Title

Novel worker-like behaviour observed in gynes of the social parasite *Tetramorium microgyna*

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Abstract

Socially parasitic ants increase their own fitness by exploiting the labour and resources of non-kin ant colonies. Here, we report a novel worker-like behaviour observed in an African workerless inquiline, *Tetramorium microgyna*, a parasite of *Tetramorium sericeiventre*. We observed several *T. microgyna* gynes excavating soil and performing nest maintenance tasks at the entrance of an established *T. sericeiventre* host colony. We photographed this event in nature, then dissected *T. microgyna* gynes to establish mating status and reproductive capacity. All *T. microgyna* gynes that participated in worker-like behaviours were unmated, with ~6 ovarioles and an estimated capacity to store 12,000 sperm cells and fertilize 3,700 eggs. We hypothesize nest excavation by inquilines represents an artefact of a non-parasitic past, where gynes that fail to mate remain in their natal colony and assume a secondary, but still mutually beneficial worker-like role. Alternatively, nest excavation by socially parasitic foundresses, could be an artefact of an ancestral behavioural repertoire associated with independent colony founding. While helping behaviour in post-reproductive inquilines does not increase personal fitness, it also does not reduce it, and may be maintained through relaxed selection. A third possibility is that putative *T. microgyna* parasites are actually microgynes of their 'host' species. Socially parasitic ants are rarely found and poorly studied compared to their non-parasitic counterparts. Our findings provide insights into how selection may act on developmental and behavioral programs during the evolution of social parasites from non-parasitic ancestors.

Keywords

Formicidae, Inquiline, South Africa, social parasitism, *Tetramorium*

Introduction

A key feature of eusocial insect societies is the division of labour between a reproductive caste, and a non-reproductive, worker caste responsible for brood care, nest maintenance, foraging and defence. Socially parasitic ants increase their own fitness by exploiting the labour and resources of established, non-kin ant colonies through temporary social parasitism, dulosis, or inquilinism (Figure 1).

Temporary social parasites exploit their hosts only during the initial stages of colony founding. Typically, a temporary social parasite queen will undertake the risky venture of invading a host nest, sometimes by first killing and interacting with a host worker's corpse, supposedly to obtain colony-specific recognition odours (Gösswald 1938; Buschinger 2009) (Fig. 1a, b, Fig. 2). Once inside the nest, the parasite exploits the host colony's workforce to raise her own worker brood, sometimes killing the resident host queen. However, once the parasite's brood mature, parasite workers take care of subsequent generations of parasite workers and thus become independent from their hosts (Buschinger 2009; Rabeling 2021). Dulotic social parasite species also usurp a host species nest by killing the resident queen, but unlike temporary parasites, they are permanently parasitic. Workers of dulotic species usually cannot forage, care for brood, perform nest maintenance tasks or even feed themselves (Buschinger 2009), but specialize on periodically conducting 'kidnapping raids' to capture worker pupae of a host species. After eclosing, these captured host workers take care of the parasitic colony's needs (D'Ettorre and Heinze 2001; Buschinger 2009).

Inquilinism is the most extreme form of social parasitism, as inquiline species typically lack a worker caste, and only produce sexuals (i.e. males and queens) (Sumner et al. 2003; Buschinger 2009; Rabeling 2021). Inquiline queens invade an established host nest, but in contrast to temporary social parasites and dulotic species, they usually coexist with the queens of the host species and rely on host queens to produce workers that rear their parasitic, sexual offspring (Buschinger 2009). Most inquilines are substantially smaller than their host queens, and some flank or even attach themselves to their host queen (Buschinger 1986; Davis and Deyrup 2006; Johnson et al. 2008; de la Mora et al. 2020).

The inquiline species *Tetramorium microgyna* (Fig. 3) is a known parasite of *Tetramorium sericeiventre* (Fig. 4) and *Tetramorium sepositum* (Bolton 1980). *Tetramorium microgyna* is listed as 'vulnerable' on the IUCN (Social Insects Specialist Group 1996a), like most other socially parasitic ants (Mabelis 2007; Alonso 2010). *Tetramorium microgyna* occurs in Angola, South Africa and Zimbabwe, which largely coincides with the distribution of its host *T. sepositum* (South Africa and Zimbabwe), and with the southernmost part of its host *T. sericeiventre*, which occurs throughout most of Africa (Bolton 1980; Janicki et al. 2016; AL-Keridis et al. 2021; Evan Economo and Benoit Guénard 2024). Besides this information, little is known about the natural history of *T. microgyna*.

