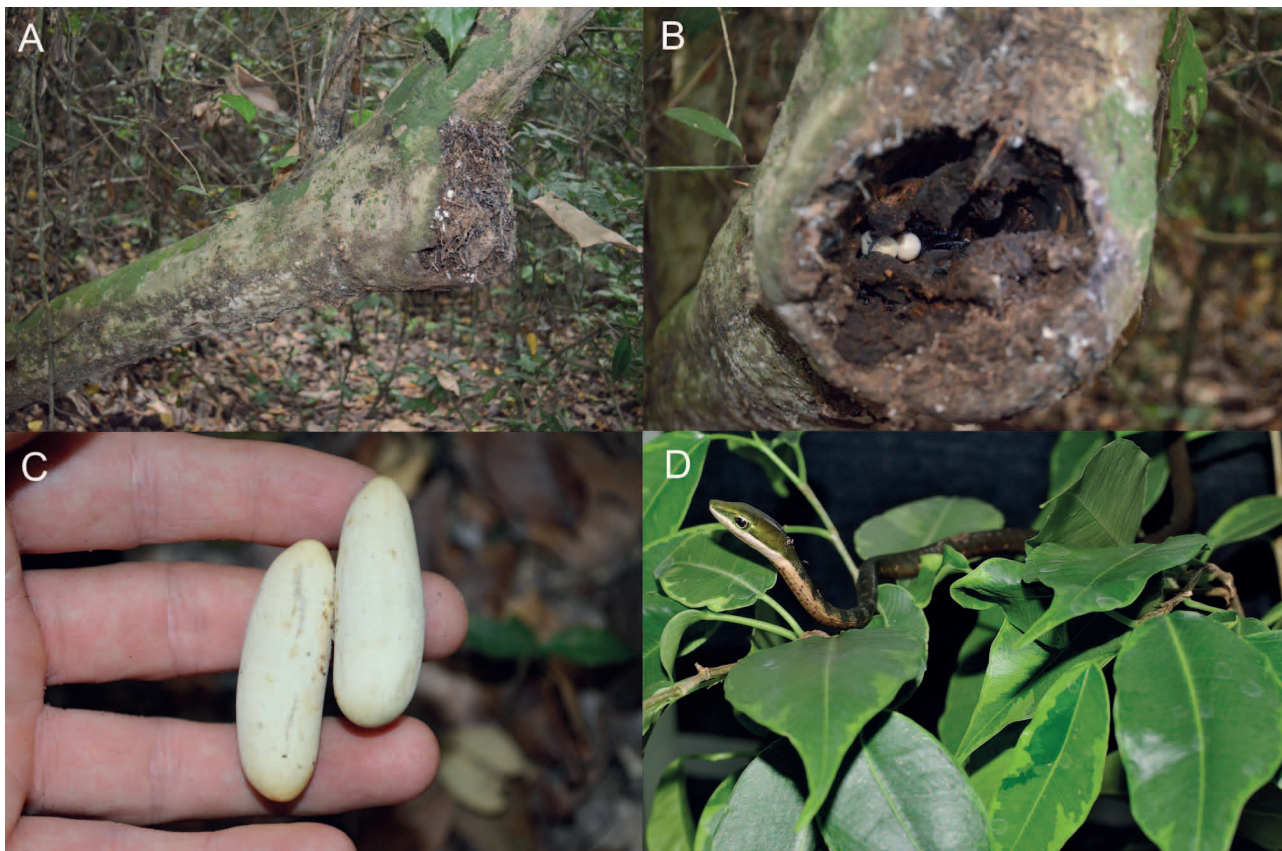


Figure 1. Oviposition of *Thelotornis kirtlandii* in a parabiotic ant nest in the Lamto Scientific Reserve, Ivory Coast. (A) Arboreal nest of the ants *Platythyrea conradti* and *Strumigenys maynei*; the entrance is plugged with soil and vegetal debris. (B) Nest opening cleared of debris; the two eggs 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 parabiocotic 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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Supplementary material

Supplementary video. Video sequence showing the parabioc 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