A new workerless inquiline in the Lower Attini (Hymenoptera: Formicidae), with a discussion of social parasitism in fungus-growing ants

CHRISTIAN RABELING[1] and MAURÍCIO BACCI Jr.[2]

[1]Section of Integrative Biology, The University of Texas, Austin, TX, U.S.A. and [2]Center for the Study of Social Insects, São Paulo State University, Rio Claro, SP, Brazil

Abstract. Ant inquilines are obligate social parasites, usually lacking a sterile worker caste, which are dependent on their hosts for survival and reproduction. Social parasites are rare among the fungus-gardening ants (Myrmicinae: tribe Attini) and only four species are known until now, all being inquilines from the Higher Attini. We describe *Mycocepurus castrator* **sp.n.**, the first inquiline social parasite to be discovered in the Lower Attini. Our study of the parasite's behaviour and life history supports the conclusion drawn from external morphology: *Mycocepurus castrator* is an evolutionarily derived inquiline parasite of *Mycocepurus goeldii*. Inquilines are of great interest to evolutionary biology because it is debated if they originated via sympatric or allopatric speciation. We discuss the life history evolution, behaviour and morphology of socially parasitic, fungus-growing ants.

Introduction

Societies of all social organisms are defined by reproductive division of labour, co-operative brood care and overlapping generations (Batra, 1966; Mitchener, 1969; Wilson, 1971; Hölldobler & Wilson, 1990). Among the social insects, ants are unique in that all extant species are eusocial, except for a few inquiline social parasites, in which eusociality has been lost secondarily, due to the marked reduction (or more often complete loss) of the worker caste. Inquiline social parasites are highly adapted to exploit their host colonies and depend completely on the host workers for food provisioning, brood care, and other colony maintenance tasks (Kutter, 1969; Wilson, 1971; Buschinger, 1986, 2009; Hölldobler & Wilson, 1990; Bourke & Franks, 1991).

Ant social parasites may be categorized according to their host interactions (Wasmann, 1891; Hölldobler & Wilson,1990). (i) In temporary social parasites, the parasite queen must be adopted by a host colony and depends on the host colony to raise the first generation of her own worker offspring. When sufficient workers of the parasitic species are produced, the colony becomes independent of the host. (ii) Dulotic or slave-making parasites steal worker brood from host colonies and the emerging host workers provide social tasks (i.e. brood care, foraging, nest hygiene) that ensure the parasite colony's survival. In addition, dulotic queens found colonies parasitically in a manner similar to temporary social parasites. (iii) Inquiline parasites depend on the host species for their entire life cycle. The inquiline foundress invades a host colony and uses the host workers to raise her sexual brood. Interestingly, the parasite queen generally does not produce a sterile worker force. Thus, her reproductive output depends entirely on the host species. In host-queen tolerant inquilines, host and parasite queens coexist, but the host queen's fitness is compromised because the parasite inhibits the production of sexual offspring, whereas the production of host workers is continued. In contrast, the inquiline queen maximizes her fitness by producing exclusively sexual offspring (Kutter, 1969; Wilson, 1971; Buschinger, 1986; Hölldobler & Wilson, 1990). Because host workers continue to be produced, the parasitized colony may survive indefinitely. In host-queen intolerant inquilines (a minority of inquiline species) the host queen is eliminated somehow and reproduction of the parasite ceases when the host workers die off. Thus, parasitized colonies have a short life span.

Correspondence: Christian Rabeling, Section of Integrative Biology, The University of Texas, One University Station C0930, Austin, TX 78712, U.S.A. E-mail: rabeling@mail.utexas.edu

Inquiline social parasites are of special interest in evolutionary biology because inquiline parasites are generally quite closely related to their host species. Indeed they may be derived from their host species – a phenomenon known as Emery's Rule (Emery, 1909). The relatedness of host and parasite has led to considerable speculation about how such host–parasite pairs might evolve. Although some have developed scenarios for allopatric speciation (e.g. Wilson, 1971; Hölldobler & Wilson, 1990), others see the evolution of inquiline social parasites as probable cases of sympatric speciation (e.g. Buschinger, 1990; Bourke & Franks, 1991).

In the fungus-gardening ants, a monophyletic group of about 230 species (tribe Attini) that depends obligately on a fungal cultivar for food (Wheeler, 1907; Weber, 1972; Mueller *et al.*, 2001), four social parasite species are known from the leafcutter ant genera *Pseudoatta* Gallardo and *Acromyrmex* Mayr. *Pseudoatta* is an obligate workerless parasite living in nests of *Acromyrmex* (Gallardo, 1916). Currently, two *Pseudoatta* species are recognized from Argentina and Brazil: first, *P. argentina* Gallardo, an inquiline of *A. lundi* (Guérin-Méneville), and its dubious subspecies *P. a. platensis* Santschi, and second, an undescribed species from Bahia, Brazil parasitizing *A. rugosus* (Smith, F.) (see Delabie *et al.*, 1993).

