

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., 1999a, 1999b; Villesen et al., 1999; Adams et al., 2000a, 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

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