Here, we report observations of *Tetramorium microgyna* gynes performing nest excavation behaviours. In non-parasitic ant lineages, gynes that fail to mate sometimes stay in their natal colony and perform worker-like tasks (Table 1), but such 'helper gyne' behaviour is unexpected and novel for a workerless, social parasite. To determine why parasitic gynes might behave like workers in host colonies, we dissected them and established their mating status, reproductive and dispersal potential.

Material and methods

We found a nest of *Tetramorium sericeiventre* parasitized by the social parasite *Tetramorium microgyna* in Skukuza, South Africa (-24.995° lat, 31.595° long) on the 3rd of March, 2023. We photographed specimens of both social parasites (*T. microgyna*) and hosts (*T. sericeiventre*) in situ, participating in nest excavations, using a DSLR camera (Nikon d500 with a 105 mm macro lens). Specimens were then hand collected at the nest entrance and put in 70% ethanol. We then mounted and imaged a specimen of each species using a Leica DMC5400 camera mounted on a Leica M205C dissecting microscope. We took image montages using the Leica Application suite v. 4.13 and stacked them in Zerene stacker version 1.04. We deposited voucher specimens in the ant collection at the Commonwealth Scientific and Industrial Research Organization in Darwin (Australia) and at the Skukuza Biological reference collection (South Africa).

Dissection:

We dissected three *Tetramorium microgyna* gynes (two with 2 or 4 wings, and one without wings) collected from a single *Tetramorium sericeiventre* nest. Dissections revealed mating status as assessed by the presence or absence of sperm in the spermatheca. We also assessed body condition including the number and condition of ovarioles to estimate the reproductive potential of *T. microgyna* gynes. Lastly, we quantified the abundance of fat body in the gaster, the nature of visible defensive glands, and the contents of the crop, midgut, and hindgut, as this may relate to a gyne's ability to disperse and invade a host colony successfully.

Gynes were dissected under deionized water. A minutin pin was used to secure the mesosoma to a wax dish, and each tergite of the gaster was removed by slipping superfine watchmaker's forceps beneath the dorsal midline, then gripping and peeling away the cuticle. The location of globular fat body, and its approximate depth (number of layers) was recorded. A stream of water was then used to remove fat that occluded visibility of other organs. The presence of liquid in the crop was noted, along with the number of Malpighian tubules, and the presence and character of particulate matter in the midgut and hindgut.

The number of ovarioles was counted *in situ* under a stereoscope, and again after removal of the entire reproductive tract for slide mounting. The condition of ovarioles and the presence or absence of vitellogenic follicles with developing ova were recorded for each gyne. Unripe ovarioles were thin and strand-like, with a uniform width across their length without girdling, and without translucent or opaque ova, or evidence of yellow bodies. Ripe ovarioles were those that contained translucent or opaque developing ova, and were plump with a beaded appearance that widened towards the calyx. Before removing the reproductive organs, we also noted the size and condition of glands associated with the sting apparatus.

To determine if *Tetramorium microgyna* gynes that performed nest excavation tasks had mated, we made wet-mount slide preparations of each gyne's entire reproductive tract. We ruptured and smeared spermathecae on the glass slide then examined the slides under a compound microscope (Nikon) at 400x magnification, to look for evidence of stored sperm. Each spermatheca was small but clearly defined by a characteristic, recurved tubule. If no sperm were visible, we made smears of the entire reproductive tract posterior to the lateral oviducts and re-examined the slide. To confirm the feasibility of visualizing sperm in specimens stored in 70% alcohol (rather than fresh frozen without preservarive), we first practiced dissections using wet mounts of mated *Solenopsis invicta* (BUREN, 1972) and *Pogonomyrmex badius* (LATREILLE, 1802) foundresses that were either frozen at -20 °C without liquid, or placed in 70% ethanol for 21 to 40 days. Sperm were visible in the spermatheca of both test species, despite the potential for wash out or clearing expected during alcohol preservation.

