

Research article

Nesting biology of the fungus-growing ant *Cyphomyrmex longiscapus* Weber (Attini, Formicidae)

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Summary

The fungus-growing ant *Cyphomyrmex longiscapus* Weber (Attini, Formicidae) exhibits one of the most peculiar nesting habits of all attine ants. It constructs semi-pendant nests resembling miniature swallow nests at vertical embankments or under overhanging logs and fashions entrances with funnel-like “auricles” constructed of clay. The entrance auricle surrounds a large nest opening that exposes the garden and renders it susceptible to desiccation. The distribution of *C. longiscapus* therefore is limited to wetter habitats. Nests are strictly monogynous. Queenright nests of *C. longiscapus* can be collected easily because of its conspicuous and shallow nest architecture, making this species an ideal candidate for population surveys of geographic variation in symbiont diversity, garden substrate, or parasitism in a lower attine ant.

Introduction

The fungus-growing ant *Cyphomyrmex longiscapus* Weber (Attini, Formicidae) is known from only five collections from Colombia and Panama, two of these from stomach contents of poison-dart frogs (Snelling and Longino, 1992). The first, rather superficial description of Colombian types was included by Weber (1940) in a taxonomic key to the genus *Cyphomyrmex*. Kempf later provided a more careful description of the *C. longiscapus* types and added a note on a worker and a female collected by W. L. Brown on Barro Colorado Island, Panama (Kempf 1965), highlighting minor morphological differences between the Colombian and Panamanian specimens. Three later collections were all from Colombia, two as mentioned from stomach contents of poison-dart frogs, one from underneath a rock in a canyon. A sketchy description of a Colombian *C. longiscapus* nest was given by Weber (1972), who found the nest “unusual in being in the form of a sack of agglutinated humus” (p. 60) that was “suspended from twigs above the soil level” (p. 57).

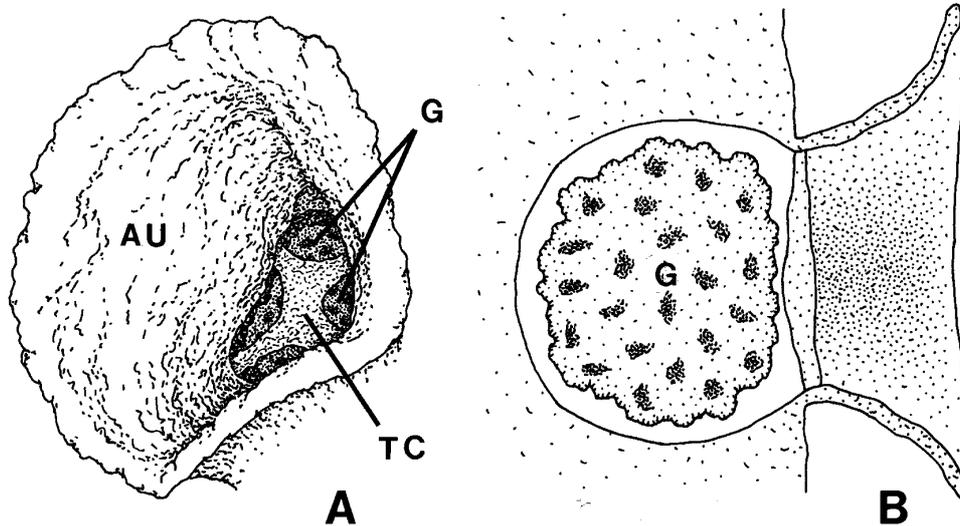


Figure 1. (A) Auricle (*AU*) surrounding the entrance of a nest of the fungus-growing ant *Cyphomyrmex longiscapus*. The outside diameter of the auricle measures 24 mm. The entrance measures 9 mm diameter and is partly obstructed by a trabecular cross (*TC*) that the ants construct from clay. The garden (*G*) is situated immediately behind the trabecular cross. (B) Cross section through a nest of *C. longiscapus*, showing the exposed position of the garden (*G*) immediately behind the wide nest entrance

We have found no additional reports of this unusual nest type. Indeed, according to available collecting records, *C. longiscapus* appears to be rare or difficult to collect.

During a year-long survey of the fungus-growing ants of Panama, *C. longiscapus* was found to be the most easily collected species of all attine ants. More than 200 queenright colonies were collected to detail the symbiont diversity cultivated by *C. longiscapus* in central Panama. These collections form the basis for the following report on the nesting habits of *C. longiscapus*. Collection of this sizable sample of colonies was facilitated by the peculiar nesting habits of *C. longiscapus*, as described below. The unusual nesting habits may explain why a century of myrmecological research on Barro Colorado Island, some of it specifically focused on fungus-growing ants, failed to discover what are surprisingly conspicuous nests (Fig. 1).

