## **Research article**

# Cryptic speciation in the fungus-growing ants *Cyphomyrmex longiscapus* Weber and *Cyphomyrmex muelleri* Schultz and Solomon, new species (Formicidae, Attini)

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Summary. Nesting in abundance on stream embankments in the wet forests of Panama, the fungus-growing ant Cypho*myrmex longiscapus sensu lato* has become a model organism for the study of behavior, ecology, mating frequency, cultivar specificity, pathogenesis, and social parasitism in the attine agricultural symbiosis. Allozyme markers, morphology, and other evidence indicate that C. longiscapus s.l. is in fact a complex of two species, one of which is new to science and described here as Cyphomyrmex muelleri Schultz and Solomon, new species. Although both species occur sympatrically in the same microhabitats and are ecologically, behaviorally, and morphologically quite similar, they consistently cultivate two distantly related fungal symbionts. Thus, each of the two sibling ant species is specialized on a distinct cultivar species, contradicting the conclusions of a previous study. Information is provided for reliably separating the two ant species; morphometrics, ecology, behavior, biogeography, and natural history are summarized. Possible evolutionary mechanisms underlying cryptic speciation in C. longiscapus s.l. are discussed.

*Key words: Cyphomyrmex,* Attini, fungus-growing ants, cryptic speciation.

### Introduction

The ant genus *Cyphomyrmex* is of special interest for understanding the evolution of agriculture in fungus-growing ants (subfamily Myrmicinae, tribe Attini) for two primary reasons:

(1) Cyphomyrmex occupies an intermediate phylogenetic position between the "lower" and "higher" (including the leafcutting) attine ants, and is possibly the sister group of the higher attine ants (Schultz and Meier, 1995; Schultz, 1998; Schultz, 2000; TRS, unpubl.); and (2) within Cyphomyrmex there are two distinct forms of fungus cultivation, typical mycelium cultivation and "yeast" cultivation (Wheeler, 1907; Weber, 1972; Mueller et al., 2001); in the latter, the cultivar is maintained in a single-celled growth phase and the garden consists of modular, easily transportable nodules. Based on morphological characters, the genus is divided into two informal subgeneric groups, the "strigatus" and "rimosus" groups (Kempf, 1966). Preliminary data indicate that the strigatus group contains only mycelium cultivators and is probably plesiomorphic and paraphyletic with respect to the rimosus group. In contrast, the rimosus group contains both mycelium and yeast cultivators, is morphologically and behaviorally derived, and is likely monophyletic (Kempf, 1966; Schultz and Meier, 1995; Meier and Schultz, 1996; TRS, unpubl.).

*C. longiscapus* is one of three species (along with *C. costatus* and *C. wheeleri*) that violate Kempf's (1966) otherwise tidy bipartite division of *Cyphomyrmex* because these species combine morphological features of both subdivisions. Although the fungal cultivars of most *Cyphomyrmex* species are unknown, Kempf (1966) points out an intriguing preliminary pattern: The three species that combine the features of both groups are also the only species in the *rimosus* group that are known to cultivate mycelium gardens, whereas, so far as is known, all other *rimosus* group species cultivate yeast gardens. This pattern suggests that *C. longiscapus* and related

species may occupy a pivotal phylogenetic position in the evolutionary transition from mycelium to yeast cultivation, recommending *C. longiscapus* as a subject for intensive biological investigation.

In the course of field studies conducted from 1995 to the present, over 550 nests and gardens that were initially identified as belonging to a single fungus-growing ant species were collected in multiple locations in Panama (see below and Appendix). These specimens key out to Cyphomyrmex longiscapus in the keys of Weber (1940), Kempf (1966), and Snelling and Longino (1992). Prior to these Panamanian field studies, C. longiscapus had been recorded from only three collection series, two from Colombia (Weber, 1940; Kempf, 1966; Snelling and Longino, 1992) and one from Panama, the latter consisting of two specimens noted by Kempf (1966: p. 167) as "aberrant" but possibly referable to the species. Working in Panama, Mueller and Wcislo (1998) found that, although nearly absent from other microhabitats, C. longiscapus nests are in fact common on steep clay embankments associated with small streams, and are easily located because they possess characteristic, ear-shaped funnel entrances ("auricles"). Thus, contrary to its previously presumed rarity, Mueller and Wcislo (1998: p. 182) call the Panamanian C. longiscapus "the most easily collected species of all attine ants," and recommend it as "an ideal candidate for a model species of lower attines" (p. 188).

The goals of this study are: (1) to summarize the molecular and morphological data that distinguish the two species currently known to comprise the Panamanian C. "longiscapus" s.l. complex; (2) to update the taxonomy of C. longiscapus sensu stricto and to describe the new species; (3) to summarize the morphometrics, distribution, and biology of both species; and (4) to facilitate easy differentiation of these species in the laboratory and the field. The goal of facilitating identification is especially important because, as noted above, C. longiscapus and its relatives are of special biological interest and, as recommended by Mueller and Wcislo (1998), have truly become model organisms in diverse behavioral, ecological, and evolutionary studies (Mueller et al., 1998; Currie et al., 1999 a, 1999b; Villesen et al., 1999; Adams et al., 2000 a, 2000b; Green et al., 2002; Mueller et al., in press; Villesen et al., in press; Adams et al., unpubl.; Mehdiabadi and Mueller, unpubl.; Villesen et al., unpubl.).

#### Methods

From 1995 to the present, over 550 queenright colonies of *Cyphomyrmex* "*longiscapus*" *s.l.* were collected in multiple locations in Panama (see Appendix). Cultivated fungi from a subset of these nests were initially screened using RFLP methods and found to fall into two distinct groups (Mueller et al., 1998). Subsequent analyses of rDNA sequences corroborated these groupings, and indicated that the two cultivar groups are phylogenetically distant, representing at least two distinct fungal species (Mueller et al., 1998). These results led to the initial conclusion that *C.*" *longiscapus*" *s.l.*, then regarded as a single ant species, was polymorphic for cultivar usage.

In a separate study of worker relatedness and queen mating frequency, a subset of ants was screened for variable allozyme loci. Allozyme analyses of selected individuals from a subset of the 80 nests were carried out with 12% horizontal starch gel electrophoresis. All gels

 Table 1. Genotype frequencies (grouped by species) for four allozyme loci

	C. longiscapus C. muelleri		Combined						
Genotype	11	12	22	11	12	22	11	12	22
IDH (n=80) GPD (n=60) HK (n=50) GPI (n=79)	1.00 1.00 1.00 0.46	0.32	0.22			1.00 1.00 1.00 1.00	0.46 0.50 0.46 0.22	0.15	0.54 0.50 0.54 0.63

were run in a Tris (27 g/l) – Citric acid (18.07 g/l) tray buffer (pH 6.3) for 4 hours at 60 mA. Four different loci were used for the analysis: Isocitrate dehydrogenase (IDH), Glycerol-3-phosphate dehydrogenase (GPD), Hexokinase (HK) and Glucose-6-Phosphate Isomerase (GPI). All four loci segregated for two alleles: slow (1) and fast (2). Three of these loci (IDH, GPD and HK) are monomorphic for alternate alleles in a consistent pattern that reliably separates the ants into two distinct groups (Table 1). Ants, including individuals of both groups collected at the same localities, were never found to be heterozygous at these three loci. With respect to the pattern supported by the first three loci, the fourth locus (GPI) is polymorphic in one group and monomorphic in the other. The pattern in the GPI locus is thus consistent with the pattern in the first three loci, but, considered alone, is useful for separating only a subset of individuals into the two allozyme groups. (Table 1). Data of this kind constitute unambiguous evidence of reproductively isolated gene pools, which, when occurring sympatrically, represent separate species (Boomsma et al., 1990; Schultz et al., 1998). Additional data from microsatellites (Villesen et al., unpubl.) and AFLP markers (Adams et al., unpubl.) corroborate this conclusion.

When the results of the allozyme analyses were compared with analyses of the fungal cultivars, it was found that they were perfectly correlated, i.e., all ants in one of the allozyme groups consistently cultivate one fungal cultivar, whereas all ants in the other group cultivate the alternate cultivar. Thus, contrary to an earlier conclusion (Mueller et al., 1998), cultivar and ant genetic markers reveal the existence of two ant species, each specialized on its own distinct fungal cultivar.