Results

Behavioural observations:

The focal nest of *Tetramorium sericeiventre* was in the soil, and host workers were performing nest maintenance and excavation behaviours at the time of our observations. We observed parasitic *T. microgyna* gynes and *T. sericeiventre* workers carrying pebbles and soil from within the nest to then deposit them at the periphery of the nest entrance (Fig. 5). Parasitic alate gynes, dealate gynes, and partially dealate gynes all appeared at the nest entrance (Fig. 6). All travelled in and out of the nest engaged in repeated, substrate deposition behaviour.

Dissection:

None of the three *Tetramorium microgyna* gynes dissected had evidence of sperm in the spermatheca (for consolidated dissection details, see Table S1 in Appendix). Gynes had 6 to 7, stout ovarioles, (3 - 4 on each side). Between 1 and 4 ovarioles were ripe, with forming eggs, while the other ovarioles were inactive and lacked yellow bodies that would suggest any eggs had recently been laid. Gynes had little to no fat body in the gaster, but were well-fed, as evidenced by the presence of clear liquid in the crop (2 of 3), fine particulate matter that filled the large round midgut, and dense consolidated material in the hindgut. Gynes had fewer than 15 Malpighian tubules, and a large, turgid venom sac/gland that covered the lower 5th of the internal gaster space and attached to the robust sting apparatus, alongside what we presume to be the Dufour's gland (colourless). Large air sacs that flanked the crop were undamaged, inflated and pearlescent, indicating that they could have provided the necessary oxygen for powered flight. All dissected gynes were free of internal parasites.

Discussion

Despite lacking a worker caste, parasitic *Tetramorium microgyna* gynes retain the ability to perform a repertoire of non-reproductive behaviours usually associated with workers or independent foundresses. To our knowledge, *T. microgyna* represents the first case of *a* workerless, inquiline performing nest excavation behaviour in a host colony. Worker-like behaviour in unmated gynes with wings (alates) is also exceptionally rare (Fresneau and Dupuy 1988; Table 1), as wing-removal can trigger physiological changes associated with worker-like behaviour (Nehring et al. 2012a; Pyenson et al. 2022). Assuming that *T. microgyna* is a distinct species from its host, several pieces of evidence suggest that gynes involved in nest excavation were failed dispersers, born in the focal nest: 1) The observed parasite gynes were likely unmated, as no sperm was present in the spermathecae of any gyne, 2) mated ant queens typically do not perform nest excavation behaviour in the presence of workers, and 3) we observed multiple, non-physogastric, winged gynes associated with the same focal nest. Although some ant colonies may be parasitized by multiple, reproducing parasite queens, these queens typically do not retain their wings (Emery 1909; Johnson 1994).

Together, our findings suggest that nest excavation by inquiline gynes likely persists due to relaxed selection on post-reproductive behaviours, which neither increase nor decrease personal fitness of the parasites, but were present in a non-parasitic ancestor. Likewise, the contributions of *T. microgyna* gynes to nest excavation are likely minimal, and unlikely to increase the fitness of the host colony or of parasite kin within the same host nest. Although helping-behaviour in *T. microgyna* might appear to benefit the host colony, the contributions of parasitic gynes could only be considered mutualistic if the benefits of these behaviours outweighed the considerable costs imposed by rearing the parasitic gynes.

Whether nest-excavation by post-reproductive inquilines echoes ancestral worker-like or queen-like behaviour is unclear. Worker-like behaviours by unmated gynes, such as foraging, allogrooming, brood-care, and nest defence, have been reported in several non-parasitic ant species, representing multiple ant subfamilies (Table 1) (Peeters 1997; Johnson et al. 2007, 2022; Nehring et al. 2012b; Vieira et al. 2012; Johnson 2021). Independent foundresses also perform all of the necessary functions of an ant colony, including nest excavation, brood care, and foraging (when not fully claustral) following dispersal. Likewise, in pleometrotic foundress associations, a division of labour among foundresses often leads one individual to take on a larger proportion of nest maintenance behaviours, which ultimately increase group survival, and fitness of the queen(s) that inherit the nest (Fewell and Page 1999; Cahan and Fewell 2004; Ostwald et al. 2021).

