SHORT COMMUNICATION

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Agro-predation: usurpation of attine fungus gardens by *Megalomyrmex* ants

Abstract A new ant species of *Megalomyrmex* conducts mass raids to usurp gardens of the fungus-growing ant *Cyphomyrmex longiscapus*, then lives in the gardens and consumes the cultivated fungus. Unlike attine ants, however, *Megalomyrmex sp.* does not forage for substrate to manure the gardens; therefore, when gardens become depleted, *Megalomyrmex sp.* must locate and usurp new gardens. *Megalomyrmex sp.* workers feed their larvae with attine brood, but only after removing the fungal mycelium that covers the attine larval integument, suggesting that this fungal coat may provide partial protection against other predators. Unlike other known *Megalomyrmex* species, which coexist as social parasites in attine colonies, *Megalomyrmex sp.* expels its attine hosts during the garden raids. *Megalomyrmex sp.* thus maintains a unique agro-predatory lifestyle that is described here for the first time.

Introduction

Fungus-growing ants (Attini: Formicidae) possess a particularly valuable resource, their fungus gardens, which they defend vigorously against each other (Weber 1972; Jaffe 1986; Whitehouse and Jaffe 1996; Adams et al. 2000). Probably because of this defense, garden stealing between fungus-growing ant colonies appears to be rare. Indeed, even in the well-studied leafcutter ants, garden stealing has only rarely been ob-

served in the form of intraspecific raids of small, incipient colonies by larger, more established ones (Autuori 1950; Rissing et al. 1989). Here we describe the first known example of routine garden usurpation by a specialized "garden predator" in the non-attine ant genus *Megalomyrmex* (Myrmicinae: Solenopsidini). Field and laboratory studies indicate that colonies of *Megalomyrmex sp.* (a new species to be described by C.R.F. Brandão, personal communication), gain possession of attine gardens by expelling the host ants from their nests.

The six species currently recognized in the *Megalomyrmex silvestrii* group are social and trophic parasites in nests of fungus-growing ants (Wheeler 1925; Weber 1941; Kempf and Brown 1968; Brandão 1990). *Megalomyrmex* workers cohabit with host workers in or near their gardens, and they harvest the cultivated fungus for food, cropping the mycelium in a manner similar to that employed by attine ants (Weber 1941). *Megalomyrmex* parasites have so far been found in nests of the diverse attine genera *Apterostigma*, *Cyphomyrmex*, *Trachymyrmex*, and *Sericomyrmex* (Brandão 1990), but they may well parasitize other attine genera.

Judging from the scant information available, the relationship between *Megalomyrmex* parasites and attine hosts seems to vary between *Megalomyrmex* species. *Megalomyrmex symmetochus* workers and queens have been found in fungus gardens peacefully cohabiting with their attine hosts (Wheeler 1925; Weber 1941; R.M.M. Adams, personal observation), whereas *Megalomyrmex silvestrii* colonies have been found occupying secondary cavities adjacent to host gardens (Brandão 1990). Seemingly healthy colonies of *M. silvestrii* have also been collected unassociated with attine hosts (Kempf and Brown 1968; Brandão 1990; R.M.M. Adams, personal observation), suggesting facultative parasitism. Whether all species of the *M. silvestrii* group are facultative garden parasites, or whether some species are obligately dependent on their attine hosts, is unknown. *Megalomyrmex sp.* apparently belongs to the latter category of obligately dependent species.

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Behavioral ecology of *Megalomyrmex sp. nov.*

