

## Scientific Note

# Ant nests found in mature pods of *Cassia fistula* L. and the extended phenotype of herbivores as house to opportunistic twig dwellers

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**Abstract.** Ants can present specialized or opportunistic nesting habits; the latter not being restricted to a single or a narrow set of host species in an ecological interaction. Herein we report ant colonies belonging to seven species and four genera inside ripe pods of *Cassia fistula* L. (Fabaceae) with signs of previous occupation by herbivores. Among the species found nesting in pods previously excavated by phytophagous insects are *Cephalotes targionii* (Emery, 1894); *Cephalotes eduarduli* (Forel, 1912); *Crematogaster* aff. *victima* Smith, 1858 and *Crematogaster* sp. (Myrmicinae); *Myrmelachista nodigera* Mayr, 1887 (Hymenoptera: Formicidae: Formicinae); *Pseudomyrmex gracilis* (Fabricius, 1804) (Hymenoptera: Formicidae: Pseudomyrmecinae) and *Tapinoma* sp. (Hymenoptera: Formicidae: Dolichoderinae), all of which showed opportunistic behavior by using pods previously hollowed and holed by phytophagous insects. This is the first record of *Cephalotes* Latreille, 1802 ants nesting in structures other than trunks and branches.

**Keywords:** Formicidae, Urban ants, Fabaceae, Pods, Plant-ant interactions.

Ants (Hymenoptera: Formicidae) are present in different environments, including trees, which are the sole nesting sites for turtle ants, *Cephalotes* Latreille, 1802 (Baccaro et al. 2015). Naturally, some plants share an intimate evolutionary history with ants and present an interaction called myrmecophily, in which the presence of inquiline ants might be favored by plant structures called domatia, cavities that serve as nesting sites. Records of ants nesting in such structures include the genera *Pseudomyrmex* Lund, 1831; *Colobopsis* Mayr, 1861; *Crematogaster* Lund, 1831; *Monomorium* Mayr, 1855 and *Cephalotes* (Hölldobler & Wilson 1990). Additionally, ants can occur in branch cavities left by xylophagous insects, as is the case for *Daceton* Perty, 1833; *Crematogaster*; *Azteca* Forel, 1878; *Lasius* Fabricius, 1804 and *Camponotus* Mayr, 1861 (Hölldobler & Wilson 1990).

Imperial cassia, *Cassia fistula* L. (Fig. 1A) is a fabaceous tree commonly used in urban environments for its shade and ornamental qualities when flowering (Boscardin et al. 2012). It is native to the tropical region of Asia, having been introduced to Brazil (Rocas 2002). Its fruit, when mature, are lignified, dark (Fig. 1B) and can reach 50 cm in length and 1.7 cm in diameter, staying attached to the tree for up to 3 months after ripening (Rocas 2002).

Some insects feed on imperial cassia's pods, such as the seed-eating beetle *Pygiopachymerus lineola* (Chevrolat, 1871) (Coleoptera: Chrysomelidae: Bruchinae) (Boscardin et al. 2012). Its larvae inhabit the seeds, and after emerging the adults drill an exit hole (Fig. 1C) through the pericarp. In addition, the pod caterpillar, *Cydia tonosticha* (Meyrick, 1922) (Lepidoptera: Tortricidae) (Bobadilla 2015) consumes the legume valves and also leaves behind not only a visible exit orifice, but also its pupal exuviae and frass inside the pod. Therefore, pods attacked by *P. lineola* are characterized by empty loci, where the seeds consumed by the beetle were once found, and those attacked by *C. tonosticha* present galleries resulting from the consumption of the fleshy tissue by the lepidopteran larvae, whilst both these herbivores leave one or more orifices that connect these cavities to the external environment. The damage caused to the pods by these insects is, in a way, an extended phenotype, which provides the larvae with the guarantee of food and protection (Pereira et al. 2015), but which is

abandoned during the herbivores' adult phase. Thereafter, the pods constitute potential shelter for other organisms.

Considering the importance of nesting sites for eusocial insects, such as ants, the present work provides evidence for the importance of herbivores as ecosystem engineers which transform plant parts into nesting sites in highly anthropized areas. It also shows a surprising diversity of ant colonies within ripe fruits.