Material and methods

Lower Attini in central Panama were surveyed from November 1995 to December 1996. The great majority of attine nests were located by carefully inspecting the ground (to identify soil dumps at nest entrances), leaf litter, or the substrate underlying and inside of logs and branches fallen to the ground. Ants were surveyed along a north-south transect spanning the Panamanian Isthmus, including, from north to south, the following collecting sites: Fort Sherman Military Reservation at the

Atlantic (off Gatun Road), Barro Colorado Island (BCI), Pipeline Road, Gamboa, Soberanía Park, and Naos Island at the Pacific. Additional but irregularly visited sites were the forests on the crest of the El Llano – Cartí Suitupo road (henceforth called El Llano), Nusagandi, Cerro Azul, and Santa Clara at the Pacific. *C. longiscapus* was found only at Pipeline Road (north of kilometer 4.5), BCI, Nusagandi, El Llano, and Ft. Sherman. A total of 203 whole nests of *C. longiscapus*, including entire gardens with workers, were collected. Collections were made between 8:00 and 16:00, a time when *C. longiscapus* workers were observed to forage; the reported colony sizes therefore underestimate actual work forces.

Because of the unusual nesting habits of *C. longiscapus*, collection of a single nest requires only about 5 minutes. *C. longiscapus* constructs shallow nests at steep river embankments, and fashions the nest entrance with a characteristic entrance “auricle” made of clay (see Fig. 1). This entrance auricle could be pried open with a pocket knife to expose the single garden of the nest. The garden was then collected *in toto* by gently rolling it into a small vial held underneath the exposed nest cavity. Workers remaining inside the cavity were collected with an aspirator. Because nests have only a single cavity and no escape tunnels, 94% of the collections were queenright; the single queen appears to remain hidden in the garden upon disturbance of the nest and is collected as part of the intact garden together with her workers, brood, and reproductive offspring. Like the pendant nests of *Apterostigma (pilosum)* group), nests of *C. longiscapus* clearly afford easy collection of large samples for population surveys.

Totals of 39, 25, 3, 19, and 117 *C. longiscapus* nests were collected respectively, at Ft. Sherman, BCI, Nusagandi, El Llano, and Pipeline Road. Table 1 lists the exact sample sizes by month of collection for each of the five sites. Nests were maintained for 3–6 weeks in the lab for fungal isolation, after which gardens were dissected under the microscope to assess the number of queens, workers, and sexuals.

Recent allozyme work (Mueller, Villesen, and Boomsma, unpublished) suggests the possible coexistence of two cryptic lineages or species of “*C. longiscapus*” in central Panama. The following report does not distinguish between the two hypothesized gel-species because the description of the nest architecture (Fig. 1) applies to both gel-species. Thus, the observations reported here on habitat use, fungiculture, social structure, colony reproduction, and colony migration are for *C. longiscapus sensu lato*.

Results

Distribution across the Panamanian Isthmus

C. longiscapus appears to be confined to the wetter parts of the Panamanian Isthmus. It occurs in the Fort Sherman Military Reservation at the Atlantic coast, on Barro Colorado Island (BCI), at Nusagandi, at the top of the ridge off the El Llano – Cartí road (El Llano), and Soberanía Park along Pipeline Road north of kilometer 4.5. Despite careful searches, it was not found in Gamboa, any more southern location in Soberanía Park, or on Naos Island at the Pacific. On BCI, a sizable population was discovered along the terminal 100 meter segment of the creek just to the west,

and paralleling, the “trolley” tracks leading up to the old station. This creek is one of the few permanent streams on the island. *C. longiscapus* was not found at seasonal creeks on BCI, or at seasonal creeks along Pipeline Road. All in all, *C. longiscapus* appears to occur in the wetter parts on the Atlantic slope of the Isthmus, but it is absent from the drier Pacific slope. The range extensions beyond the surveyed area (bordered by the Canal to the west and Nusagandi to the east) are unknown. The existence of *C. longiscapus* in Colombia suggests contiguity between Panamanian and Colombian populations.

Habitat

C. longiscapus is a forest-dwelling species. It is most predictably found on steep embankments along creeks in wetter forests. Occasionally it nests on steep clay cliffs, or on short vertical steps in the forest that are somewhat distant to streams, in such cases usually as an extension of a population associated with a creek gully or canyon. It is apparently absent, or extremely rare, in those microhabitats typical for most lower attines, the more level or moderately sloped forest floor. Whether along creeks or in forest, nests of *C. longiscapus* are generally situated underneath overhangs, or set back in a crevice underneath a sheltering root or rock, thus gaining protection from the heavy rains. Nesting in these protected locations underneath overhangs or in crevices may allow construction of the fragile entrance “auricle” and the sometimes semi-pendant, “swallow-nest” architecture (see next section).