Four colonies of *Megalomyrmex sp.* were collected in a survey of the fungus-growing ant *Cyphomyrmex longiscapus* in central Panama during June 1998 and September 1999. A total of 262 whole *C. longiscapus* nests, including intact fungus gardens, were collected at two forested sites, one site near the crest of the El Llano – Cartí Suitupo Road (77 nests in total), the other site at Pipeline Road, Km 6, Parque Soberanía (185 nests in total). Mueller and Wcislo (1998) provide detailed descriptions of the collection sites. *C. longiscapus* constructs shallow cavities in clay embankments along permanent streams, and its nests are characterized by a unique and easily recognizable nest architecture, consisting of a single large entrance (5–10 mm diameter) surrounded by an auricle constructed of clay (1–4 cm diameter) (Mueller and Wcislo 1998). The currently recognized *C. longiscapus* (Snelling and Longino 1992) actually comprises two cryptic sister species (*C. longiscapus sensu stricto* and *Cyphomyrmex sp. nov.*; T.R. Schultz et al., in preparation; R.M.M. Adams, U.G. Mueller, M.M. O'Herron, in preparation), each cultivating its own distinct fungus (Mueller et al. 1998). The two species will be referred to here as *C. longiscapus sensu lato*, unless otherwise indicated, because the absence of *Cyphomyrmex* workers in most *Megalomyrmex*-occupied gardens did not allow determination of the *Cyphomyrmex* hosts to species, as explained below.

Most nests contained typical colonies and gardens of *C. longiscapus* (Mueller and Wcislo 1998), but two nests at the El Llano site in 1998 and one nest at the Pipeline Road site in 1999 lacked *C. longiscapus* ants and instead contained fungus gardens occupied by queens and workers of *Megalomyrmex sp.* The *Megalomyrmex sp.* colonies contained between 12 and 22 workers. Two of the three *Megalomyrmex sp.* colonies were monogynous while the third was polygynous with two queens.

The fourth nest, from the 1999 Pipeline Road series, contained a single *Megalomyrmex sp.* queen and three workers of *C. longiscapus sensu stricto*. At the time of discovery, the three *C. longiscapus* workers were in the process of dismantling the garden, discarding small garden fragments just outside the entrance. The lone *Megalomyrmex sp.* queen in this fourth nest may have been in the process of usurping a *C. longiscapus* colony without the help of daughter workers, or aided by only a very few workers that may have been missed during collection, while the *C. longiscapus* ants in the first three parasitized nests, found solely occupied by *Megalomyrmex sp.*, had apparently been entirely displaced by *Megalomyrmex sp.* invaders. This interpretation of garden usurpation by *Megalomyrmex sp.* ants is supported by the behavioral experiments described below.

In contrast to the typical *C. longiscapus* nest architecture (Mueller and Wcislo 1998), the exterior auricles of the three nests occupied solely by *Megalomyrmex sp.* ants appeared to have been washed away by rain and only the entrance had been manipulated and was obstructed by soil except for a small entrance hole. Such nests are not easily recognized, and most certainly were missed in earlier surveys (Mueller and Wcislo 1998). Thus, the observed frequency of 1.53% (four of 262 nests) for *Megalomyrmex sp.* parasitism in the *C. longiscapus* population no doubt represents an underestimate. *Megalomyrmex sp.* colonies were brought to the laboratory and placed in clear plastic boxes (8 × 8 × 3 cm) for observation and video recording. Behavioral experiments were conducted on the three worker-right colonies over a period of 5–10 months after collection. No laboratory experiments were conducted with the fourth colony containing the single *Megalomyrmex sp.* queen, because it took several months before the queen reared her own workers.

Fungus harvesting

Like attine ants, *Megalomyrmex sp.* workers harvest fungal substrate by cropping the mycelium from the garden surface, masticating it into a pulp with their mandibles and ingesting it. Because *Megalomyrmex sp.* colonies can be maintained for months in the laboratory solely on gardens, attine fungi appear to provide a sufficient diet for *Megalomyrmex sp.* ants. Thus, like attine ants but unlike any other known ant, *Megalomyrmex sp.* can subsist exclusively on a fungal diet, suggesting considerable adaptation for fungivory (Mueller et al. 2000). However, because many attine ants supplement their diet with nectar and other plant juices (Littledyke and Cherrett 1976; Cherrett et al. 1989; Murakami and Higashi 1997), it remains possible that *Megalomyrmex sp.* also seeks such non-fungus supplements in the field.