Working separately from and without direct reference to the allozyme results, morphological examination of individuals from a subset of Panamanian C. "longiscapus" s.l. nests (workers, females, and males; see Appendix) was undertaken in order to identify morphological character-state differences, if any, that reliably separate C. "longiscapus" s.l. into two or more species. Specimens were also measured for three parameters commonly employed in ant systematic studies (Brown, 1953): head length (HL), head width (HW), and Weber's length (WL) (Table 2). In the course of the morphological research, all known specimens of C. "longiscapus" s.l. (including material from Costa Rica, Colombia, and Ecuador) were assembled and compared to specimens from Panama. This material was borrowed from the following collections: Museum of Comparative Zoology, Harvard (MCZ); National Museum of Natural History (USNM); Los Angeles County Museum (LACM); Muséu de Zoología da Universidade de São Paulo, Brazil (MZSP, including the W.W. Kempf Collection); and Instituto Nacional de Biodiversidad, Heredia, Costa Rica (INBC). Measurements follow Brown (1953); terminology follows Bolton (1994), Harris (1979), Kempf (1966), and Snelling and Longino (1992). Two species were identified, C. longiscapus s.s. and a second, undescribed species. The name and description of the new species were produced by a subset of the authors (TRS and SAS); the description appears below under the heading "Cyphomyrmex muelleri Schultz and Solomon, new species.'

#### Taxonomy

Data from morphological characters and from three allozyme loci are perfectly correlated and distinguish the same two distinct ant species.

	HL (mm)	HW (mm)	WL (mm)
Workers			
<i>C. longiscapus</i> workers (Panama) N=52 (26 nests, 2 workers per nest)	$\begin{array}{c} 0.80 \pm 0.005 \\ (0.70 - 0.86) \end{array}$	$\begin{array}{c} 0.62 \pm 0.004 \\ (0.55 - 0.67) \end{array}$	$\begin{array}{c} 1.03 \pm 0.007 \\ (0.90 {-} 1.12) \end{array}$
<i>C. longiscapus</i> workers (Panama) N=20 (1 nest)	$\begin{array}{c} 0.80 \pm 0.005 \\ (0.75 - 0.84) \end{array}$	$\begin{array}{c} 0.64 \pm 0.003 \\ (0.60 - 0.66) \end{array}$	$\begin{array}{c} 1.03 \pm 0.003 \\ (0.96 - 1.10) \end{array}$
<i>C. longiscapus</i> workers (Colombia) N=21 (minimum of 3 nests)	$\begin{array}{c} 0.92 \pm 0.006 \\ (0.85 - 0.97) \end{array}$	$\begin{array}{c} 0.72 \pm 0.010 \\ (0.64 - 0.79) \end{array}$	$\begin{array}{c} 1.24 \pm 0.013 \\ (1.11 - 1.37) \end{array}$
<i>C. longiscapus</i> worker (Costa Rica) N=1	0.91	0.72	1.22
<i>C. muelleri</i> workers (Panama) N=56 (28 nests, 2 workers per nest)	$\begin{array}{c} 0.82 \pm 0.004 \\ (0.74 - 0.88) \end{array}$	$\begin{array}{c} 0.63 \pm 0.004 \\ (0.58 - 0.70) \end{array}$	$\begin{array}{c} 1.08 \pm 0.007 \\ (0.96 - 1.18) \end{array}$
<i>C. muelleri</i> workers (Panama) N=20 (1 nest)	$\begin{array}{c} 0.85 \pm 0.004 \\ (0.80 - 0.89) \end{array}$	$\begin{array}{c} 0.64 \pm 0.004 \\ (0.61 - 0.67) \end{array}$	$\begin{array}{c} 1.12 \pm 0.007 \\ (1.06 - 1.18) \end{array}$
<i>C. muelleri</i> worker (Ecuador) N=1	0.79	0.61	1.06
Gynes			
C. longiscapus gynes (Panama) N=25 (20 nests)	$\begin{array}{c} 0.89 \pm 0.003 \\ (0.85 - 0.93) \end{array}$	$\begin{array}{c} 0.72 \pm 0.005 \\ (0.67 - 0.79) \end{array}$	$\begin{array}{c} 1.23 \pm 0.008 \\ (1.15 - 1.29) \end{array}$
<i>C. longiscapus</i> gynes (Colombia) N=2 (1 nest) (alate, dealate)	0.97, 0.99	0.76, 0.76	1.37, 1.38
<i>C. muelleri</i> gynes (Panama) N=23 (20 nests)	$\substack{0.90 \pm 0.003 \\ (0.87 - 0.93)}$	$\begin{array}{c} 0.70 \pm 0.003 \\ (0.68 - 0.73) \end{array}$	$\begin{array}{c} 1.23 \pm 0.006 \\ (1.18 - 1.27) \end{array}$
Males			
<i>C. longiscapus</i> males (Panama) N=25 (23 nests)	$\begin{array}{c} 0.60 \pm 0.005 \\ (0.55 - 0.66) \end{array}$	$\begin{array}{c} 0.56 \pm 0.009 \\ (0.46 - 0.62) \end{array}$	$\begin{array}{c} 1.05 \pm 0.010 \\ (0.94 - 1.18) \end{array}$
<i>C. longiscapus</i> male (Colombia) N=1	0.68	0.62	1.22
<i>C. muelleri</i> males (Panama) N=29 (28 nests)	$\begin{array}{c} 0.63 \pm 0.005 \\ (0.58 - 0.69) \end{array}$	$\begin{array}{c} 0.56 \pm 0.011 \\ (0.46 - 0.62) \end{array}$	$\begin{array}{c} 1.07 \pm 0.010 \\ (0.99 - 1.19) \end{array}$

Table 2. Mean values, standard errors, ranges (in parentheses), and in four cases, individual measurements of head length (HL), head width (HW), and Weber's length (WL), in millimeters, for workers, gynes (queens), and males of the two species from the Panamanian nest series, and for workers and gynes of all known non-Panamanian specimens. Eyes were included in the measurement of head width in males

Fortunately, the two species are consistently distinguishable on the basis of discrete morphological character states, and these provide the most practical means for differentiating the two species. The species are also distinguishable based on statistically significant body size differences, although, because of broad overlap in these parameters between individuals of the two species, these differences are of little practical value for specimen identification. The two species also differ in nest entrance "auricle" morphology (Fig. 8; Table 3). One of the species corresponds to *Cyphomyrmex longiscapus* Weber; the other is new to science.

#### Cyphomyrmex longiscapus Weber 1940

LECTOTYPE (reexamined by TRS): Worker (MCZ). Designated by Kempf (1966). Colombia: Rio Porce; 1020 m (3200'); nesting in rain forest; Weber No. 1088; 3 Aug 1938; N.A. Weber, collector. Based on a handwritten note accompanying Weber-collected vials in the MCZ, the type series was collected at 6°40'N, 75°10'W. Measurements (in mm, remeasured by TRS): HL=0.90; HW=0.64; WL=1.26; SL=0.79; maximum diameter of eye=0.15; hind femur length=1.14. Note that in the original description, Weber (1940, p. 407) reports a collection time period of June-August 1938 and that Kempf (1966) mistakenly reports the collection date as "Nov. 1938."

Originally described by Weber (1940), *C. longiscapus* is unique within the genus. Of its distinctness, Kempf (1966, p. 167) cited, in addition to the uniquely elongate scapes (which surpass the occipital corners), the characters of the weakly expanded frontal lobes, elongate mandibles, and neck-like collar on the occiput. Unfortunately, all of these character states are shared with the new species described below, and thus both species run to *C. longiscapus* in the keys of Weber (1940), Kempf (1966), and Snelling and Longino (1992). The information provided here supplements information provided by these authors and is sufficient for distinguishing *C. longiscapus s.s.* from *Cyphomyrmex* new species.