Data was collected from five different sites between February and June of 2023. Two of the sites were located at the "Luiz de Queiroz" campus (ESALQ) of the Universidade de São Paulo, while the other three were in urban areas within the city of Piracicaba, São Paulo, Brazil. The sites were not chosen based on landscape characteristics, but the urban areas had cemented sidewalks around the trees. Sample sizes were determined based on the availability of ripe pods with holes (Fig. 1C). Each site included pod collections from the same tree. The numbers of pods collected were as follows: 30 hanging and 15 fallen pods in ESALQ I (22°42'34"S 47°37'52"W), 15 hanging and 23 fallen pods in ESALQ II (22°42'00"S 47°37'58"W), 12 hanging pods in URBAN I (22°42'57"S 47°38'15"W), 27 hanging pods in URBAN II (22°43'01"S 47°38'53"W) and 22 hanging pods in URBAN III (22°44'27"S 47°37'26"W). We collected ripe pods of *C. fistula* directly from the trees and those on the ground, all of which had visible orifices, different types of collection were used to maximize sampling. In urban sites, we generally found the ground to have been swept of any pods, making it impossible to collect fallen ones. In order to examine the presence of ant colonies (Fig. 1D-F), the pods were opened with the use of a hammer and pliers. Colonies found were carefully collected and stored in plastic containers (10 cm height × 6 cm width) and then killed by freezing at -20 °C, to be later analyzed in the laboratory. Genera were identified based on Baccaro et al. (2015) and Feitosa & Dias (2024), and for species-level identification we used Ward (2017) as reference for *Pseudomyrmex*; Smith (1858) for *Crematogaster* and Oliveira et al. (2021) for *Cephalotes*. All specimens were then stored in 3 mL tubes filled with 70% ethanol. Voucher specimens were deposited at the "Luiz de Queiroz" Entomology Museum MELQ-ESALQ/USP.



**Figure 1.** Imperial cassia, *Cassia fistula* L., and ants' colony with larvae, pupae and adults found in pods. A) Plant in urban area used for shade and ornamental qualities when flowering. B) Ripe fruits. C) Holes in fruits. D-F) Colony of ants, D) *Cephalotes targionii*; E) *Pseudomyrmex gracilis*; F) *Crematogaster* aff. *victima*.

Colonies belonging to 7 species in 4 genera of ants were found inside the ripe pods of *C. fistula*. No different species were found sharing a pod, and different entry holes in the same pod were considered from the same nest. The species are *Pseudomyrmex gracilis* (Fabricius, 1804) (Fig. 2A) (Hymenoptera: Formicidae: Pseudomyrmecinae), *Cephalotes eduarduli* (Forel, 1912) (Fig. 2B), *Cephalotes targionii* (Emery, 1894) (Fig. 2C), *Crematogaster* sp. (Fig. 2D) and *Crematogaster* aff. *victima* Smith, 1858 (Fig. 2E) (Myrmicinae), *Myrmelachista nodigera* Mayr, 1887 (Fig. 2F) (Hymenoptera: Formicidae: Formicinae), and *Tapinoma* sp. (Fig. 2G) (Hymenoptera: Formicidae: Dolichoderinae). The number of nests found was three of *P. gracilis*, two of *C. eduarduli*, nine of *C. targionii*, one of *Crematogaster* sp., nine of *C. aff. victima*, one of *M. nodigera* and eight of *Tapinoma* sp. (Tab. 1). Adult *P. lineola* beetles and the larvae of the moth *C. tonosticha* could be found in most pods sampled, always accompanied by the characteristic exit hole and the absence of an intact seed in the nest-forming cell. However, these herbivores and any materials associated with their activity were absent in cells occupied by ant colonies. Hence, exuvia, frass, silk and the herbivores themselves were probably removed by the ants when establishing the nests.

Our data indicates that the sampled ants are dependent on prior occupation by herbivores, which transform their food sources into suitable shelters. This process involves endophytic oviposition and cryptic feeding by beetles and moths, a critical ecological characteristic for these species. This can be considered an example of what Dawkins (1989) defines as an "extended phenotype," where the phenotype encompasses all the effects genes have on the world, including those extending beyond the development of the organism's own body.

On the other hand, there are no records on the natural history of *C. targionii*, and the present study is the first one regarding its host, since Andrade & Urbani (1999) did not assess this aspect when describing the