Nest architecture

Figure 1A shows the typical frontal aspect of a *C. longiscapus* nest. The entrance is flanged with a broad, funnel-like auricle that the ants construct of soil or clay. The auricle is more or less symmetrical along the vertical axis and may be either vertically elongated (ellipsoid) or round. The overall size of the auricle appears to correlate with the size (and presumably the age) of the colony, with larger colonies showing larger auricles. The smallest auricles discovered (entrance auricle of about 4 mm diameter, round) were those of single foundress queens, the largest auricles (about 50 mm vertical \times 25 mm horizontal) those of nests with extreme work forces of around 100 workers. On average, auricles measure 23 mm along the vertical axis and 19 mm along the horizontal axis ($n=47$ nests, measured at Pipeline Road, km 6). Auricle walls vary between 0.5–5.0 mm in thickness, with the thinnest parts toward the margin. Auricles are therefore extremely fragile.

The entrance auricle surrounds the single nest entrance, which is surprisingly large (Fig. 1). Even though the broad entrance is partly closed by irregularly shaped “trabeculae” that the ants construct of clay and that zig-zag across the entrance and thus separate the cavity from the auricle (Fig. 1A), the spaces left within the trabecular framework are large and leave the single garden, situated immediately behind the trabeculae, partly exposed. In fact, in most nests it is possible to view the garden, and even observe gardening workers, through the large auricular opening (Fig. 1A). This exposure of the garden is atypical for attine ants; it should render

gardens of *C. longiscapus* prone to desiccation and may explain why *C. longiscapus* occurs in wetter habitats and predominantly along moist gullies of permanent streams.

The single nest cavity is situated immediately behind the entrance auricle (Fig. 1A, B). It is generally more or less spherical with an approximate average volume between 1–3 cc, but roots or rock protrusions into the cavity may pose physical constraints forcing the construction of non-spherical gardens (e.g., a thin elongate garden partly wedged into the crack between two rocks). Parts of the cavity walls, especially those supporting the entrance auricle, may be constructed by the ants. Like the auricle, these walls are constructed by layering bits of clay; they are, however, invariably thicker and more robust than the auricle and may even be supported by rootlets that happen to become incorporated in the walls. In extreme cases, ant-constructed walls may surround almost the entire nest cavity, and the garden then appears to be hanging in a “bag” of clay reminiscent of miniature swallow nests. Such nests were found in all locations studied, but they were particularly abundant at El Llano, where numerous nests were found to be attached in swallow-nest fashion below logs and suspended close to the ground. Weber (1972) also described such a swallow-nest architecture for a Colombian nest. At the other extreme, some colonies of *C. longiscapus* appear to utilize preexisting cavities (e.g., cracks in rock) that the ants close off by constructing a front wall saddled with an entrance auricle.

Fungiculture

C. longiscapus cultivates its fungal symbiont as a mycelium growing on a mixture of substrates. Identifiable substrates include insect droppings (probably from beetles and caterpillars), small flower parts, seeds, seed pods or husks, and an occasional small piece of green leaf. Like many other attines, *C. longiscapus* also incorporates parts of insect exoskeletons, such as heads or thoraxes of other ants, but it is unknown whether these exoskeletal parts serve any nutritive function for the symbiont. Exoskeletal parts are found incorporated in the garden, but also strewn around the nest cavity. Symbionts cultured by *C. longiscapus* were found to be exclusively G3-symbionts (Chapela et. al., 1994), that is lepiotaceous symbionts closely related to species of free-living *Leucocoprinus*.

Social structure

Nests of *C. longiscapus* are monogynous. Of 203 nests examined, all but one nest had a single dealate female. The single exceptional nest had two dealate females. It is unknown whether both these females were functional reproductives at the time of collection. Work forces of collected colonies averaged 29.4 workers, with median and maximum size at 24 and 109 workers, respectively.

Colony reproduction and sex ratio

Adult sexuals were present throughout the year. Specifically, all samples contained at least some nests with reproductives (Table 1), with the exception of one small sample of 3 nests collected in April 1995 at El Llano. Thus *C. longiscapus* appears to belong to those lower attine ants with a life history of more or less continuous production of sexuals, rather than the life history of many other Panamanian attines with exclusive production of sexuals timed to coincide with the onset of the rainy season in April/May. The numerical sex ratio varied between 20% and 100% male production, and there were no clear annual changes in the numerical sex ratio (Table 1). Most samples showed slightly male-biased numerical sex ratios of 45–75%. This suggests that investment ratios may well be female-biased, given the substantially larger size of females and the possible existence of fissioning as a mechanism of colony reproduction (see next section) (Crozier and Pamilo, 1996).