**Table 3.** Mean values, standard errors, ranges (in parentheses), and calculated ratios (auricle height/auricle width), in millimeters, for nest entrance auricles of the two species taken from Panamanian nests

	Auricle height (mm)	Auricle width (mm)	Height/Width
C. longiscapus N=55	$23.10 \pm 0.98 \\ (9.7 - 41.4)$	$\begin{array}{c} 19.05 \pm 0.75 \\ (9.2 - 33.55) \end{array}$	$\begin{array}{c} 1.24 \pm 0.04 \\ (0.68 - 1.96) \end{array}$
C. muelleri N=24	$\begin{array}{c} 16.65 \pm 0.88 \\ (8.15 - 27.05) \end{array}$	$20.03 \pm 1.13 \\ (10 - 33.45)$	$\begin{array}{c} 0.90 \pm 0.08 \\ (0.36 - 1.86) \end{array}$



Figure 1. Worker head, frontal (dorsal) view. VC=vertexal carina. (a.) C. longiscapus (Panama) (b.) C. muelleri (Panama)

Figure 2. Worker, lateral view. PT=posterior metanotal tubercles; MG=metanotal groove; IG=third intersegmental groove of the thorax. (a.) *C. longiscapus* (Panama). (b.) *C. muelleri* (Panama)

Figure 3. Worker postpetiole, dorsal view. PT=postpetiolar tubercle. (a.) C. longiscapus (Panama). (b.) C. muelleri (Panama)

Figure 4. Worker posterior leg, posterior view. FL=metafemoral lobe. (a.) C. longiscapus (Panama). (b.) C. muelleri (Panama)

WORKERS (diagnosis): Measurements as in Table 2. Possessing 11 antennal segments and palpal formula 4, 2 (features plesiomorphic for, and widespread in, the Attini). Color ranging from yellow to testaceous to fuscous brown. Head and alitrunk uniformly foveate, each fovea usually surrounded by a circlet of whitish "bloom" that resembles the attine actinomycete symbiont (Currie et al., 1999a), the extent of this bloom highly variable across individuals: It may be entirely absent; it may be present as small, separate circlets, creating a discontinuous pattern; or it may be present as a continuous integumental blanket formed of larger, overlapping circlets. Pilosity inconspicuous, fine, thin, silvery, and decumbent.

The following characters reliably separate *C. longiscapus* from its cryptic sister species (described below): Vertexal carinae (i.e., paired carinae on the vertex, running parallel to and on either side of the mid-

line) strongly produced (Fig. 1a, VC). Metanotal groove ("mesoepinotal impression" of Kempf) deep, clearly interrupting the continuity of the alitrunk in lateral view (Fig. 2a, MG). Third intersegmental groove of the thorax, separating the mesopleuron from the metapleuron, complete, extending from the metanotal groove to between the coxae, although obscured in some specimens by the whitish integumental "bloom" (the attine actinomycete symbiont?) (Fig. 2a, IG). Posterior tubercles on the postpetiolar dorsum forming low, rounded tumuli (Fig. 3a, PT); in dorsal view, the postpetiole only slightly emarginate and broadly and shallowly impressed posteriorly (Fig. 3a). Hind femur lacking ventral carinae and a ventral lobe (Fig. 4a, arrow).

The following characters are generally useful for distinguishing *C*. *longiscapus* from the new species, but, because a minority of specimens in both species possess intermediate states, they are less reliable than the



Figure 5. Gyne head, frontal (dorsal) view. VC=vertexal carinae. (a.) *C. longiscapus* (Panama). (b.) *C. muelleri* (Panama)
Figure 6. Male, lateral view. PS=propodeal spine. (a.) *C. longiscapus* (Panama). (b.) *C. muelleri* (Panama)
Figure 7. Male postpetiole, dorsal view. PT=postpetiolar tubercle. (a.) *C. longiscapus* (Panama). (b.) *C. muelleri* (Panama)

characters listed above and, therefore, should be used for identification only with caution: Whitish integumental "bloom" (actinomycete symbiont?), when present, rarely occurring within the antennal scrobe. Frontal triangle usually broad, usually forming a finger-shaped area between the frontal lobes that is rounded posteriorly. Posterior mesonotal tubercles usually strongly produced (Fig. 2a, PT), but variable, more weakly produced in some specimens. Propodeal angle usually present, with dorsal and declivous faces usually separated by a distinct shoulder.

GYNES (diagnosis): Measurements as in Table 2. Like the workers, possessing 11 antennal segments, palpal formula 4, 2, and the unique *C. longiscapus s.l.* characters of the elongate antennal scapes and weakly expanded frontal lobes. Color, microsculpture, integumental bloom, and pilosity as in the workers.

*C. longiscapus* gynes generally differ from those of the new species in the same character states as the workers except, obviously, for those of the alitrunk. The most reliable characters include: Vertexal carinae strongly produced (Fig. 5 a, VC). Posterior tubercles on the postpetiole forming low, rounded tumuli (as in worker, Fig. 3 a, PT); in dorsal view, the postpetiole only slightly emarginate and broadly and shallowly impressed posteriorly (as in worker, Fig. 3 a). Hind femur lacking ventral carinae and lobe (as in worker, Fig. 4 a, arrow).

Somewhat less reliable, more continuously varying characters include: Frontal triangle usually broad, usually forming a finger-shaped area between the two lobes that is rounded posteriorly. Whitish integumental "bloom" (actinomycete symbiont?) rarely present within the antennal scrobe.

MALES (diagnosis): Measurements as in Table 2. Possessing 13 antennal segments and palpal formula 4, 2. Mandibles with four or five teeth, the basal (fifth) tooth sometimes reduced to a rounded basal angle. As in the workers and gynes, head and alitrunk rather uniformly foveate, the foveae occasionally surrounded by a whitish "bloom"; pilosity as in the other castes. Based on three dissections (one male from each of three Panamanian nests), male genitalia conform closely to the plesiomorphic attine pattern: parameres simple, forming short, rounded, concave lobes; aedeagus simple, forming a broad rounded lobe with minute teeth along the ventral edge; and volsella with digitus simple, long, narrow, and strongly recurved. The only departure from the plesiomorphic attine pattern (in which the volsellar cuspis is absent) is that the cuspis is present as a short, simple, rounded lobe.

In general, males of *C. longiscapus* are difficult to distinguish from males of the new species, but the most reliable characters include: Postpetiole in dorsal view weakly emarginate posteriorly (Fig. 7a); and the propodeal spines short, the width at the base of the spine exceeding the total spine length (Fig. 6a, PS). In all *C. longiscapus* males examined, the head and usually the dorsum of the alitrunk are more darkly pigmented (testaceous to fuscous brown) than the rest of the body, which is yellow, a pattern suggesting a day-flying species.



Figure 8. Nest entrances. (a.) C. longiscapus (Panama): "swallow's-nest" form. (b.) C. longiscapus (Panama): auricle form. (c.) C. muelleri (Panama)

NEST ARCHITECTURE (diagnosis): Nest entrance architecture differs enough between *C. longiscapus* and the new species to constitute a generally useful character for distinguishing between nests of the two species in the field. *C. longiscapus* constructs nest entrances of two kinds: the swallow's-nest type, reported from Colombia and Panama (Fig. 8 a; Weber, 1972; Mueller and Wcislo, 1998), and the auricle type, reported from Panama (Fig. 8b). Auricle dimensions are summarized in Table 3. In *C. longiscapus*, nest entrance auricles are usually higher than wide (i.e., longer in the vertical than in the horizontal dimension), and auricles are strikingly flared, i.e., they are much broader across the rim than across the base.

Distribution: *C. longiscapus* is currently known from the wet forests of Colombia, Panama, and Costa Rica.

Paratypes examined (10 total): 2 workers (LACM), 4 workers (MCZ), 1 worker (MZSP, W.W. Kempf Collection), 1 alate gyne (MCZ), 1 dealate gyne (MCZ), 1 male (MCZ): Colombia: Rio Porce; 1020 m; nesting in rain forest; No. 1088; 3-viii-1938; N.A. Weber, coll.