maintenance of this species in a laboratory. However, it is known that ants in this genus nest in pre-existing cavities (Powell 2008), a behavior in accordance with the present work. *Crematogaster* is a genus found throughout Brazil, nesting in the soil or inside trees, in cavities built by other animals or in myrmecophytic plant domatia (Baccaro et al. 2015). *Crematogaster* aff. *victima* inhabits myrmecophytic plants (Oliveira et al. 2015) and, like *P. gracilis*, visits flowers and is a predator, attacking herbivores that feed on its host plant (Byk & Del-Claro 2010). The species *P. gracilis* has a wide geographical distribution and has already been reported to be an opportunist, nesting in stems and twigs, live and dry, in building cracks and in *Acacia* Mill., where this species can be considered invasive (Wetterer 2010). In the latter case, *P. gracilis* competes for nesting sites with *Pseudomyrmex ferrugineus* (Smith, 1877), in a way that affects the biology of the plant (Clement et al. 2008). This species also occurs in urban environments, such as hospitals (Lima et al. 2013). *Myrmelachista* Roger, 1863 is known to nest in hollow fallen twigs, including our sampled species, *M. nodigera*, which were found in twigs measuring 10-30 cm (Nakano et al. 2012), bearing a striking resemblance to the pods of the imperial cassia. Another important aspect to discuss regarding our findings is the pods' hanging time and the lifespan of ant colonies, which often exhibit considerable longevity. However, in some genera, the queens' lifespan can be ephemeral. For instance, in *Tapinoma*, a queen may last only a few weeks, contrasting with the longevity seen in other species (Keller 1998). We propose that the sampled species exhibit ephemeral colonies and/or rapid production of reproductive castes, as their nests inhabit a structure with a lifespan of only four months.



**Figure 2.** Ants (workers) collected in ripe pods of *Cassia fistula*. A = *Pseudomyrmex gracilis* complex; B = *Cephalotes eduarduli*; C = *Cephalotes targionii*; D = *Crematogaster* sp.; E = *Crematogaster* aff. *victima*; F = *Myrmelachista nodigera*; G = *Tapinoma* sp. Scale bars = 1 mm.

Other ant species dwelling in fruits, which can be ephemeral resources, have been reported in the literature. In Ghana, *Cataulacus guineensis* Smith, 1853 was observed nesting in wood and pods of cocoa trees (Ackonor 1983), while in Israel, *Cardiocondyla wroughtonii*



**Table 1.** Ant species of the colonies sampled on the different points. Only one colony was considered for the same pod, considering the possibility of polydomy.

Subfamily	Species	Sampling points				
		ESALQ I	ESALQ II	URBAN I	URBAN II	URBAN III
Dolichoderinae	<i>Tapinoma</i> sp.	0	3	0	4	1
Formicinae	<i>Myrmelachista nodigera</i>	0	1	0	0	0
	<i>Cephalotes eduarduli</i>	1	0	1	0	0
Myrmicinae	<i>Cephalotes targionii</i>	3	0	2	0	4
	<i>Crematogaster</i> aff. <i>victima</i>	9	0	0	0	0
	<i>Crematogaster</i> sp.	0	1	0	0	0
Pseudomyrmecinae	<i>Pseudomyrmex gracilis</i>	3	0	0	0	0

(Forel, 1890) was found nesting in figs of *Ficus sycomorus* L. (Lupo & Galil 1985). Numerous other cases of ants nesting in fabaceous pods are documented in the literature, including five in *Canavalia* spp. (Yamashiro & Yamashiro 2008) and two in *Stryphnodendron adstringens* (Mart.) Coville (Souza et al. 2022). Seven species were reported to inhabit hickory nuts, with two of them identified in the *Crematogaster* genus, *Crematogaster lineolata* (Say, 1836) and *Crematogaster minutissima* Mayr, 1870 (MacGown 2006). *Crematogaster* is also reported to inhabit pods, such as *Crematogaster carinata* Mayr, 1862 in *Macropsychnanthus comosus* (G.Mey.) L.P.Queiroz & Snak (Fonseca-Cortés & Sanabria-Baquero 2022) and *Crematogaster* sp. in the external domatia of *Mucuna interrupta* Gagnep (Kato et al. 2004).

*Cassia fistula* and, most likely, the insects that feed on it, are introduced species from Indochina. Thus, ant species sampled did not coevolve with these organisms due to their distinct geographical distributions. We hypothesize that these ants exhibit opportunistic nesting behavior, being able to occupy a wide range of suitable shelters, natural or manmade, present in their environment. Structures like branches or twigs attacked by xylophagous insects, as well as domatia, may have played a significant role in the evolutionary history of these ants, particularly *Cephalotes*. Nevertheless, they can also nest in a twig-like pod.

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## Authors' Contribution

FGG: Conceptualization, Data curation, Investigation, Visualization, Writing – Original draft; MRS: Data curation, Methodology, Project administration, Supervision, Writing – Review & editing; GT: Validation, Writing – Review & editing; JMSB: Funding acquisition, Resources; MS: Conceptualization, Formal analysis, Supervision, Validation, Writing – Review & editing.

## Conflict of Interest Statment

The authors declare no conflicts of interest.

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