Lifetime reproductive capacity and body condition of inquiline gynes:

Across ant subfamilies, the number of sperm that ant queens store increases as a function of ovariole number (Tschinkel 1987). *Tetramorium microgyna* gynes have just six ovarioles, which is exceptionally low for the subfamily Myrmicinae. Using Tschinkel's (1987) equation, *T. microgyna* gynes are estimated to store just 2,000 sperm cells per ovariole, for a total of 12,000 sperm cells. Ants are conservative with sperm use, expending approximately 3.2 sperm cells per fertilized egg (Tschinkel 1987; Tschinkel and Porter 1988). Given *T. microgyna* ovariole number, and assuming 3.2 sperm are spent per fertilization, gynes would have the capacity produce ~3,700 fertilized eggs per lifetime, and an unknown number of unfertilized, haploid, male eggs.

Dissected gynes had little to no accumulated fat body, but were well fed, as evidenced by the presence of liquid in the crop and particulate matter in the midgut and hindgut. Independently founding ant queens are typically endowed with large fat reserves amassed during the weeks preceding nuptial flights. Up to 61% of foundresses body weight is made up of fat (Keller and Passera 1989), with fat stores located in the mesosoma and gaster (Boomsma and Isaaks 1985). Semi-claustral independent foundresses, dependent foundresses, and parasitic foundresses are expected to have much less fat than fully claustral, independent foundresses that provision their first larvae from their own fat reserves. For instance, in an interspecific context, semi-claustral species have on average 18% less body fat than claustral foundresses, whereas dependent foundresses have on average 47% less body fat than claustral foundresses (Keller and Passera 1989).

It is difficult to determine if the *T. microgyna* gynes in our study had a typical amount of fat for parasitic founding, or if they were in such poor condition that they would have been unable to produce more than a few eggs. We can gain some insight from species that employ both independent and parasitic founding strategies. The red imported fire ant, *Solenopsis invicta,* produces a combination of fat, independently founding queens and lean, dependently founding queens on a seasonal schedule. Dependent foundresses parasitize orphaned conspecific colonies and have 5-15% less body fat than conspecific independent foundresses produced earlier in the year (Tschinkel 1996). At least one study reports on the fat content of dependent foundresses of *Formica rufa*, which participate in either colony budding or temporary social parasitism of *Formica fusca*. In *F. rufa*, both alate and dealate gynes have almost no fat in their gaster prior to founding, and ovary activation only begins following the histolysis of wing muscles after dispersal (Fedoseeva and Grevtsova 2020).

All 3 *T. microgyna* gynes dissected in our study had at least one active ovariole, with 1-4 eggs forming. It is possible that these eggs were produced in anticipation of mating, and less probable that they were trophic eggs (given that inquilines do not feed their own young). Some ant queens do begin to produce eggs prior to dispersing, and developing oocytes have been found in unmated, dealate gynes (Vieira et al. 2012). Although we do not know if *T. microgyna* gynes mate before dispersing, or if they use their wings to disperse, 2 of the 3 parasitic gynes observed performing nest maintenance behaviour were partially or fully dealate, but with an intact tracheal system and no other internal anomalies that would prevent powered flight. If the premature loss of wings alone prevented participation in mating or dispersal flights, then this reinforces our hypothesis that these gynes failed to mate and disperse, then assumed worker-like roles. Describing the mating behaviour and dispersal mode of *T. microgyna* will help resolve this question, as it is possible that queens disperse on foot, without the aid of their wings.

***Social parasitism in Tetramorium microgyna*:**

The gynes of *Tetramorium microgyna* have several morphological attributes that are typical of inquilines: they are smaller than their host workers, and their cuticle is paler and more delicately sculptured than the cuticle of their host workers (Bolton 1980, Fig. 3-4). However, other *Tetramorium* inquilines have much more derived morphologies, with mostly edentate mandibles, a lack of propodeal spines, extremely small sizes, physogastric queens, and pupoid males (Bolton 1980; Francoeur and Pilon 2011; Wagner et al. 2018, 2021; Vankerkhoven and Dekoninck 2022). In species with more extreme parasitic traits, edentate mandibles and small body size could physically prevent gynes from performing tasks that require gripping, like nest excavation or host brood care. Given that *T. microgyna* has relatively well-developed mandibles, the observed excavation behaviors may be unique among inquilines from the same clade.