Other (non-type) non-Panamanian specimens examined (14 total): 5 workers (LACM): Colombia: Choló Quebrada Bolindramá; 1968; Coll. Silverstone; LACM 43800; stomach contents *Phyllobates aurotaenia* (Amphibia); No. M29. 2 workers (LACM) and 6 workers (MZSP): Colombia, Valle; Anchicayá, Municipo Buenaventura; ca. 2000 m; rain forest, under rock in canyon; 19-vi-1971; W.L. Brown, Jr. collector. 1 worker (INBC): Costa Rica: Herédia; Est. Biol. La Selva; 50–150 m; 10°26'N, 84°01'W; Sept 1992; INBio-OET; INBio CRI001237776; Longino No. 3328; J. Longino, Collector. Snelling and Longino (1992) list a second Colombian series (LACM) from the stomach contents of the frog *Dendrobates histrionicus*, which was not located for this study.

Panamanian specimens examined: 931 workers (105 nests), 192 gynes (72 nests), and 180 males (49 nests); see Appendix.

#### Cyphomyrmex muelleri Schultz and Solomon, new species

HOLOTYPE (worker): Republic of Panama: Barro Colorado Island; 14 February 1996; U.G. Mueller, collector; Nest series: UGM960214–05. Measurements (in mm): HL=0.85; HW=0.65; WL=1.08; SL=0.79; hind femur length=1.02; greatest diameter of eye=0.17. USNM.

WORKERS (diagnosis): Measurements as in Table 2. Possessing 11 antennal segments and palpal formula 4, 2. Color ranging from yellow to testaceous to fuscous brown. Head and alitrunk uniformly foveate, each fovea usually surrounded by a circlet of whitish "bloom" that resembles the attine actinomycete symbiont (Currie et al., 1999a), the extent of this bloom highly variable across individuals as described above for *C. longiscapus*. Pilosity inconspicuous, fine, thin, silvery, and decumbent.

Keying out to C. longiscapus, to which it is very closely related, in the keys of Weber (1940), Kempf (1966), and Snelling and Longino (1992), and sharing with that species the uniquely elongate antennal scapes and weakly expanded frontal lobes. Distinguishable by the following criteria: Vertexal carinae (i.e., paired carinae on the vertex, running parallel to and on either side of the midline) vestigial or absent (in C. longiscapus, strongly produced) (Fig. 1b, VC). Dorsum of the alitrunk in lateral profile smoother and more continuous than in C. longiscapus. In particular, the metanotal groove ("mesoepinotal impression" of Kempf) obsolete, present only as a shallow transverse line (Fig. 2b, MG), so that, in lateral view, the dorsum of mesonotum and propodeum are continuous and uninterrupted by a deep suture such as is present in C. longiscapus. Third intersegmental groove of the thorax (separating the mesopleuron from the metapleuron) incomplete, present dorsally as the vestigial metanotal groove and laterally just above the coxae, but absent in between (Fig. 2b, IG); in C. longiscapus the groove is complete (Fig. 2a, IG). Posterior tubercles of the postpetiole produced into strong denticles (Fig. 3b, PT), noticeably protruding posterad such that the postpetiole is strongly posteriorly emarginate in dorsal view (Fig. 3b); in C. longiscapus, the postpetiole is weakly emarginate (Fig. 3 a). Hind femur with a pair of strong ventral carina, forming a groove for the reception of the tibia and produced in the basal one-third into a strong ventro-posterior lobe (Fig. 4b, FL), apparently for the protection of the joint between the tibia and tarsus. The ventral femoral groove thus receives the tibia distally and the tarsus basally. Carinae and lobe absent in C. longiscapus (Fig. 4a, arrow).

The following characters are generally useful for distinguishing *C. muelleri* from *C. longiscapus*, but, because a minority of specimens in both species possess intermediate states, these additional characters are somewhat less reliable for the identification of *C. muelleri:* Whitish integumental "bloom" (actinomycete symbiont?), when present elsewhere on the body, also occurring within the antennal scrobe (usually absent in *C. longiscapus*). Frontal triangle usually laterally compressed, forming a narrow acute triangle or a linear impression (broad and finger-shaped in *C. longiscapus*). Posterior mesonotal tubercles absent or present as weak carinae (Fig. 2b, PT); usually well developed in *C. longiscapus*. Propodeal angle usually absent; the dorsal and declivous faces merged into a continuous curve; propodeal angle usually present in *C. longiscapus*.

GYNES (diagnosis): Measurements as in Table 2. As in the worker, possessing 11 antennal segments, palpal formula 4, 2, and the *C. longiscapus s.l.* worker/gyne characters of the elongate antennal scapes and weakly expanded frontal lobes. Color, sculpture, integumental bloom, and pilosity as in the workers.

Generally differing from *C. longiscapus* in the same character states as the workers except, obviously, for those of the alitrunk. Vertexal carinae vestigial or absent (Fig. 5 b, VC). Postpetiole in dorsal view strongly emarginate posteriorly (as in worker, Fig. 3b). Hind femur with strong ventral carinae and lobe (as in worker, Fig. 4b, FL).

MALES (diagnosis): Measurements as in Table 2. Antennae with 13 antennal segments, palpal formula 4, 2. Mandibles with four or five teeth, the basal (fifth) tooth sometimes reduced to a rounded basal angle. As in the workers and gynes, head and alitrunk rather uniformly foveate, the foveae occasionally surrounded by circlets of whitish "bloom"; pilosity as in the other castes.

Males of *C. muelleri* are generally difficult to distinguish from males of *C. longiscapus*, but they differ in the following characters: As in workers and gynes, the postpetiole of *C. muelleri* males in dorsal view is strongly emarginate posteriorly (Fig. 7b); in *C. longiscapus* males, the postpetiole is very weakly emarginate (Fig. 7a). Propodeal spines longer than those of *C. longiscapus* males (Fig. 6a, PS), the total spine length exceeding the spine width at the base (Fig. 6b, PS). All males of *C. muelleri* examined are uniformly yellow/orange in color, with darker pigmentation restricted to the integument immediately surrounding the ommatidia, as might be expected from a nocturnal or crepuscular flier. Based on three dissections (a male from each of three nests), there is no difference between *C. muelleri* and *C. longiscapus* in male genitalic morphology (as described above for *C. longiscapus*).

NEST ARCHITECTURE (diagnosis): Nest entrance architecture constitutes a generally useful field character for distinguishing between nests of *C. muelleri* and *C. longiscapus*. All nest architecture observations for *C. muelleri* originate from Panama and, as far as is known, *C. muelleri* constructs nest entrances of the auricle type only (Fig. 8 c), and not of the swallow's-nest type (Fig. 8 a). Auricle dimensions are summarized in Table 3. In *C. muelleri*, nest entrance auricles are "mouthlike," usually wider than high (i.e., longer in the horizontal than in the vertical dimension), and, rather than flared, the auricle rim is merely swollen or thickened beyond the circumference of the base (Fig. 8 c).

In his discussion of C. longiscapus, Kempf (1966, p. 167) mentions two specimens collected by W.L. Brown, Jr., and E.S. McCluskey in January 1960 on Barro Colorado Island, Panama (collection no. B-55), which "although basically resembling, I hesitate in definitely ascribing to the present species" [i.e., to C. longiscapus]. Although these specimens (a worker and a gyne) could not be located and thus could not be examined for the present study, they are assignable to C. muelleri based on: (1) the fact that so far only C. muelleri – and not C. longiscapus has been collected on Barro Colorado Island (UGM, pers. obs.); and, more importantly, (2) Kempf's succinct description of the worker: "Mesonotum having only the anterior pair of tubercles developed, the rest being flat ... Mesoepinotal impression obsolete ... Hind femora ventrally lobate and carinate on basal third ... [Postpetiole] with a deeper middorsal impression, stronger posterior paired tubercles, which project beyond the mesially deeply excised posterior border" (Kempf, 1966: p. 167). Kempf's reported worker head measurements (HL=0.72 mm, HW=0.56 mm) fall slightly outside the observed lower range for C. muelleri, but reported worker Weber's length (1.01 mm) and all gyne measurements (HL=0.88 mm; HW=0.69 mm; WL=1.22 mm) fall within the observed ranges (Table 2).