Garden maintenance

Attine fungus gardens require constant maintenance by their resident ants; in the absence of attine gardeners, gardens are quickly overrun by contaminants (Weber 1972; Cherrett et al. 1989; Currie et al. 1999a, b). Fungus-growing ants have evolved specialized behaviors associated with garden maintenance, including the harvesting and processing of appropriate nutritional garden substrates, defecation on the garden to disseminate fungus-derived enzymes that pass unmodified through the ants' guts (Martin 1984, 1987), and the use of growth-promoting substances, antibiotics, and fungicides (Schildknecht and Koob 1971; Martin 1987; Jaffe et al. 1994; Knapp et al. 1994). *Megalomyrmex sp.* may have evolved a similar repertoire of maintenance behaviors and chemical secretions, because gardens occupied exclusively by these ants remain healthy, whereas they quickly deteriorate when abandoned.

Megalomyrmex sp. workers hollowed out a cavity at the center or the bottom of the fungus garden mass and used mycelial fragments to seal off the many passages in the spongelike garden of *C. longiscapus*, but left a small entrance hole near the top of the garden (Fig. 1a). This cavity housed the queens and the brood. *Megalomyrmex sp.* workers manipulated the sticky fungal substrate by ripping off mycelial fragments from one area, carrying them to a new location, then "patting" the mycelium into place with their forelegs. This behavior closely resembles that of attine ants when they add substrate to or reconstruct gardens (Weber 1972; Murakami 1998), suggesting that it has been convergently derived in *Megalomyrmex sp.* ants.
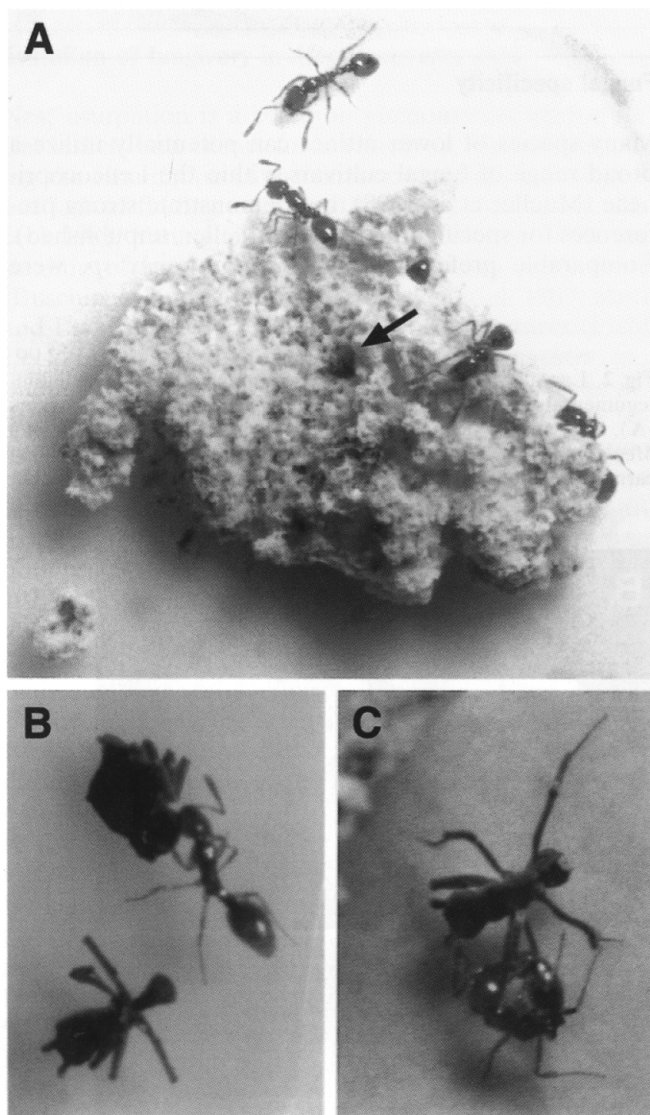