Two species of recently evolved inquiline social parasites are known among attine ants. *Acromyrmex insinuator* Schultz, Bekkevold & Boomsma parasitizes *A. echinatior* (Forel) in Panama (Schultz *et al.*, 1998), whereas the recently discovered *A. ameliae* Souza, Soares & Della Lucia is a social parasite of *A. subterraneus* (Forel) and *A. s. brunneus* (Forel) in Minas Gerais, Brazil (de Souza *et al.*, 2007). In contrast to the highly derived *Pseudoatta* inquilines, these social parasites still produce a worker caste. In addition, parasite queens and males lack the morphological specializations indicative of the inquiline syndrome, or anatomical parasitic syndrome (Kutter, 1969; Wilson, 1971, 1984). Because of their lesser degree of morphological specialization, recently evolved social parasites are thought to be recently derived from their ancestors, whereas the workerless inquilines probably represent a highly derived group of generally greater evolutionary age (see Discussion).

Here we describe a new species of workerless inquiline in the fungus-gardening ant genus *Mycocepurus* Forel. It is the first inquiline species discovered in the Lower Attini. Based on morphological, behavioural and life history characteristics, this new species is categorized as an evolutionarily derived inquiline. To gain some insight into the behavioural biology and life history evolution of inquiline parasites, we observed the parasite's behaviour in laboratory colonies. The parasite's invasion behaviour was studied by introducing newly mated inquiline queens into nonparasitized laboratory and field colonies of the host. Subsequently, host and parasite queens were dissected to confirm their reproductive status. The results are discussed in the context of inquiline morphology, behaviour and life history evolution.

Materials and methods

Field site

Mycocepurus castrator **sp.n.** and *Mycocepurus goeldii* (Forel) were collected on the Campus of São Paulo State University (UNESP) in Rio Claro, Brazil (22.3955°S, 047.5424°W; elevation 608 m). Fieldwork was conducted between 29 September and 14 October 2006, and between 29 September and 20 October 2008.

Material examined

Museum specimens of additional *Mycocepurus* species from the following collections were examined for this study:

AMNH	American Museum of Natural History, New York, NY, U.S.A.
BMEL	M. Bacci Jr. Molecular Evolution Laboratory, São Paulo State University, Rio Claro, Brazil
CRC	C. Rabeling Collection, Austin, TX, U.S.A.
INBC	Instituto Nacional de Biodiversidad, San José, Costa Rica
INPA	Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil
JTCL	J.T. Longino Collection, Evergreen State College, Olympia, WA, U.S.A.
LACM	Los Angeles County Museum of Natural History, Los Angeles, CA, U.S.A.
MCSN	Museo Civico di Storia Naturale, Genoa, Italy
MCZC	Museum of Comparative Zoology, Harvard University, Cambridge, MA, U.S.A.
MHNG	Muséum d'Histoire Naturelle, Geneva, Switzerland
MZSP	Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil
SMNK	Staatliches Museum für Naturkunde, Karlsruhe, Germany
SSPC	Sergio Sánchez-Peña collection, Monterrey, Mexico
UCDC	Bohart Museum of Entomology, University of California, Davis, CA, U.S.A.
UFU	Heraldo Vasconcelos collection, Uberlândia, Brazil
USNM	National Museum of Natural History, Washington, DC, U.S.A.
WPMC	W.P. Mackay Collection, El Paso, TX, U.S.A.

Morphological examination

Morphological examinations were undertaken using a Leica MZ16 stereo-microscope at magnifications of maximally 100×. Measurements were completed at 100× using a calibrated ocular micrometer. All metric measurements were recorded to the nearest 0.001 mm and rounded to two decimal places. Measurements are given in millimetres. Scanning electron micrographs were taken with a Zeiss SUPRA 40 VP scanning electron microscope. Morphological terminology follows Bolton (1994). Anatomical abbreviations and measurements are defined in Rabeling *et al.* (2007a).

Behavioural biology and natural history

Alates of *M. castrator* were observed for the first time on a nest mound of a colony of *M. goeldii* on 29 September 2006. Upon recognition of these small specimens as inquiline parasites, the host nest was excavated by digging a trench of approximately 250 cm depth, 50 cm distance from the first nest entrance. Other *M. goeldii* nest excavations on UNESP campus showed that the ants excavate nest chambers as deep as 190 cm below the surface (Table 1; see Rabeling *et al.*, 2009). Working gradually towards the nest entrance, vertical slivers of soil were sliced away with a spade and the position, dimension and content of every nest chamber were recorded. The digging continued 50 cm past the last excavated chamber. All *Mycocepurus* specimens were stored in 95% ethanol (see Tables 1, 2).