So far, we have assumed that *T. microgyna* and *T. sericeiventre* are a distinct species. It is possible that putative *T. microgyna* gynes are actually *T. sericeiventre* microgynes (i.e. an alternative, miniature queen morph). This possibility provides an intriguing alterative explanation for the worker-like behavior observed *T. microgyna*. In 1990, Professor Philip S. Ward collected in Madagascar what he called microgynes of *Tetramorium quadrispinosum* (now synonymized with *Tetramorium sericeiventre*, see Garcia and Fisher 2012). This specimen differs from *Tetramorium microgyna*, but show similar morphological features associated with social parasitism, such as a small size, a pale and less sculptured cuticle, and small propodeal spines (Fig. 7), especially compared to “typical” queens of *T. sericeiventre* (Fig. 8). Microgynes have been described as independent social parasite species in the past, to then later be synonymized with their putative host species, although this has led to disagreements, such as in *Myrmica rubra* and *Myrmica microrubra* (Seifert 1994; Steiner et al. 2006; Vepsäläinen et al. 2009). In one case, the putative social parasite *Manica parasitica* was found to be nothing more than a cestode infected morph of its presumed host, *Manica bradleyi* (Prebus et al. 2023). We hope that future molecular phylogenetic analyses of *T. microgyna*, typical *T. sericeiventre* and *T. sericeiventre* microgynes will one-day clarify the species-level relationships between these organisms.

Conclusion:

In ants, social parasitism is known from 6 subfamilies, 42 genera and 401 species. It occurs predominantly in the diverse subfamilies Dolichoderinae, Formicinae and Myrmicinae (Rabeling 2021), but is also found in relatively species-poor lineages such as Myrmeciinae (Mera-Rodríguez et al. 2023). Social parasitism in ants evolved independently at least 91 times across the world (Gray and Rabeling 2023). Despite the rich diversity of socially parasitic ant species, our knowledge of the intricacies of social parasite behaviours mostly comes from a few well studied species of Northern latitudes belonging to the genera *Formica*, *Polyergus*, *Temnothorax* and *Harpagoxenus* (Creighton 1927, Talbot 1967, Talbot 1976, Cool-Kwait & Topoff 1984, Heinze & al. 1994, Foitzik &

al. 2001, Bauer & al. 2009, Chernenko & al. 2013 but see Sumner & al. 2003). This geographic focus on social parasitism may result from its increased frequency at higher latitudes (i.e., it follows an inverse latitudinal diversity gradient), especially in the Northern hemisphere (Gray and Rabeling 2023). For instance, up to 30% of the Swiss ant diversity consists of social parasites, compared to roughly 2% of the world's species (Kutter 1968). Nevertheless, social parasitism does occur in the southern hemisphere, albeit in relatively less taxa. Most social parasite species are rare and remain poorly known, with most species lacking natural history observations (Rabeling 2021; Gray and Rabeling 2023). As such, there is likely a range of novel behaviours to record in social parasites, including those described in the current study.

As a clade, *Tetramorium* is fascinating because it contains species that span the whole spectrum of the social parasite syndrome. Most social parasite species are extremely rare, but *Tetramorium* is a diverse and ubiquitous genus throughout the Old World. As such, it is likely that many more socially parasitic species are currently unknown. Moreover, our knowledge of the behavior and life history of described social parasites is patchy at best. Gaining more information on social parasitism in *Tetramorium* would better our understanding of the evolution and ecology of ants as a whole. As such, we encourage fellow myrmecologists to prospect for such species, and to record their life history traits.

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Table and figures

Table 1. Worker-like or independent foundress-like behaviours performed by non-reproductive gynes (not comprehensive). N.R. = not reported.