While the production of male and female alates, and direct observation of many incipient single-foundress nests, may indicate that *C. longiscapus* reproduces largely via mating flights and dispersal of winged foundresses, the following observation suggests that colony fission could represent a second mode of reproduction. The single pleometrotic nest described above was part of a colony transfer experiment (Mueller, unpublished) in which colonies were collected, maintained in the lab for

Table 1. Numerical sex ratio (in % male), percent of nests with sexual adults (% nests), and the number of nests collected (n), listed by month of collection for each of the five study sites

		Ft. Sherman	BCI	Nusagandi	El Llano	Pipeline Rd.
Dec 95	sex ratio	–	72.0	–	–	90.9
	% nests	–	80.0	–	–	43.8
	n	–	5	–	–	16
Jan 96	sex ratio	–	–	–	82.6	45.8
	% nests	–	–	–	82.6	50.0
	n	–	–	–	16	8
Feb 96	sex ratio	–	53.1	–	–	51.9
	% nests	–	65.0	–	–	63.3
	n	–	20	–	–	49
Apr 96	sex ratio	61.1	–	20.0	no alates	–
	% nests	20.0	–	33.3	0	–
	n	10	–	3	3	–
July 96	sex ratio	100	–	–	–	45.7
	% nests	36.4	–	–	–	50.0
	n	11	–	–	–	20
Aug 96	sex ratio	46.9	–	–	–	77.4
	% nests	50.0	–	–	–	28.6
	n	14	–	–	–	14
Dec 96	sex ratio	58.3	–	–	–	48.5
	% nests	50.0	–	–	–	60.0
	n	4	–	–	–	10

three weeks to obtain an isolate of the symbiont, then reintroduced to the field (placing the colony and garden into a small artificial cavity pressed into a clay embankment). Immediately after reintroduction of the polygynous nest, some workers moved part of the garden to a small crevice about 20 cm distant from the artificial cavity. The other garden half remained in the artificial cavity, and the ants constructed auricles at both nest sites. The two subnests coexisted for 12 weeks until termination of the experiment, at which point each of the two subnests was found to contain a single queen. Even though these observations were made on experimentally manipulated colonies, they suggest that *C. longiscapus* is capable of colony fission and may reproduce via such a process at least on occasion.

Colony migration

Observations on this polygynous and many additional monogynous experimental colonies further revealed that *C. longiscapus* is capable of relocating the nest. Specifically, some experimental nests abandoned their artificial cavities and moved their garden short distances, generally less than 30 cm, to construct a nest at a new location. Construction of a moderately sized entrance auricle generally took less than two days, with most colonies showing a typical large auricle after a week. Migration in *C. longiscapus* is certainly facilitated by the simple, superficial nest architecture. In fact, the observations on *C. longiscapus* suggest that other attines with shallow nests, specifically those nesting underneath logs or in leaf litter, may occasionally or routinely relocate their gardens (e.g., *C. minutus* nesting between leaves). Among the attines, colony migration has been observed so far in the higher attines *Trachymyrmex* (Weber, 1941), *Acromyrmex* (Fowler, 1981), and *Atta* (Rockwood, 1973; Porter and Bowers, 1980; Fowler, 1981), but, to our knowledge, not in any lower attine.

Discussion

C. longiscapus exhibits one of the most unusual nest architectures of all fungus-growing ants (Fig. 1). The most distinctive features include the entrance auricle, the large entrance opening exposing the garden, the frequent construction of clay walls surrounding the nest cavity in swallow-nest fashion, and the predominant location of the nest at vertical embankments along permanent streams. The only other attine known to construct similar entrance structures is another Panamanian species of *Trachymyrmex* (cf. *zeteki*) (Mueller, pers. obs.), but the entrance structure is shaped more like a vertically elongated funnel, and it leads to a narrow tunnel (about 3–8 cm long), rather than to a broad entrance opening typical for *C. longiscapus*. Interestingly, *Trachymyrmex* cf. *zeteki* also nests almost exclusively at vertical embankments, frequently in mixed aggregations with *C. longiscapus*. Unlike *C. longiscapus*, however, *T. cf. zeteki* exists in considerably drier habitats (e.g., along seasonal streams) and ranges well into the drier Pacific slope in Panama. This further supports the hypothesis that the large entrance opening renders *C. longiscapus* susceptible to desiccation and thus limits its distribution to moist habitats, while the

entrance tunnel and the subterranean cavity construction of *T. cf. zeteki* protects its gardens from desiccation and allows existence in relatively dry habitats. It is unclear, however, why *C. longiscapus* fails to protect its garden from desiccation by facultative closing of the large auricle opening, or by construction of slightly less superficial nest cavities.