To study the mating and nest founding behaviour of *M. castrator*, the same locality was visited in 2008 to search for parasitized *M. goeldii* colonies. To collect live colonies before the sexual offspring dispersed and to census the complete offspring produced by the parasite queen(s), fieldwork started before the onset of the rainy season, which usually marks the beginning of the mating season in many ant species in seasonal, subtropical habitats. *Mycocepurus goeldii* colonies were marked and random nests were excavated following the description above. It was not possible from above-ground observations to detect whether a colony was parasitized. Upon encountering a parasitized colony (3 October), the fungus garden and all ants of each chamber were transferred to separate, transparent plastic boxes, which were lined with a plaster bottom for moisture regulation [described in Schultz (1993)]. In the first 36 h after collecting (3 October), the colony was observed for a total of 12 h. On subsequent days, observations were more sporadic, ranging between 30 min and 2.5 h per day. Observations ended on 18 October.

Twelve dealate *M. castrator* queens were present in a single nest chamber (CR081003-04). To test for functional polygyny, all dealate *M. castrator* queens ($n = 12$) were dissected to determine their reproductive status, and alate queens ($n = 3$) served as a negative control for virgin, noninseminated queens. In addition, seven *M. goeldii* queens (two dealate, five alate) from two colonies were dissected to ensure that the reproductively active queens of both colonies were studied during the laboratory observations and parasite introduction experiment. The methodology and results of the dissections were published in the context of a study about asexual reproduction of *M. smithii* (Forel) queens (Rabeling *et al.*, 2009) and *M. castrator* was referred to as 'undescribed species' in that study.

In contrast to Rio Claro colonies, some *M. goeldii* colonies studied in the Amazonas Basin contained multiple dealate queens per chamber (Rabeling *et al.*, 2007b). To test whether these *M. goeldii* colonies were functionally polygynous we

Table 1. Census of two *Mycocepurus goeldii* colonies parasitized by *M. castrator*.

	M. castrator queens[a] (n)	*M. castrator* males (n)	*M. goeldii* workers (n)	Chamber depth (cm)	Chamber dimension (cm) (width × height)	Collection ID
2006						
Individuals on nest mound day 1	31	1	37	n/a	n/a	CR060929-14
Individuals on nest mound day 2	24	72	283	n/a	n/a	CR061002-09
Chamber 1 – fungus chamber	—	—	5	93	10 × 7.5	CR061002-01
Chamber 2 – fungus chamber	32	1	302	32	9 × 7	CR061002-02
Chamber 3 – empty chamber	3	3	12	5	1 × 2	CR061002-03
Chamber 4 – empty chamber	—	—	—	84	7 × 6.5	CR061002-04
Chamber 5 – fungus chamber	—	—	2	118	6 × 5.5	CR061002-05
Chamber 6 – empty chamber	7	1	1	31	5 × 2.5	CR061002-06
Chamber 7 – fungus chamber	8	—	97	54	7 × 6	CR061002-07
Chamber 8 – fungus chamber	—	—	32	69	11 × 9.5	CR061002-08
Total	105	78	771	n/a	n/a	n/a
2008						
Chamber 1 – fungus chamber	3 dealate and 13 alate	3	149	56	9 × 7	CR081003-01
Chamber 2 – fungus chamber	—	—	52	66	11 × 7	CR081003-02
Chamber 3 – fungus chamber	20	1	154	120	10 × 7	CR081003-03
Chamber 4 – queenright fungus chamber	12 dealate and 20 alate	1	339	160	10 × 9	CR081003-04
Chamber 5 – fungus chamber	13	1	340	190	10 × 8	CR081003-05
Total	81	6	1034	n/a	n/a	n/a

The colony studied in 2008 had a single reproductively active *M. goeldii* queen (chamber 4). The reproductive *M. goeldii* and *M. castrator* queens of the 2006 colony were not collected, indicating that the queenright chamber was either missed during excavation or that the queens escaped.
[a]Parasite queens were alate, if not noted otherwise.

Table 2. Census of an unparasitized *Mycocepurus goeldii* colony.

	M. goeldii queens[a] (*n*)	*M. goeldii* males (*n*)	*M. goeldii* workers (*n*)	Chamber depth (cm)	Chamber dimension (cm) (width × height)	Collection ID
2008						
Chamber 1 – fungus chamber	–	–	10	110	6 × 6	CR081007-01
Chamber 2 – fungus chamber	–	–	30	54	11 × 6	CR081008-01
Chamber 3 – queenright fungus chamber	1 dealate and 9 alate	–	193	34	8 × 6	CR081008-02
Chamber 4 – fungus chamber	6	–	153	45	6 × 4.5	CR081008-03
Chamber 5 – fungus chamber	2	–	82	49	7 × 7	CR081008-04
Chamber 6 – fungus chamber	16	–	28	160	8 × 6	CR081008-05
Total	34	0	496	n/a	n/a	n/a

The colony had a single reproductively active queen (chamber 3). Alate males were not present in the colony, which indicates that the colony was excavated during or just after the nuptial flight.
[a]*Mycocepurus goeldii* queens were alate, if not noted otherwise.

dissected 11 queens from six queenright colonies (the number of dealate queens is given in parentheses): Caldeirão 3 (*n* = 2), Caldeirão 8 (*n* = 2) and Pilz Präp 22 (*n* = 4). Three queens of monogynous colonies served as positive controls for reproductively active queens: Pilz Präp 23 (*n* = 1), laboratory colonies A5-2 (*n* = 1) and A5b (*n* = 1).