Subfamily	**Species**	**Parasitic?**	**Location**	**Wings**	**Behavior**	**Source**
Myrmicinae	*Tetramorium microgyna*	**Yes**	Field	**Alate, partially dealate**, dealate	Excavating soil alongside *T. sericeiventre* host workers	This study
	Acromyrmex echinatior	No	Lab	Dealate	Brood care, defence	Nehring et al. 2012
	Acromyrmex niger	No	Lab	Dealate	Foraging	Della Lucia et al. 1993
	Acromyrmex octospinosus	No	Lab	Dealate	Brood care, defence	Nehring et al. 2012
	Acromyrmex subterraneus	No	Lab	Dealate	Foraging	Della Lucia et al. 1993
	Mycetomoellerius turrifex	No	Field, Lab	Dealate	Excavation, guarding, carcass tending, brood care, fungus garden care	Murakami 2020
	Mycetomoellerius urichii (formerly *Trachymyrmex fuscus*, see Weber 1958; Solomon et al. 2019)	No	Field	N.R.	Foraging	Araújo et al. 2015
	Pogonomyrmex pima	No	Field	Dealate	Foraging	Johnson et al. 2007
	Veromessor andrei	No	Field	Dealate	Foraging, midden work	Creighton 1953; Brown 1999
Ponerinae	*Harpegnathos saltator*	No	Lab	Dealate	Worker-like dominance behaviors	Pyenson et al. 2022
	Neoponera apicalis	No	Lab	**Alate**	Foraging	Fresneau and Dupuy 1988
	Odontomachus rixosus	No	Field, lab	Dealate	Foraging, allogrooming, larval care	Ito et al. 1996
Ectatomminae	*Ectatomma tuberculatum*	No	Lab	N.R.	Patrolling, foraging	Hora et al. 2005
	Ectatomma vizottoi	No	Lab	Dealate	Brood care, grooming, foraging	Vieira et al. 2012

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Figure captions

Fig. 1: Types of social parasitism are a-b) temporary social parasitism, c-d) dulosis and e) inquilinism. In a-b) two gynes of temporary social parasite species in the genus *Lasius* captured and chewed workers of a host *Lasius* species, likely to gain their odour before invading the host colony. In c-d) the host species are either *Leptothorax acervorum* or *Leptothorax muscorum*, whereas the parasite is the European robber ant *Harpagoxenus sublaevis*, which is listed as vulnerable by the IUCN (Social Insects Specialist Group 1996b). In d), a worker of the *Leptothorax* host species transports a worker of the Harpagoxenus parasite species. In e), a wingless gyne of the inquiline species *Tetramorium microgyna* is about to enter the nest of its host species *Tetramorium sericeiventre*. Photos by François Brassard.

Fig. 2: Instances of failed invasion attempts by gynes of temporary social parasites. Here, all parasites and hosts belong to the genus *Lasius*. Photos by François Brassard.

Fig. 3: Photos of a) head view, b) *dorsal* view *and* c) *lateral* view *of a g*yne of *Tetramorium microgyna*. Photos by François Brassard.

Fig. 4: Photos of a) head view, b) *dorsal* view *and* c) *lateral* view *of a w*orker of *Tetramorium sericeiventre*. Photos by François Brassard.

Fig. 5: *Tetramorium microgyna* gyne and *T. sericeiventre* workers cooperating on nest maintenance by carrying pebbles and soil within the nest to then deposit them at the periphery of the nest entrance. Photos by François Brassard.

Fig. 6: Gynes of *T. microgyna* at the nest entrance were either winged, wingless, or partially dealate. Photos by François Brassard.

Fig. 7: Photos of a) head view, b) lateral view, c) dorsal view and d) labels of specimen (CASENT 0102386) collected by Philip S. Ward in 1990 in Madagascar. It was then labelled as a microgyne of *Tetramorium quadrispinosum* (now *Tetramorium sericeiventre*). Photos by April Nobile and taken from AntWeb.org.

Fig. 8: Photos of a) head view, b) lateral view, c) dorsal view and d) labels of specimen (CASENT 0101997), a typical queen of *Tetramorium sericeiventre*. Photos by April Nobile and taken from AntWeb.org.