Auricle function

C. longiscapus and *T. cf. zeteki* are the only two attines known to build funnel-like entrance structures. Both of these species are also obligate cliff dwellers, suggesting that auricle and funnel construction are related functionally to the vertical habitat of these species. Obviously, nesting on level ground precludes the construction of funnel-like entrances, because nests would become inundated by the intense tropical rains. But while flooding danger explains the absence of funnel entrances in species nesting on level ground, and rather predicts more protective entrance structures like turrets to guard against flooding (as in *Acromyrmex landolti*; Navarro and Jaffe, 1985), flooding danger does not explain the presence in cliff dwelling species. Smoothed down-facing entrance funnels appear to lower rates of parasitism in some eumenid wasps because the smoothed inside surface of the funnel affords no grip to parasitoids (Iwata, 1976; Smith, 1978), but this explanation does not apply to *C. longiscapus* because the entrance auricle is not smoothed and provides good footholds to potential invaders, as it does to the ants themselves. The function of the entrance funnels in *C. longiscapus* remains unclear at this point.

The model species C. longiscapus

Lower attine nests are generally cryptic, hidden below leaf litter, and may extend up to several meters deep into the ground. Collection of queenright nests or entire gardens of most lower attines therefore is difficult, and consequently little is known about the nesting biology and life history of most lower attine species (Weber, 1972, 1982; Hölldobler and Wilson, 1990). In contrast, the ease of collection and the small colony size of *C. longiscapus* facilitates population surveys and thus make this species an ideal candidate for a model species of lower attine. Colonies of *C. longiscapus* can also be maintained in the laboratory with relatively little labor. Detailed studies of geographic variation in symbiont diversity, substrate use, levels of associated pathogens, or other features of the attine symbiosis thus seem feasible in the lower attine *C. longiscapus*.

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References

- Chapela, I.H., S.A. Rehner, T.R. Schultz and U.G. Mueller, 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* 266:1691–1694.
- Crozier, R.H. and P. Pamilo, 1996. *Evolution of Insect Colonies*. Oxford University Press, Oxford. 306 pp.
- Hölldobler, B. and E.O. Wilson, 1990. *The Ants*. Harvard University Press, Cambridge. 732 pp.
- Iwata, K., 1976. *Evolution of Instinct: Comparative Ethology of Hymenoptera*. Amerind Publishing Company, New Delhi. 535 pp.
- Kempf, W.W., 1965. A revision of the Neotropical fungus-growing ants of the genus *Cyphomyrmex* Mayr. Part II: Group of *rimosus* (Spinola) (Hym. Formicidae). *Studia Entomologica* 8:161–200.
- Navarro, J.G. and K. Jaffe, 1985. On the adaptive value of nest features in the grass-cutting ant *Acromyrmex landolti*. *Biotropica* 17:347–348.
- Porter, S.D. and M.A. Bowers, 1980. Emigration of an *Atta* colony. *Biotropica* 12:232.
- Rockwood, L.L., 1973. Distribution, density, and dispersion of two species of *Atta* (Hymenoptera: Formicidae) in Guanacaste Province, Costa Rica. *J. Anim. Ecol.* 42:803–817.
- Smith, A.P., 1978. An investigation of the mechanisms underlying nest construction in the mud wasp *Paralastor* sp. (Hymenoptera: Eumenidae). *Anim. Behav.* 26:232–240.
- Snelling, R.R. and J.T. Longino, 1992. Revisionary notes on the fungus-growing ants in the genus *Cyphomyrmex*. In: *Insects of Panama and Mesoamerica* (D. Quintero and A. Aniello, Eds.), Oxford University Press, Oxford, pp 481–494.
- Weber, N.A., 1940. The biology of the fungus-growing ants. Part VI. Key to *Cyphomyrmex*, new Attini and a new guest ant. *Revista de Entomologia* 11:406–427.
- Weber, N.A., 1972. *Gardening Ants: The Attines*. American Philosophical Society, Philadelphia. 146 pp.
- Weber, N.A., 1982. Fungus Ants. In: *Social Insects, IV* (H. Hermann, Ed.), Academic Press, New York, pp. 255–363.

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