Results

Taxonomy

Mycocepurus castrator Rabeling & Bacci, sp.n.

(Figs 1A, C, E, G; 2A, C, E, G)

Holotype, ♀, BRAZIL: São Paulo, Rio Claro, Campus of São Paulo State University (UNESP), 22.3955°S, 047.5424°W, elevation 608 m, 29.ix.2006, C. Rabeling acc. no. CR060929-14, ex *Mycocepurus goeldii* nest. Holotype deposited at MZSP. Measurements (in millimetres): HW 0.6, HL 0.64, SL 0.76, WL 1.07, PPW 0.62, PW 0.21, PL 0.24, PPL 0.19, CI 94, SI 127.

Paratypes, 104 ♀, 78 ♂, BRAZIL: same nest as holotype, 29.ix.2006–02.x.2006, col. C. Rabeling. Paratypes deposited at: AMNH, BMEL, CRC, MCZC, MZSP, UCDC, USNM.

Holotype, ♀ (queen). *Diagnosis.* Small species (WL 1.07) with a unique morphology reflecting the parasitic life history. In full face view, head rectangular (CI 94); sides approximately parallel, slightly tapering above mandibular insertions; head widest directly above the eyes; posterior margin of the head heart shaped, with a slight but distinct median concavity; posterolateral corners rounded, in lateral view drawn out to form a short, rounded lobe forming the ventrolateral corner of the head. Antennae with 11 segments; antennal scapes extremely long (SL 0.76), surpassing the posterior margin of the head by nearly half their length (SI 127). In full face view, frontal carinae and antennal scrobes absent. Frontal lobes small and rounded, barely covering the antennal sockets in frontal view. Median triangular portion of clypeus raised

between the antennal insertions. Mandibles reduced, narrow, elongate, blade-like terminating in a pointed tooth; otherwise lacking teeth except for a small basal denticle. Maxillary palps reduced, with only three segments, labial palps with two segments. Ocelli slightly raised above the surface of vertex. Mesosoma with characteristic morphology related to wing bearing. Pronotal spines absent; propodeal spines well developed, stout, as wide as long at the base and sharply pointed; metapleural gland orifice very large and circular in oblique view, ventral margin forming small, vertical tooth. Petiole with a short peduncle; node triangular in side view, with sharp crest terminating in two thick pointed teeth. In dorsal view, postpetiole approximately 3× as wide as long (PPL 0.19, PPW 0.62); lateral borders tapering into pointed angles; translucent area near posterior margin forming broad u-shaped invagination. First gastric tergite strikingly concave in lateral view. Entire body surface more or less smooth and shiny, in most areas with hexagonal microsculpture resembling a honeycomb. Body sparsely covered with stiff setae; setae erect on vertex and frontal lobes, sub-decumbent on mesoscutum and scutellum, and appressed on postpetiole and metasoma. Wings infuscated with reduced venation, densely covered with setae; clear spot or fenestra in apical part of forewing absent; rsf1 faint, hardly visible. Colour: light to dark reddish-brown. – Paratype ♀♀. Measurements (*n* = 15). HW 0.6–0.65, HL 0.63–0.64, SL 0.73–0.8, WL 1.07–1.23, PPW 0.62–0.65, PW 0.21–0.25, PL 0.24–0.28, PPL 0.18–0.2, CI 94–104, SI 115–128.