*Distribution: C. muelleri* is currently known almost entirely from the wetter forests of central Panama, but a single specimen collected in wet forest in Ecuador (see below) indicates that this species (or a cryptic, closely related species) also occurs in South America.

*Paratypes*: Panama: 868 workers (67 nests), 253 gynes (52 nests), and 385 males (40 nests); see Appendix. Ecuador: 1 worker.

*Collection data*: Panamanian specimens: see Appendix. Ecuadorian specimen: 1 worker (MZSP): Ecuador: Esmeraldas; 10 km. south of Atacames; isca no solo ("bait on ground"); 7-xi-1987; C.R.F. Brandão and C.D. Bastidas, collectors. This specimen was taken in wet forest at sardine bait (C.R.F. Brandão, pers. comm.).

Specimen deposition: USNM (holotype, paratypes), MCZ (paratypes), LACM (paratypes), MZSP (paratypes), BM (paratypes), CASC (paratypes).

*Etymology*: It gives us great pleasure to name this species in honor of our friend and colleague Ulrich G. Mueller, who, through diligent field work and exemplary biological study, has pioneered this ideal group of model organisms for attine research.

#### Results

#### **Morphometrics**

WORKERS: Based on measurements taken from 52 C. longiscapus workers from 26 nests and from 56 C. muelleri workers from 28 nests from Panama (Table 2), C. muelleri workers are on average significantly larger than C. longiscapus workers in both head and body length, but only marginally larger in head width (two-tailed t test on average values: HL: t=3.959, df=105, P<0.001; HW: t=1.975, df=104, P=0.0510; WL: t=4.435, df=105, P<0.0001). A comparison of 20 workers from a single nest of C. longiscapus (Panama) with 20 workers from a single nest of C. muelleri (Table 2) follows the same pattern (two-tailed t test on average values: HL: t=6.804, df=37, P<0.0001; HW: t=0.458, df=35, P=0.650; WL: t=7.542, df=36, P<0.0001). Though demonstrably real and biologically interesting, size difference is of little practical value for separating the two species, at least in Panama.

Based on measurements taken from 21 Colombian C. longiscapus workers from three nests and from 52 Panamanian C. longiscapus workers from 26 nests (Table 2), Colombian C. longiscapus workers are significantly larger than Panamanian C. longiscapus workers (two-tailed t test on average values: HL: t=15.815, df=46, P<0.0001; HW: t=9.178, df=27, P<0.0001; WL: t=14.798, df=32, P < 0.0001). Based on measurements from 56 C. muelleri workers taken from 28 nests (Table 2), Colombian C. longiscapus workers are also larger than C. muelleri workers (twotailed t test on average values: t=12.848, df=43, P<0.0001; HW: t=8.214, df=26, P<0.0001; WL: t=11.685, df=33, P < 0.0001). There is minimal overlap in the ranges of these three parameters between the known Colombian and Panamanian C. longiscapus populations, and a greater overlap between the ranges of the Colombian C. longiscapus and C. muelleri. Curiously, the single known Costa Rican specimen of C. longiscapus, a worker, is much closer in size to the Colombian C. longiscapus than it is to the Panamanian C. longiscapus specimens (Table 2). Measurements from the single known non-Panamanian C. muelleri worker specimen, from Ecuador, fall within the ranges of the Panamanian specimens of this species (Table 2).

Visual inspection suggests that, of the three Colombian *C. longiscapus* specimen series, the Silverstone series (5 workers taken from the stomach of a poison-dart frog) are consistently larger than the other 16 Colombian worker specimens. Statistical comparisons indicate that, indeed, the Silverstone workers have longer heads and bodies than the other Colombian worker specimens (two-tailed t test on average values: HL: t=6.951, df=17, P<0.0001; HW: t=1.203, df=18, P=0.245; WL: t=3.700, df=15, P<0.01). GYNES: Based on measurements of 25 Panamanian *C. longiscapus* gynes from 20 nests and of 23 *C. muelleri* gynes from 20 nests (Table 2), Panamanian *C. muelleri* gynes have longer and slightly wider heads than do *C. muelleri* gynes, but there is no difference in body length (two-tailed t test on average values: HL: t=2.562, df=45, P=0.014; HW: t=2.041, df=35, P=0.049; WL: t=0.070, df=45, P=0.944). As with worker measurements, these marginal size differences are obviously of little practical value for identifying specimens. Based on measurements taken from two gynes from the same nest (Table 2), Colombian *C. longiscapus* gynes from Panama, a pattern parallelling that in the workers.

MALES. Based on measurements of 25 Panamanian *C. longiscapus* males from 23 nests and of 29 *C. muelleri* males from 28 nests (Table 2), *C. muelleri* males have on average longer heads than Panamanian *C. longiscapus* males, but do not differ in other dimensions (two-tailed t test on average values: HL: t=3.762, df=52, P<0.001; HW: t=0.244, df=52, P=0.808; WL: t=1.023, df=51, P=0.311.) In parallel with workers and gynes, the only known non-Panamanian male of *C. longiscapus*, from the paratype series, is slightly larger than the largest *C. longiscapus* and *C. muelleri* males from Panama.

NEST ARCHITECTURE: Based on measurements of 55 C. longiscapus and 24 C. muelleri nest entrances from Panama (Table 3), C. longiscapus entrance auricles are on average higher than wide (i.e., vertically elongate), whereas C. muel*leri* auricles are wider than high (i.e., horizontally elongate) (t=-3.862, df=37, P<0.001) (Figs. 8b and 8c). Because there is some overlap in the ratio of auricle height to width across the two species (Table 3), this difference obviously needs to be used with caution as a field character for distinguishing nests of the two species. However, of 54 additional nests of C. longiscapus and C. muelleri examined in spring of 2001 at Pipeline Road in Panama, 50 were classified in the field as either C. longiscapus (22 nests assigned) or C. muelleri (28 nests assigned); the auricles of the remaining four of the 54 nests were judged to be "intermediate" in shape and thus did not allow clear assignment to either species. Using only auricle architecture as a criterion, all of the 22 C. longiscapus nests were correctly identified to species, whereas 27 of the 28 C. muelleri nests were correctly identified. In practice, therefore, auricle shape (summarized as the ratio of vertical to horizontal diameter; Table 3 and Figs. 8a and 8b) is quite reliable for species identification, especially when combined with the other differences discussed above.

#### Ecology and behavior

What little knowledge we have of the ecology of *C. longiscapus* in Colombia is consistent with the ecology of the far better studied Panamanian *C. longiscapus* and *C. muelleri* (Mueller and Wcislo 1998; UGM, unpublished). Although no ecological data are reported in Weber's original (1940) description of *C. longiscapus*, a subset of the paratype

labels indicates that the colony was taken at 1020 m elevation (another label indicates 3200 ft); one paratype label specifies "nesting in rain forest"; and Weber's notes indicate a collection location of 6°40'N, 75°10'W. Weber (1972) supplies a verbal description and a photograph of the Colombian C. longiscapus type series nest, and provides the additional information that the nest was collected in "a deep, moist ravine in the Andes of Colombia" (p. 57), consistent with Mueller and Wcislo (1998), who surveyed 203 nests of C. longiscapus s.l. in Panama and documented a nesting preference for steep embankments along permanent streams. Label data accompanying Brown's 1971 Colombian collection of C. longsicapus indicate that the nest was encountered at 2000 m elevation in rain forest. The only known Costa Rican specimen, a lone worker, was found inside a dead stick on the ground of an old alluvial terrace close to a river (J. Longino, pers. comm.). Colombian C. longiscapus specimens have been identified from the stomach contents of two poisondart frogs, Phyllobates aurotaenia and Dendrobates histrionicus (Snelling and Longino, 1992). The only known non-Panamanian C. muelleri specimen was taken in wet forest at sardine bait in Ecuador (C.R.F. Brandão, pers. comm.).