Fig. 1 Attine garden occupied by *Megalomyrmex sp.* (**A**), and aggressive interactions between *Megalomyrmex sp.* and *Cyphomyrmex longiscapus* workers during garden raids (**B**, **C**). Attacking *Megalomyrmex sp.* workers bite (**B**) and sting (**C**) the *Cyphomyrmex* host workers and thus expel them from the garden. The host workers show the curled-up defense position typical for *Cyphomyrmex* ants (**B**)

While *Megalomyrmex sp.* thus possesses the behavioral repertoire necessary for harvesting the fungus and for modifying the garden architecture, they were, unlike their attine hosts, not observed to add new substrate to the garden. First, the gardens of the field-collected nests showed no sign of recent addition of new substrate; and second, suitable substrates such as oak catkins, crushed oats, and caterpillar frass, which are readily taken by attine workers in the laboratory, were ignored by captive *Megalomyrmex sp.* workers. Indeed, compared to attine ants, *Megalomyrmex sp.* workers devoted little time to garden maintenance, largely limiting their garden interactions to harvesting. This continuous harvesting, coupled with the lack of substrate addition, gradually reduced the size of the gardens.

Unlike other known *Megalomyrmex* parasites (Wheeler 1925; Weber 1941; Kempf and Brown 1968; Brandão 1990), *Megalomyrmex sp.* colonies do not coexist with attine hosts and do not rely on them for garden maintenance. Gradual depletion of unmanured gardens thus eventually forces *Megalomyrmex sp.* colonies either to switch to a non-parasitic existence or to locate a new garden. The ability to locate and usurp new gardens from neighboring attine colonies was confirmed by the following experiments.

Garden usurpation

In 24 experiments, a *Megalomyrmex sp.* colony was presented with a healthy garden maintained by *C. longiscapus* ants. *Megalomyrmex sp.* worker scouts quickly located the new garden if their own garden was old and depleted, but scouting behavior could also be induced in workers on partially depleted gardens by physically disrupting the garden and exposing the internal cavity. After discovering the new garden, *Megalomyrmex sp.* scouts laid scent trails back to their nest, and nestmates immediately followed the trails to the new garden. The *Megalomyrmex sp.* workers attacked the resident *C. longiscapus* workers using the highest levels of aggression as measured by the Carlin and Hölldobler scale (1986) (Fig. 1b, c). Although *Megalomyrmex sp.* workers did not sever *C. longiscapus* antennae and legs, they relentlessly pursued *C. longiscapus* workers, pulling at appendages to drag them from the fungus garden. A stinging posture was occasionally assumed (Fig. 1c), but cuticular penetration of the sting was not observed, suggesting that *Megalomyrmex sp.* may use a contact venom like the one described for *Megalomyrmex foreli* (Jones et al. 1991).

Beleaguered *C. longiscapus* ants tried to escape or "play dead" by curling up, protecting legs and antennae (Fig. 1b), a typical defense posture of *Cyphomyrmex* species (Wheeler 1907; Weber 1957, 1972). This passive posture enabled *Megalomyrmex sp.* workers to "sting" *C. longiscapus* workers, or carry them away from the garden (Fig. 1b). *Cyphomyrmex longiscapus* workers that escaped *Megalomyrmex sp.* attack sometimes tried

to retrieve some of their brood and small garden fragments. Such rescue attempts were usually not successful, and *C. longiscapus* colonies lost their entire gardens. This suggests that, in addition to pathogen-driven garden loss (Currie et al. 1999a, b), raids by *Megalomyrmex sp.* in the field may be a second cause of cultivar loss that forces *C. longiscapus* to switch to a novel cultivar, either by acquiring a replacement fungus from a neighboring attine colony (Adams et al. 2000), or by de novo domestication of a free-living fungus as implicated by phylogenetic analysis by Mueller et al. (1998).

During *Megalomyrmex sp.* raids, *C. longiscapus* workers showed no signs of an effective defense; those that continued to return to the garden eventually died, possibly from repeated exposure to *Megalomyrmex sp.* venom. In contrast, *Megalomyrmex sp.* ants did not appear to be harmed by *C. longiscapus* workers; they consistently usurped healthy *C. longiscapus* gardens and pursued the escapees to retrieve attine brood for consumption.