Paratype ♂♂ (males). *Diagnosis.* Remarkably similar to female, not resembling any other *Mycocepurus* male; characters as in female diagnosis with the following exceptions: head size of males smaller (HL 0.58–0.6, HW 0.58–0.6), whereas body length similar (WL 1.1–1.2). Mandibles reduced, narrow, elongate, blade-like, which do not terminate in a pointed tooth; otherwise lacking any teeth or denticles. Number of antennal segments reduced to 11; funicular segments approximately as long as broad, slowly increasing in length towards apex to 1.5× their width, only apical segment 5× as long as
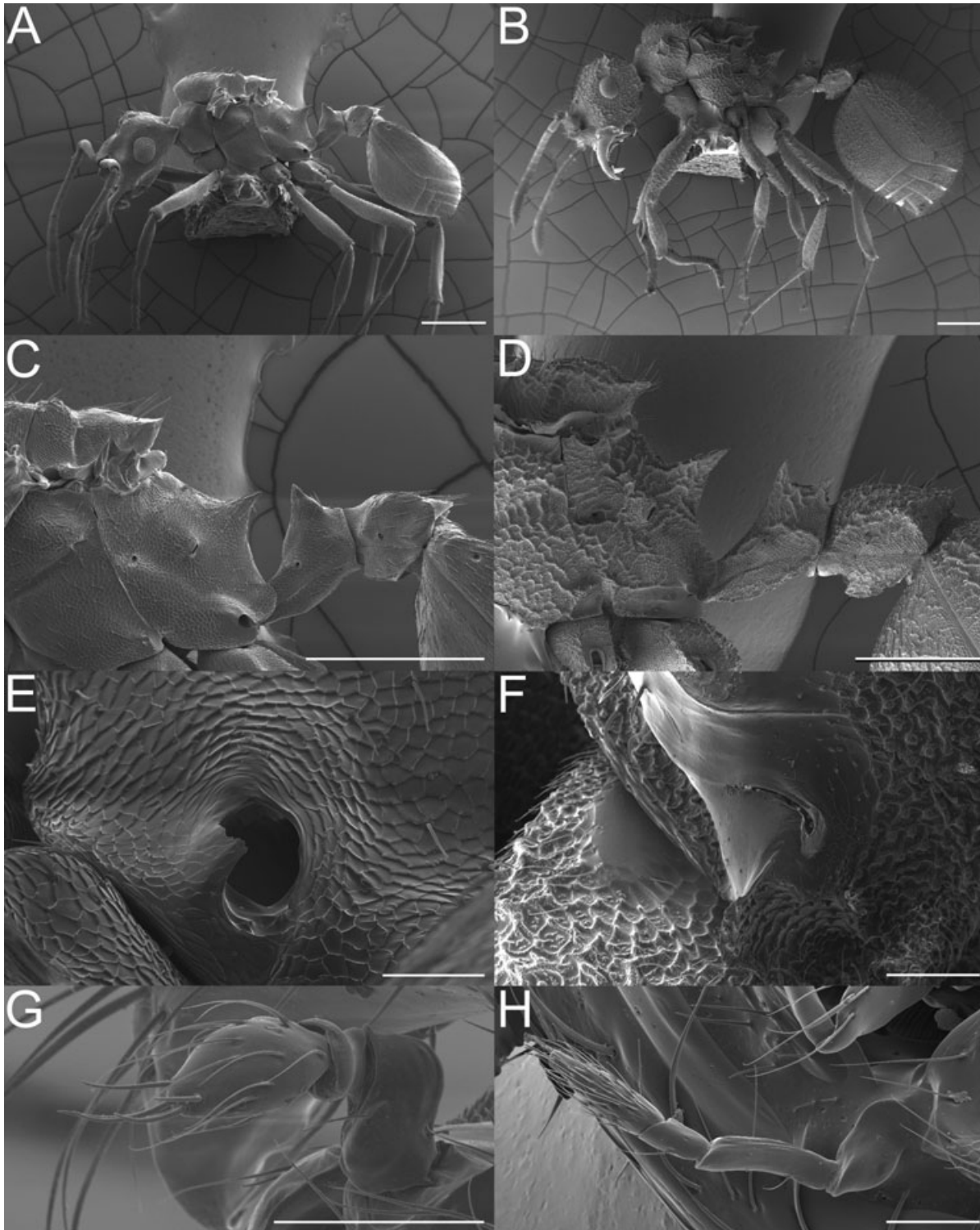
Fig. 1. Queens of *Mycocepurus castrator* (A, C, E, G) and *Mycocepurus goeldii* (B, D, F, H). (A, B) Habitus in lateral view; scale bar represents 0.5 cm. (C, D) Posterior part of the mesosoma, petiole and postpetiole in lateral view; scale bar represents 0.5 cm. (E, F) Metapleural gland orifice in oblique view, metacoxa is visible in the lower left corner of the image; scale bar represents 50 μm. (G, H) Maxillary palp, scale bar represents 50 μm.
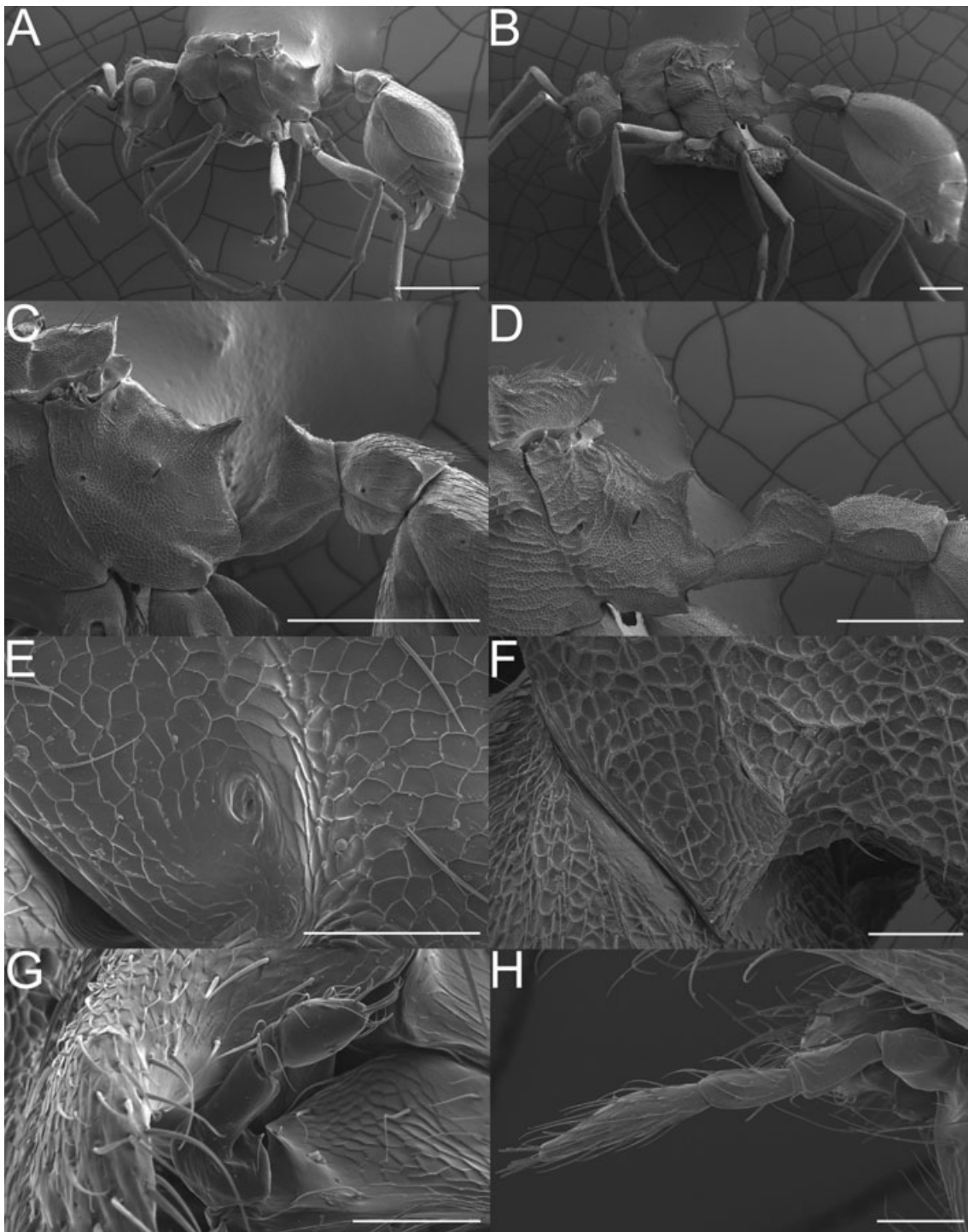
Fig. 2. Males of *Mycocepurus castrator* (A, C, E, G) and *Mycocepurus goeldii* (B, D, F, H). (A, B) Habitus in lateral view; scale bar represents 0.5 cm. (C, D) Posterior part of the mesosoma, petiole and postpetiole in lateral view; scale bar represents 0.5 cm. (E, F) Metapleural orifice in oblique view, metacoxa is visible in the lower left corner of the image; scale bar represents 50 μm. (G, H) Maxillary palp, scale bar represents 50 μm.