Fig. 1

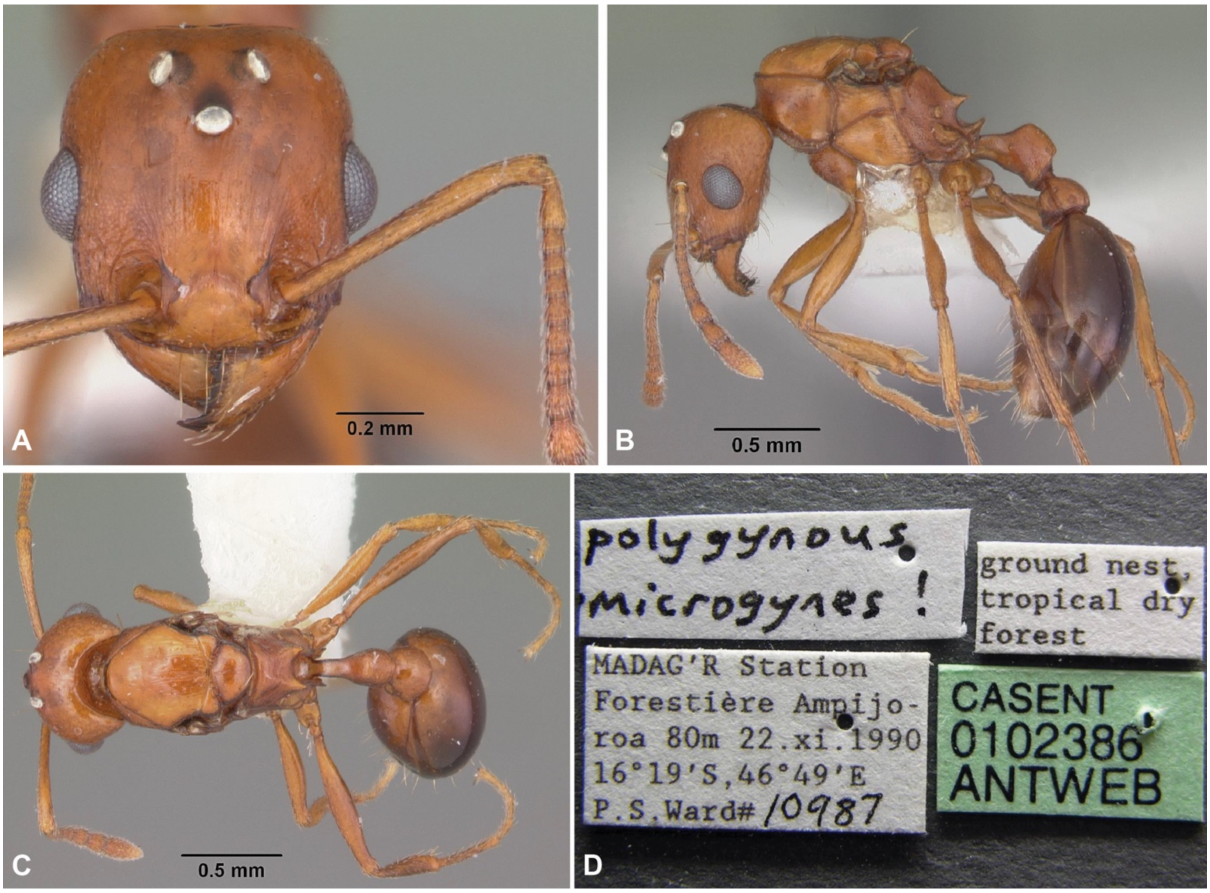
Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6



polygynous
microgynes !

ground nest,
tropical dry
forest

MADAG'R Station
Forestière Ampijo-
roa 80m 22.xi.1990
16°19'S,46°49'E
P.S.Ward#10987