Treatment of attine larvae

Attine larvae are generally enveloped by a blanket of living mycelium (Weber 1972; Schultz and Meier 1995) (Fig. 2b). This mycelial blanket is presumably beneficial to attine larvae and may afford physical or chemical protection against microbial pathogens, parasites, parasitoids, or predators such as army ants (Weber 1972; Swartz 1998).

After stealing the brood of *C. longiscapus*, *Megalomyrmex sp.* workers carefully removed the mycelial blanket from the larvae and pupae (Fig. 2a, b). Following the removal of the mycelial blanket, *Megalomyrmex sp.* workers continued to lick the brood for some time, then placed the *C. longiscapus* larvae and pupae with their own brood, presumably for larval consumption. Indeed, in one case *Megalomyrmex sp.* workers were observed cutting up *C. longiscapus* larvae, and *Megalomyrmex sp.* larvae were observed consuming a white material that resembled larval tissue. This suggests that *Megalomyrmex sp.* larvae may be at least partly carnivorous, which is consistent with the carnivorous rather than fungivorous morphology of the larval mouthparts (Fig. 2c, d) (Wheeler and Wheeler 1976; Schultz and Meier 1995).

Fungal specificity

Many species of lower attines can potentially utilize a broad range of fungal cultivars within the Leucocoprineae (Mueller et al. 1998), but demonstrate strong preferences for specific fungi (U.G. Mueller, unpublished). Comparable preferences in *Megalomyrmex sp.* were

Fig. 2 Larvae of *Cyphomyrmex longiscapus* with the mycelial integumental cover stripped away by *Megalomyrmex sp.* workers (**A**), and with a typical, intact mycelial coat (**B**). Frontal view of a *Megalomyrmex sp.* larva (**C**) showing the mouthparts typical of carnivorous but not fungivorous ants such as *C. longiscapus* (**D**)
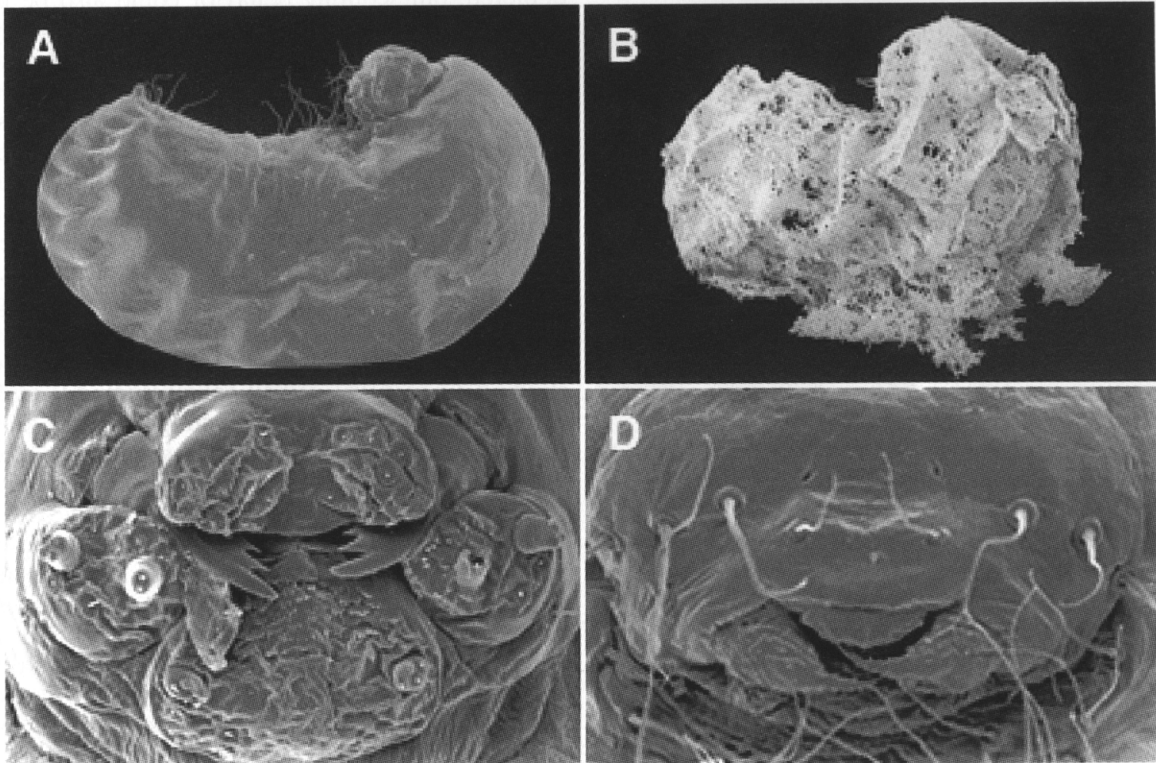
tested by experimental presentation of garden fragments from a diversity of Panamanian attine species. *Megalomyrmex sp.* ants readily consumed a variety of fungal cultivars, including cultivars from gardens of *Cyphomyrmex costatus, Cyphomyrmex longiscapus, Cyphomyrmex sp. nov.,* and even the phylogenetically distant fungal cultivars of *Acromyrmex octospinosis, Trachymyrmex cornetzi* and *Trachymyrmex cf. zeteki,* all highly derived fungi exclusively associated with the higher attines (Chapela et al. 1994; S.A. Rehner, personal communication). One *Megalomyrmex sp.* colony was maintained on a *Trachymyrmex* garden for nearly 4 months. This suggests that a diversity of attine fungi can meet the nutritional demands of *Megalomyrmex sp.*