wide. Mesosoma lower and narrower; tiny opening present at the metapleuron, corresponding to the position of a metapleural gland opening in the female. First gastric tergite flat to slightly concave; male genitalia projecting forward from tip of metasoma. Basal apodeme lobed, separated from aedeagus by a deep constriction; ventral border lacking serration. Wing colour: medium to dark brown. *Measurements* ($n = 15$). HW 0.58–0.6, HL 0.58–0.6, SL 0.73–0.75, WL 1.1–1.2, PPW 0.63–0.65, PW 0.23–0.3, PL 0.25–0.28, PPL 0.18–0.2, CI 96–104, SI 121–126.

Worker. The worker caste is unknown and probably nonexistent.

Additional material examined. BRAZIL: São Paulo, Rio Claro, Campus of São Paulo State University (UNESP), 22.3955°S, 047.5424°W, elevation 608 m, 03.x.2008, C. Rabeling acc. no. CR081003-01, CR081003-02, CR081003-03, CR081003-04, CR081003-05; ex *Mycocepurus goeldii* nest.

Comments. Mycocepurus castrator is an obligate, workerless social parasite of *M. goeldii* and is so far known only from Rio Claro, São Paulo State, Brazil. *Mycocepurus castrator* occurs sympatrically with *M. smithii* and *M. obsoletus*, but cannot be confounded with any other *Mycocepurus* species because of its multiple morphological adaptations for a parasitic lifestyle (Table 3). *Mycocepurus castrator* can be recognized by the following characteristics: (i) the long antennal scapes surpassing the posterior margin of the head by half their length; (ii) reduced, blade-like mandibles lacking dentition of masticatory margin; (iii) concave shape of first gastric tergite; (iv) smooth and shiny body sculpture with hexagonal microsculpture; (v) reduced palpal formula (3,2); (vi) females and males with 11 antennal segments; (vii) males lacking serrated ventral border of aedeagus; (viii) absence of clear fenestra from forewings of queens and males; (ix) worker caste presumably absent; (x) metapleural gland orifice enlarged in females, and potentially present in males. *Mycocepurus castrator* males and females look extremely similar to each other, and males are distinguished most easily from the females by the genitalia protruding from the tip of the metasoma and their darker brown colour (vs reddish brown in the queens).