Both species are monogynous and perennial (Mueller and Wcislo, 1998; Villesen et al., unpubl.), queens are singly mated (Villesen et al., 1999; Adams et al., unpubl.; Villesen, unpubl.), and both cultivate mycelium gardens (Mueller and Wcislo, 1998; Mueller et al., 1998). Nests consist of a single garden chamber and are usually constructed in steep embankments under the shelter of overhangs or set back underneath the shelter of a rock or root. Nest entrance architecture is complex and characteristic (Fig. 8). In Panama, C. longiscapus constructs nests of two distinct, but intergraded, morphologies: (1) the hanging, baglike "swallow's nest" type (Fig. 8a), sometimes suspended from rock faces; and (2) the "auricle" type (Fig. 8b). Both of these nest types have pronounced, vertically elongate auricle-shaped entrances, but, whereas the "swallow's nest" type is suspended and the garden chamber is surrounded at the sides by thin (approximately 2 to 5 mm thick) walls constructed by the ants from clay, the simpler "auricle" type nest is set into the soil, the lateral walls of the excavated garden chamber are natural, and only the front walls surrounding the auricle are constructed by the ants. The single described non-Panamanian C. longiscapus nest, from Colombia, was of the swallow's-nest type (Weber, 1972; Mueller and Wcislo, 1998). C. muelleri constructs nests of the auricle type only, but significant differences separate the auricle-type nests of the two species: Only C. longiscapus constructs large, flaring nest auricles that are generally longer in the vertical than in the horizontal dimension (Fig. 8b; see also Fig. 1 in Mueller and Wcislo, 1998). C. muelleri, in contrast, constructs "mouthlike" auricles that possess swollen or thickened rather than flared rims and that are generally longer in the horizontal rather than vertical dimension (Fig. 8c). Neighboring nests of the two species almost invariably maintain these species-specific features, indicating that differences in auricle architecture are not microhabitat-dependent and confirming that the two species have diverged with respect to nest construction behavior.

Mueller and Weislo (1998) report an average colony size of 29.4 workers for a mixed sample of C. longiscapus and C. muelleri. However, when the colony sizes of these nests are recalculated by species, C. muelleri nests are found to contain twice as many workers (average  $43.8 \pm 27.57$  s.d. workers; range 6-109; N=106) than C. longiscapus nests (average  $22.7 \pm 12.33$  s.d. workers; range 4–58; N=67). This difference in observed nest size may be due in part to sampling bias: Because C. muelleri nests possess less conspicuous entrance auricles than do C. longiscapus nests (Figs. 8b and 8c), smaller colonies of C. muelleri may be less frequently noticed and collected in the field relative to smaller colonies of C. longiscapus. However, in a more recent survey of three populations in central Panama where both species occur sympatrically, conducted in June 1998, a special effort was made to locate and collect smaller nests. In this case the average number of workers per nest was  $14.6 \pm 9.73$  s.d. (range 0-40; N=76) for C. longiscapus and  $29.4\pm24.24$  s.d. (range 4–117; N=42) for C. muelleri. Thus, when the problem of size-biased sampling error is addressed and when nests of all sizes are sampled, sympatrically occurring C. muelleri nests are found to contain about twice as many workers as C. longiscapus nests.

For the subset of colonies with alates reported in Mueller and Weislo (1998), C. muelleri averaged 12.4 alates per nest (N=30), whereas C. longiscapus averaged only 7.8 alates per nest (N=56), a pattern paralleling the average worker colony-size differences between the two species. At locations where both species occur in mixed aggregations, alates were found in nests of both species during the early dry season (December to February) of 1996, and also during the wet season in July and August of 1997, suggesting temporal overlap of alate production between the two species at these times. However, collections of both species taken at the same sites in June 1998, at the start of the wet season, yielded only a single alate (a male) from 29 nests of C. muelleri and 101 alates of both sexes from 72 nests of C. longiscapus (Villesen et al., unpubl.). In addition, late dry-season collections from April 2001 yielded only six males from 34 nests of C. muelleri and 111 alates of both sexes from 32 nests of C. longiscapus (UGM, unpubl.). Although these data are inadequate for drawing firm conclusions, they suggest a scenario of partial reproductive isolation in which both C. longiscapus and C. *muelleri* produce alates during the wet season and early dry season (July to February), but in which only C. longiscapus (and not C. muelleri) produces sexuals during the late dry season and early rainy season (March to June). Additional data are needed from more extensive nest surveys conducted throughout the year, as well as from observations of mating flight times in both species. Pigmentation differences between males of the two species, noted above, may indicate time-ofday separation in alate flight times, a phenomenon known to occur between sympatric, closely related species of Atta (Mariconi, 1970; Weber, 1972; TRS and UGM, pers. obs.). Specifically, the lighter pigmentation in males of C. muelleri suggests nocturnal mating flights, whereas the darker pigmentation in males of C. longiscapus suggests diurnal mating flights.

#### Discussion

C. longiscapus and C. muelleri are obviously very closely related, and are remarkably similar in terms of ecology, behavior, and morphology. The primary morphological differences separating these species suggest a common evolutionary pattern: Relative to C. longiscapus, C. muelleri appears to be more specialized for cryptic defense. Specifically, the surfaces of the head and alitrunk of C. muelleri are smoother and more rounded than are those of C. longiscapus. Carinae and tubercles are more reduced and the dorsal profile is less interrupted by sutures and grooves (Fig. 2b). This "streamlined" morphology in C. muelleri plausibly reduces the available points of purchase for the mandibles or grasping organs of an attacking, similarly-sized predator (e.g., another arthropod). In contrast to this general trend toward reduced sculpture, but in agreement with the general trend toward more efficient cryptic defense, sculpturing in two features in C. muelleri is increased over that found in C. longiscapus: the posterior postpetiolar tubercles are produced into teeth (Fig. 3b) and the hind femur is equipped with a pair of ventral carinae, forming a ventral groove, and with a ventral lobe (Fig. 4b). These features, which occur independently in other Cyphomyrmex species (Kempf, 1966), serve to protect vulnerable body parts that are commonly attacked by arthropod predators, particularly other ants; specifically, the postpetiolar tubercles protect the point of articulation between the postpetiole and the gaster; the metafemoral groove receives the tarsus and tibia when the leg is folded in the cryptic-defensive posture; and the metafemoral lobe protects the point of articulation between metatibia and metatarsus.

Thus, morphological features of *Cyphomyrmex* spp. in general and of C. muelleri in particular suggest adaptations to predation pressure from arthropod-sized predators. Such predation pressure, at least from above-ground (rather than subterranean) hunters, is also suggested by the unusual "auricle" nest-entrance morphology of C. longiscapus and C. muelleri, which may serve as a partial physical or even chemically protected barrier to surface-raiding arthropods, particular predatory ants. Likely examples of such predators include army ants in the subfamily Ecitoninae, which are known to significantly impact Neotropical ant colonies in general (Schneirla, 1971; Rettenmeyer, 1983; Gotwald, 1995; Kaspari, 1996). Published records of army ant predation specifically on fungus-growing ants other than Atta spp. are rare and include no raids on Cyphomyrmex spp. (Cole, 1939; Weber, 1945; Schneirla, 1958, 1971; Fowler, 1977; Mirenda et al., 1980; summarized in LaPolla et al., 2002). That the auricle nest entrance could serve to deter the entry of surface-raiding army (and other) ants is suggested by a single observation in Panama in 1996 in which a *Neivamyrmex sp.* raiding column, consisting of many thousands of workers, swarmed past the entrances of two C. longiscapus nests. Although many dozens of army ant workers climbed up the outside (i.e., ground-facing) surfaces of the auricles, none ventured onto the auricle rim or onto the frontal (outward) face adjacent to the nest opening (UGM, pers. obs.). If nest-entrance auricle morphology of C. longiscapus (Figs. 8a and 8b) is more efficient

at repelling army ants than the auricle morphology of *C*. *muelleri* (Fig. 8c), this could account for stronger selection for the seemingly more effective anti-predatory body morphology in the latter species.