CASENT
0102386
ANTWEB

Fig. 7

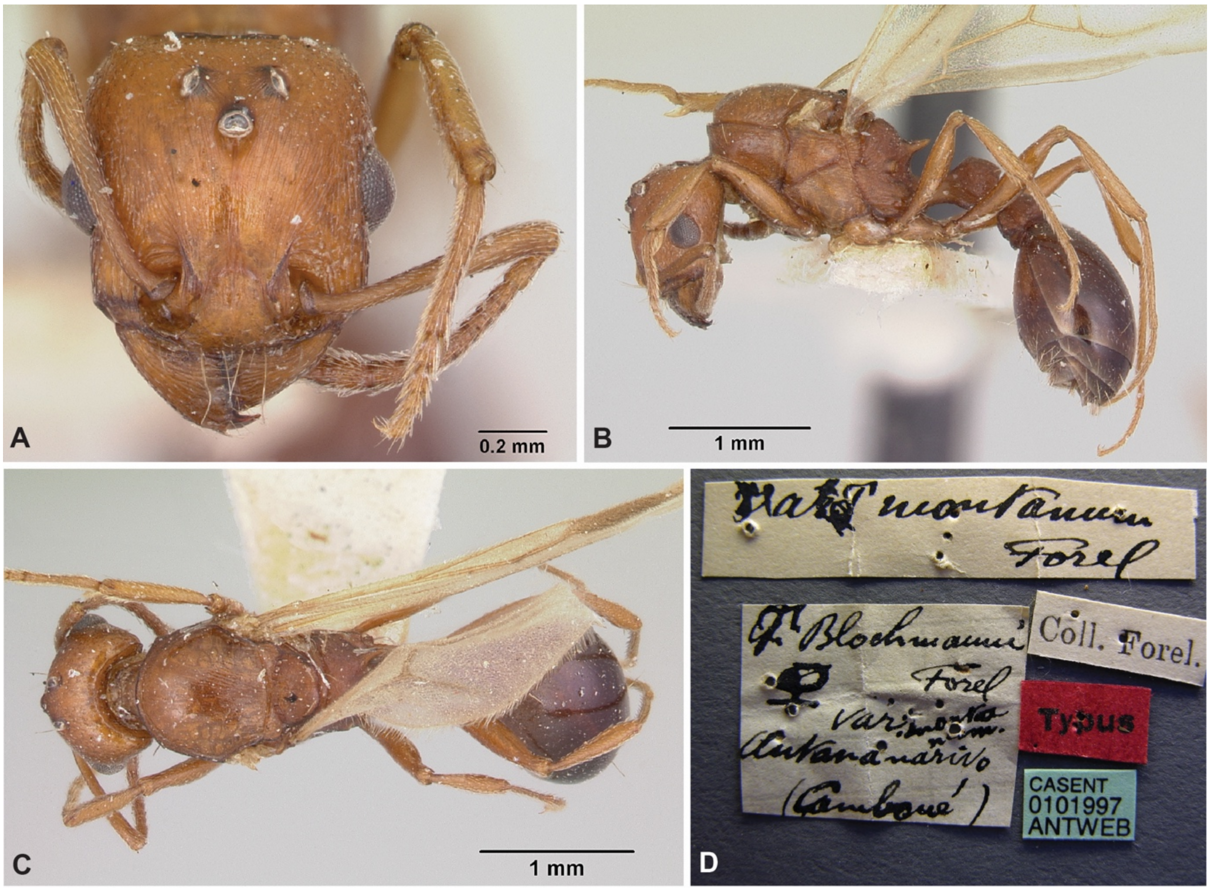

A
0.2 mm
B
1 mm
C
1 mm
D
Plat. montanum Forel
C. Blochmanni Forel ♀ var. montanum Antananarivo (Camboué)
Coll. Forel.
Typus
CASENT 0101997 ANTWEB

Fig. 8

Appendix

Table S1 Dissection details for three gynes of *Tetramorium microgyna*.

Gyne ID	Wings	Sperm in spermatheca?	Number of ovarioles	Number of ripe ovarioles	Fat body	Air sacs	Crop	Midgut	Malpighian tubules	Hindgut	Poison gland/sac	Dufour's gland
Tmicro01	Both wings persent on right side of body, wings absent on left side	No	6 (3 left, 3 right)	3 moderately ripe (two left ovary, 1 right ovary)	none, completely devoid of fat body	inflated	Full, clear liquid	Full, with fine, dark particulate matter	<15	Full, single large oblong pellet. Very hard and comprised of small, compressed tubules of particulate matter	Present, large and turgid	visible
Tmicro02	Dealate, fresh wing scars with frayed wing membrane	No	6 (3 left, 3 right)	4 moderately ripe (three left ovary, 1 right ovary)	1-layer thick, minimal coverage with some anterior and dorsal to crop, and ventral near distal end of the gaster	inflated	deflated, empty but not perforated	Full and round with pale, particulate matter	<10	Minimal brown hindgut material, tubular under magnification as in Tmicro01	Present, large and turgid	visible
Tmicro03	4 wings, intact	No	7 (3 left, 4 right)	1 ripe with well-developed, opaque egg (right ovary)	1-layer thick, ventral, beneath all organs, no dorsal fat observed	Deflated or perforated during dissection	Full, clear liquid	Full, with fine, dark particulate matter	Not counted	Empty	Present, large and turgid	visible