Etymology. During collections of *M. castrator*, the host colonies were not observed to produce any alate queens and males, although sympatrically nesting *M. goeldii* colonies released alates. Therefore, we assume that the inquiline inhibits the host queens' production of sexual offspring, allowing only for the production of the sterile worker caste. This is essentially 'social castration', hence the specific name 'castrator'.

Host species. Mycocepurus castrator has been found only in nests of *M. goeldii* and is so far only known from the type locality (Rio Claro, SP). *Mycocepurus goeldii* is a conspicuous, widely distributed species ranging approximately from the 40th to the 67th meridian west and from the 2nd to the 31st latitude south, an area covering most of Brazil, parts of Bolivia, Paraguay and northern Argentina. The range of habitats occupied by *M. goeldii* is remarkably diverse and ranges from Amazon rainforest, savannahs (Cerrado) to the fertile South American lowlands (Pampas), and secondary habitats disturbed by human activities. It does not occur in elevated sites of the South American Cordilleras. *Mycocepurus goeldii* workers can be distinguished clearly from its congeners based on the size and spine pattern of the mesosoma: it is the largest species in the genus and has the most complete set of spine pairs on the mesosoma (Kempf, 1963: figs 2, 3). The natural history of this species has been studied near São Paulo City (Luederwaldt, 1918, 1926) and in the Manaus region of the Amazon Basin (Rabeling *et al.*, 2007b), but these studies do not report the presence of a social parasite attacking *M. goeldii*. Like most inquilines for which we have data, *M. castrator* probably has a patchy and locally restricted distribution. In addition, it is probable that *M. castrator* is host specific, occurring only in nests of *M. goeldii*. Despite extensive excavation of nests of sympatrically occurring *Mycocepurus* species, the parasite was never encountered in the nests of *M. smithii* (Rabeling *et al.*, 2009) or any other *Mycocepurus* species in Latin America (Rabeling, unpublished).

Natural history and nest biology. Mycocepurus castrator has been found twice in adjacent nests of *M. goeldii*. The two host nests had five and eight chambers, respectively, which were distributed between 5 and 190 cm depth (Table 1). The colony studied in 2006 contained 105 alate queens and 78 alate males of *M. castrator*, and 771 workers of *M. goeldii* (Table 1). Dealate queens of either species could not be encountered, suggesting that the queenright chamber was either missed during the excavation or that the queens escaped into adjacent tunnels.

The 2008 colony contained 15 dealate and 66 alate *M. castrator* queens, only six alate males, 1034 *M. goeldii* workers, a single dealate *M. goeldii* queen and worker pupae (Table 1). The parasite's numerical male/female sex ratio was strongly female biased ($6/66 = 0.09$). Twelve of the 15 dealate *M. castrator* queens were encountered in the same fungus garden chamber as the reproductively active female of *M. goeldii*. Thus, *M. castrator* is host-queen tolerant (Table 1). The other three dealate *M. castrator* queens were found together in a separate fungus chamber (chamber 1; Table 1). The 12 queens encountered with the *M. goeldii* queen showed different reproductive activities: three were active egg layers, showing developed ovaries, yellow bodies and sperm-filled spermathecae. Thus, the parasite can be polygynous. In contrast, the remaining nine queens were prereproductive with filled spermathecae, but the ovaries were still developing, and yellow bodies were absent. The three dealate queens from chamber 1 were also prereproductive. The single *M. goeldii* queen was reproductively active.

The unparasitized *M. goeldii* colony studied in 2008 contained a single reproductively active queen, 33 alate queens,

Table 3. Life history and morphological characters of inquiline parasites in fungus-gardening ants.

Life history and morphological traits	Incipient inquiline parasites		Evolutionarily derived inquiline parasites	
	Acromyrmex insinuator	*Acromyrmex ameliae*	*Mycocepurus castrator* [3]	*Pseudoatta argentina*
Parasite association with host	Obligate [1, 6]	Obligate [2]	Obligate	Obligate [7,8]
Parasite tolerant/intolerant of host queen	Tolerant [1,6]	Tolerant [2]	Tolerant	Intolerance suggested [8]
Worker caste present/absent	Minors present, very rarely media or large workers [1,6]	Minors present [2]	Absent	Absent [9]
Number of reproductively active parasite queens	Facultative polygyny [6]	Facultative polygyny [2]	Polygyny	Unknown
Production of host alates in parasitized nests	Host and parasite alates co-occur [6]	Parasite alates precede or follow host alates [2]	Host alates absent when parasite alates are present	Host alates absent when parasite alates are present [7,8]
Mating flights (compared with host)	Present (normal) [1,6]	Present (normal) [2]	Absent	Absent in ♂; ♀ perform dispersal flight [7,8]
Nest founding strategy	Invasion of established nests; pleometrosis with host ♀ suggested [1,6,16]	Invasion of established host colonies suggested [2]	Invasion of established host colonies	Invasion of established host colonies suggested [7,8]
Reproductive strategy (semelparity vs iteroparity)	Semelparity [6]	Unknown	Iteroparity suggested	Semelparity suggested (host colony died after nuptial flight) [7,8]
Mating strategy (intranidal vs outside nest)	Possibly mixed: inside and outside nest mating [6]	Unknown	Intranidal mating	Intranidal mating [7,8]
Host species	*A. echinatior* [1,6]	*A. subterraneus subterraneus, A. subterraneus brunneus* [2]	*M. goeldii*	*A. lundi* [9]
Number of reproductively active host queens	Facultative polygyny [12]	Facultative polygyny [10,11]	Monogyny in Rio Claro [3]; facultative polygyny in Manaus [3,13]	Unknown
Reduction of parasite body size	Both sexes [1]	Both sexes [2]	Both sexes	Both sexes [9]
Gynomorphism of parasite males	Absent [1]	Absent [2]	Present	Present [9]
Integument sculpturing of parasite	Well developed, matt [1]	Well developed, matt [2]	Smooth, shiny	Smooth, shiny [9]