Another known predator of both C. longiscapus and possibly of C. muelleri is the semi-nomadic, socially parasitic, agropredatory ant species Megalomyrmex sp. nov. (Formicidae: Solenopsidini) (Adams et al., 2000b). Based on field and laboratory data, Megalomyrmex sp. nov. colonies aggressively raid C. longiscapus and C. muelleri nests, biting and stinging host-species workers. The raiders eject the resident Cyphomyrmex colony and then occupy and consume the fungus garden over a period of weeks or months, depending on garden size. Unfortunately, this species is known from only a few collections (5 colonies of Megalomyrmex sp. nov. from 344 C. longiscapus/C. muelleri nests collected during 1999 and 2001) (Adams et al., 2000b), and so, without more research, it is impossible to accurately assess the (possibly differential?) predation pressure exerted by this species on C. longiscapus and C. muelleri.

Perhaps the most remarkable difference between C. *longiscapus* and *C. muelleri* is that, even though these two species are quite similar biologically and even though they occur sympatrically in the same microhabitats, they consistently employ two very different, distantly related fungal cultivar species. Each of these fungal cultivars is also employed by other, distantly related attine ants that occupy different microhabitats and that are otherwise quite dissimilar biologically (Mueller et al., 1998; Green et al. 2002). Specifically, C. longiscapus shares a narrow group of cultivars of the "Clade 1" type (Mueller et al., 1998) with the sympatric fungus-growing ant Apterostigma auriculatum; molecular data indicate that in one case a cultivar clone has been transferred recently between nests of these two ant species (Mueller et al., 1998). Similarly, C. muelleri shares a narrow group of cultivars of the "Clade 2" type with the sympatric C. costatus, and multiple cultivar exchanges have occurred between these two ant species (Green et al. 2002). Both A. auriculatum and C. costatus are commonly encountered under logs and rocks on the rain forest floor (UGM, pers. obs.), a very different microhabitat from the embankments preferred by C. longiscapus and C. muelleri. Based on these microhabitat differences, we might expect cultivar exchanges between C. longiscapus and A. auriculatum, or between C. muelleri and C. costatus, to occur at very low frequencies relative to exchanges between C. longiscapus and C. muelleri, which often occur in mixed aggregations. Yet such across-microhabitat exchanges between the more distantly related attine species are well documented (Mueller et al., 1998; Green et al. 2002). In contrast, in over 400 nest collections in which cultivar species could be identified, C. longiscapus was invariably associated with its own Clade 1 type cultivar, and C. muelleri was invariably associated with its own Clade 2 type cultivar. Thus, cultivar exchanges apparently do not occur between nests of the closely related and physically more proximate C. longiscapus and C. muelleri.

This strikingly precise pattern, in which two closely related ant species consistently cultivate two distantly related fungal species, prompts the obvious question of whether a shift in an ancestral C. "longiscapus" s.l. population to a new cultivar may have somehow initiated the divergence of these two species. For example, under an allopatric scenario, an isolated population of C. "longiscapus" s.l. could have switched to and become specialized on a new fungal cultivar. Subsequent secondary contact between ant populations specialized on different cultivars could have produced hybrids that were inferior in their ability to cultivate either fungus, leading to selection for prezygotic reproductive isolation and completing the speciation process. Whatever the prezygotic isolating mechanism, it probably did not involve genitalic evolution (Eberhard 1985, 1996), because the genitalia of C. longiscapus and C. muelleri males appear to be identical (see above). A more likely mechanism might be temporal separation of mating flights, known to isolate other closely related, sympatric Attini (Mariconi 1970; Weber, 1972; Schultz et al., 1998). A scenario of behavioral isolation through differential timing of mating flights is consistent both with observed partial time-of-year differences in alate production between C. longiscapus and C. muelleri and with possible time-of-day flight-time differences implied by differences in male body pigmentation (described above). Alternatively, under a sympatric-speciation scenario, a single nest of an ancestral C. "longiscapus" s.l. could have switched to a new cultivar, instantaneously generating a tendency toward reproductive isolation due to an unknown, cultivar-specific mechanism such as mating pheromones or other mate-recognition factors derived from, or otherwise correlated with, fungal cultivar type. These allopatric and sympatric hypotheses generate different testable predictions. For example, under the sympatric scenario, genetic diversity should be depressed in the more recently derived species relative to the "ancestral" species, and the biogeographic distribution of the recently derived species may be nested within the larger range of the ancestral species. Testing such predictions will require comprehensive sampling across the complete ranges of C. longiscapus and C. muelleri, coupled with detailed analyses of genetic diversity using highresolution molecular markers such as microsatellites.

As summarized in Table 2 and in our discussion of morphometrics, Colombian C. longiscapus workers are significantly larger than workers from Panama, with virtually no overlap in the ranges of the three measurement parameters. The two known Colombian gynes and the single known male reinforce this pattern. This correlation between biogeography and body size suggests the possibility that C. longiscapus may actually comprise two species: a smaller, Panamanian species and a larger, Colombian species. Contradicting this biogeographic scenario, however, is the single known Costa Rican worker specimen, which is more similar in size to the Colombian than to the Panamanian specimens (Table 2). Because the Colombian, Panamanian, and Costa Rican specimens are identical in all studied discrete morphological character states, and because allozyme profiles from Colombian specimens are unknown, no additional character data corroborate this division of C. longiscapus into two species based on size differences. In the absence of such data, and because the Colombian specimens are known from as few as three nest series, we must adopt, for now, the taxonomically conservative null hypothesis that these size differences represent population-level variation in a poorly understood within-species morphocline. Such a pattern could be maintained, for example, by a form of character displacement (Brown and Wilson, 1956) in which there is selection for smaller body size in *C. longiscapus* where it occurs in sympatry with *C. muelleri*, but in which such pressure is released in areas where *C. muelleri* does not occur.

Obviously, additional data from new collections are required, particularly from western Colombia, eastern Panama, and Costa Rica. Parallel attempts must be made to locate populations of C. muelleri in these regions. Only a broad biogeographic sample of ant colonies and their symbionts will provide the phylogeographic data necessary for understanding: (1) whether the ranges of the two species are broadly continuous or whether they consist of isolated, potentially diverged populations; (2) whether or not C. longiscapus and C. muelleri are sympatric throughout their ranges, or whether the range of one species is nested within the range of the other; (3) whether there are additional cryptic species within the C. longiscapus s.l. complex; and (4) whether the two species consistently cultivate the same two distinct fungal cultivars throughout their ranges. Increased understanding of these factors will in turn allow us to better evaluate hypotheses about the mechanisms that have precipitated cryptic speciation as well as other important evolutionary transitions in Cyphomyrmex "longiscapus" s.l.

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#### Appendix

Panamanian specimens examined

W=workers; G=gynes; M=males. In most cases, collection numbers incorporate collection dates.

#### Cyphomyrmex longiscapus Panamanian nest series examined:

Republic of Panama: Pipeline Road, Km 4, La Seda River; June 16 1998; U.G. Mueller, C.R. Currie, and P. Villesen, collectors: SD-8 (1W), SD-16 (1W).

Republic of Panama: Pipeline Road, Km 6; U.G. Mueller, collector: UGM960104-04 (2W), UGM960125-04 (10W, 3G), UGM960208-24 (1W, 1G, 2M), UGM960208-25 (2W, 14G, 9M), UGM 960208-26 (12G, 7M), UGM 960208-27 (16W, 1G), UGM 960208-28 (23W, 1G, 16M), UGM960208-29 (1M), UGM 960208-30 (8W, 4G), UGM960208-31 (4W, 1G), UGM 960222-10 (6W, 1G), UGM960222-12 (2 M), UGM 960222-14 (40W, 1G, 1M), UGM 960222-15 (2W, 1G), UGM 960222-18 (29W, 4G, 12M), UGM960222-19 (2W), UGM 960222-20 (44W, 17G, 1M), UGM960712-01 (1G, 1M), UGM960712-02 (1G, 1M), UGM960712-10 (1M), UGM 960712-12 (4G, 1G, 3M), UGM 960803-01 (6G, 9M), UGM 960803-11 (2G, 11M), UGM980528-01 (8W), UGM 980528-04 (1W), UGM980618-10 (18W, 9G, 10M), UGM980618-12 (12W, 1G), UGM 980618-16 (2W, 1G), UGM 980618-18 (1W), UGM980618-25 (8W), UGM980618-26 (20W, 2G, 3M), UGM 980618-28 (1W), UGM980618-29 (9W).