Evolution of fungivory in *Megalomyremx* ants

Nest usurpation is a common phenomenon among social insects and occurs for a variety of reasons, including the elimination of the time-consuming and dangerous task of finding or excavating a new nest cavity (Alcock 1981; Yamaguchi 1992; Field and Foster 1995), the stealing of brood to increase the colony work force (Buschinger and Klump 1988; Rissing et al. 1989; Mori and Le Moli 1998), or the looting of food stores (Field 1994; Field and Foster 1995). In *Megalomyrmex sp.,* raiding of attine colonies occurs for trophic reasons. *Megalomyrmex sp.* "agro-predators" simultaneously acquire two kinds of food, fungus gardens and attine brood. While attine brood represents a protein- and lipid-rich food source that is quickly depleted, the fungus gardens are a more long-lasting resource, providing both proteins and carbohydrates (Quinlan and Cherrett 1979; Mueller et al.2000). However, in the absence of attine hosts that maintain gardens by adding substrate, gardens are eventually depleted, necessitating periodic raids on new attine colonies.

The agro-predatory behavior of *Megalomyrmex sp.* suggests a possible sequence of evolutionary transitions during the behavioral diversification of the *Megalomyrmex silvestrii* group, starting with an initial non-parasitic, predatory existence, represented by extant *Megalomyrmex* species outside the *silvestrii* group, and leading to: (1) an agro-predatory existence, raiding and completely eliminating the attine hosts, represented by *Megalomyrmex sp.,* described here for the first time; (2) a parasitic existence inside attine nests, perhaps in a chamber somewhat removed from the garden, exemplified by *M. silvestrii* (Kempf and Brown 1968; Brandão 1990); and (3) a parasitic existence inside attine nests characterized by peaceful cohabitation with the host ants directly in the garden, exemplified by *M. symmetochus* (Wheeler 1925; Weber 1941). This hypothesis views nest usurpation as a modified predatory behavior, and social parasitism as an additional modification of the nest usurpation behavior. Alternatively, the raiding behavior of *Megalomyrmex sp.* may have been de-

rived from an ancestral parasitic state in which attine hosts and *Megalomyrmex* parasites coexisted in attine nests. A well-supported phylogeny of the genus *Megalomyrmex,* combined with a reliable reconstruction of ancestral behavioral character states (Schultz et al. 1996; Cunningham et al. 1998), will provide the necessary means for distinguishing between these two hypotheses for the evolution of social parasitism in the genus *Megalomyrmex.*

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