Republic of Panama: Pipeline Road, Km 8; U.G. Mueller, collector: UGM960208-19 (2W, 1M), UGM960208-20 (2W, 1G), UGM960208-21 (2W, 1G, 1M), UGM960208-22 (2W, 1G), UGM960208-23 (2W, 1G, 1M).

Republic of Panama: Pipeline Road, Km 10.5 (site of PA 464); U.G. Mueller, collector: UGM970508-02 (3W, 1G), UGM970508-06 (6W, 1G, 7M), UGM970508-09 (17W, 12G, 21M), UGM970508-11 (8W, 1M), UGM970521-14 (10W)

Republic of Panama: Pipeline Road, Km 12; U.G. Mueller, collector: UGM951211-01 (9W), UGM951211-02 (26W, 1G, 1M), UGM951211-03 (7W, 8G, 7M), UGM951211-04 (22W, 1G), UGM951211-06 (7W, 1G), UGM951211-07 (15W, 1G, 1M), UGM951211-08 (10W, 1G), UGM951211-09 (9W, 1G).

Republic of Panama: Pipeline Road, Km 14; U.G. Mueller, collector: UGM951227-03 (10W, 1G, 1M), UGM951227-04 (10W, 1G), UGM951227-06 (12W, 1M), UGM951227-07 (18W, 1G), UGM951227-08 (11G, 3M).

Republic of Panama: Pipeline Road, Km 19; U.G. Mueller, collector: UGM960208-01 (2W), UGM960208-02 (2W, 1G), UGM960208-03 (2W, 1G), UGM960208-04 (2W), UGM960208-05 (2W, 1G, 1M), UGM960208-06 (2W, 1G), UGM960208-07 (2W, 2G, 1M), UGM960208-08 (2W, 1G, 1M), UGM960208-09 (2W, 1G, 1M), UGM960208-10 (2W, 1G), UGM960208-11 (2W, 1G), UGM960208-12 (2W, 1G, 1M), UGM960208-13 (2W, 1G, 1M), UGM960208-14 (2W, IG, 1M), UGM960208-15 (2W, 1M), UGM960208-16 (1W, 1G), UGM960208-17 (2W, 1G, 1M), UGM960208-18 (2W, 3G, 1M), UGM970505-11 (2W).

Republic of Panama: Crest of El Llano-Carti Suitupo Road (~Km 6 from El Llano); U.G. Mueller, collector: UGM960107-03 (1W, 1M), UGM960107-08 (12W, 1G), UGM960107-10 (17W, 1G), UGM960107-11 (4W), UGM960128-06 (9W, 1G), UGM960128-07 (17W), UGM960128-08 (32W, 1G), UGM960128-09 (12W, 1G, 2M), UGM960128-11 (20W, 5G, 5M), UGM960128-12 (23W, 3G), UGM960128-13 (21W, 3G, 2M), UGM960427-06 (2W), UGM960427-07 (2W), UGM960427-08 (2W, 1G), UGM 980607-08 (1W), UGM 980607-09 (1W), UGM 980607-11 (1W), UGM980607-12 (15W, 2M), UGM980607-13 (3W), UGM980607-15 (20W, 5G, 4M), UGM980617-05 (24W, 1G), UGM980617-08 (19W, 1G), UGM 980617-09 (2W), UGM 980617-11 (2W), UGM980617-13 (14W, 1G, 5M), UGM 980617-17 (2W).

Republic of Panama: Nusagandi Biological Station, 1.4 km on Nusagandi-Markisgandi trail at Markisgandi River; U.G. Mueller, collector: UGM960426-07 (7W, 3G, 1M).

Republic of Panama: Fort Sherman Military Reservation, off Gatun-Fort Sherman Road; U.G. Mueller, collector: UGM960428-10 (29W, 7G, 11M).

Republic of Panama: Fort Sherman Military Reservation, STRI canopy crane site; U.G. Mueller, collector: UGM970520-02 (5W), UGM970520-03 (7W).

Republic of Panama: Fort Sherman Military Reservation, off main road about halfway to STRI canopy crane site; U.G. Mueller, collector: UGM980616-14 (26W), UGM980616-17 (16W).

Cyphomyrmex muelleri Panamanian paratype nest series examined:

Republic of Panama: Pipeline Road, Km 4, La Seda River; June 16 1998; U.G. Mueller, C.R. Currie, and P. Villesen, collectors: SD-114 (1W), SD-11A (6W), SD-14 (1W).

Republic of Panama: Pipeline Road, Km 6; U.G. Mueller, collector: UGM960104-01 (2W), UGM960222-13 (19W, 1G), UGM960222-16 (47W, 1G, 1M), UGM980618-22 (18W), UGM980618-27 (25W, 1M), UGM980618-32 (9W, 3G), UGM980618-34 (15W, 9M), UGM980618-36 (1W), UGM960712-07 (1G, 1M). Republic of Panama: Pipeline Road, Km 10.5 (site of PA 464); U.G. Mueller, collector: UGM970508-03 (20W, 21G, 10M), UGM970508-07 (15W, 32G, 9M), UGM970521-13 (5W, 39G, 54M), UGM 970521-16 (2W, 1G), UGM970521-21 (16W, 6M), UGM970521-24 (16W, 6M), UGM970521-25 (2W), UGM970521-27 (1W, 9G), UGM970521-31 (11W, 4G, 37M).

Republic of Panama: Pipeline Road, Km 14; U.G. Mueller, collector: UGM951227-02 (12W), UGM951227-05 (3W).

Republic of Panama: Fort Sherman Military Reservation, off Gatun-Fort Sherman Road; U.G. Mueller, collector: UGM960428-11 (36W, 1G), UGM960428-12 (65W, 1G), UGM960428-13 (25W, 1G, 1M), UGM960428-14 (12W, 1G), UGM 960428-15 (2W), UGM960428-16 (2W, 1G), UGM960428-17 (20W, 1G), UGM960428-18 (8W, 1G), UGM960710-03 (8W, 1M), UGM960710-18 (6M), UGM960712-05 (1G, 1M), UGM960802-01 (2G, 15M), UGM960802-02 (2G, 15M), UGM960802-04 (14M), UGM960802-05 (7G, 7M), UGM960802-07 (1G), 960802-09 (1G, 1M), UGM960802-14 (1G, 1M), UGM980611-01 (11W, 1M), UGM980611-02 (20W, 1G), UGM980611-03 (9W, 2G, 5M), UGM980611-10 (10W, 4G, 1M), UGM980611-11 (3W), UGM980611-12 (6W), UGM980611-13 (3W). Republic of Panama: Fort Sherman Military Reservation, STRI canopy crane site; U.G. Mueller, collector: UGM970520-07 (0W, 0G, 0M), UGM970520-14 (3W, 39G, 11M), UGM970520-19 (4W), UGM970520-28 (11W, 1G, 31M), UGM970520-31 (1W, 1G, 32M).

Republic of Panama: Fort Sherman Military Reservation, off main road about halfway to STRI canopy crane site; U.G. Mueller, collector: UGM980616-16 (5W, 6M), UGM980616-21 (9W).

Republic of Panama: Barro Colorado Island; U.G. Mueller, collector: UGM951221-04 (4W, 5G, 4M), UGM951221-05 (12W, 1G), UGM951221-06 (46W, 1G, 10M), UGM951221-07 (4W, 1G), UGM951221-08 (4W, 2G, 2M), UGM960214-01 (42W, 1G), UGM960214-02 (27W, 1G), UGM960214-03 (2W, 3G, 2M), UGM960214-04 (22W, 3G, 9M), UGM960214-05 (76W, 1G, 18M; holotype nest series), UGM960214-06 (5W, 11G, 2M), UGM960214-07 (13W, 10G, 9M), UGM960214-06 (5W, 11G, 2M), UGM960214-07 (2G), UGM960214-12 (9W, 6G, 24M), UGM960214-15 (3G), UGM960214-16 (10W, 1G, 3M), UGM 960214-21 (1G), UGM960224-02 (19W, 6G, 4M), UGM960224-03 (2W, 6G, 12M), UGM960224-04 (2W), UGM960224-05 (6W, 1G), UGM960308-01 (2W, 1G).



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