Table 3. Continued

Life history and morphological traits	Incipient inquiline parasites		Evolutionarily derived inquiline parasites	
	Acromyrmex insinuator	*Acromyrmex ameliae*	*Mycocepurus castrator* [3]	*Pseudoatta argentina*
Pilosity (relative to host)	Similar [1]	Similar [2]	Reduced	Reduced [9]
Mandibular dentition	Well developed [1]	7–8 denticles in ♂ [2]	Reduced	7–8 denticles in ♀ [9]
Number of antennal segments	♀: 11; ♂: 13; funicular segments 4 and 5 partly fused in some ♂ individuals [1]	♀: 11; ♂: 13; funicular segments 4 and 5 partly fused in some ♂ individuals [2,14,15]	♀: 11; ♂: 11	♀: 11; ♂: 11 [9]
Palp formula (maxillary, labial palp)	4,2 [1]	4,2 [2]	3,2	3,2 [1,14,15]
Size and shape of metapleural gland opening in ♀	Similar to host [4]	Similar to host [5]	Enlarged, circular, pointing backwards	Enlarged, circular, pointing backwards [4]
Size and shape of metapleural opening in ♂ (existence of gland not confirmed with histological methods)	Absent [17]	Absent [17]	Tiny, circular opening on metapleuron, congruent with position of MPG opening in female	Enlarged opening, similar to the queen [17]
Postpetiole width	Similar to host [1]	Similar to host [2]	Enlarged	Enlarged [9]
First gastral tergite of ♀ with concave, median impression	Absent [1]	Absent [2]	Present	Present [9]

All four species originated independently, but evolved similar traits convergently, allowing for classifying them as incipient and evolutionarily derived social parasites. A second, hitherto undescribed *Pseudoatta* species is not considered in this table, because only few morphological characteristics are described in the literature (Delabie *et al.*, 1993; see also Schultz *et al.*, 1998). Some life history information summarized here is derived only from single observations or stems from indirect evidence, and therefore should be considered tentative.
Numbered references in this table refer to the following sources (please see reference list for complete citations): [1] Schultz *et al.* (1998); [2] Souza *et al.* (2007); [3] this study; [4] S.H. Yek & U.G. Mueller (personal communication); [5] Rabeling (personal observation); [6] Bekkevold & Boomsma (2000); [7] Gallardo (1929); [8] Bruch (1928); [9] Gallardo (1916); [10] Della Lucia & Vilela (1986); [11] Delabie (1989); [12] Bekkevold *et al.* (1999); [13] Rabeling *et al.* (2007b); [14] Kusnezov (1951); [15] Kusnezov (1954); [16] J. J. Boomsma & V. Nehring (personal communication); [17] T.R. Schultz (personal communication).

496 workers and no males (Table 2). During the excavation, males and queens were leaving the maternal colony for their nuptial flight, which started on 7 October.

A natural history study of *M. goeldii* in the Amazon Basin (Rabeling *et al.*, 2007b) showed that some colonies had a single queen, whereas others were occupied by as many as four queens. Dissection of eight individuals from three separate colonies revealed that all of them were inseminated and had fully developed ovaries, demonstrating that these colonies were functionally polygynous.

The observations on nesting biology and colony counts suggest that *M. castrator* is polygynous, host tolerant and allows for the production of sterile *M. goeldii* workers, whereas the production of host sexual offspring is suppressed in the presence of the parasite. The host, *M. goeldii*, appears to be monogynous in the Rio Claro population, but both mono- and polygynous colonies co-occur in the Brazilian Amazon.

Behaviour. In the late afternoon of 29 September 2006, *M. castrator* was discovered when 31 queens and a single male left the host colony to aggregate on the nest mound. The dispersal activity was interrupted by rain, but continued on 2 October, when 24 queens and 72 males emerged. No further behavioural observations were made that year.

In 2008, *M. goeldii* colonies were excavated at the end of the dry season in order to study parasitized colonies before the nuptial flight. An approaching mating flight is easily identified in *M. goeldii* colonies, because the workers increase the number of nest entrances per soil mound to maximally 30 entrances, giving the nest mound a sponge-like appearance (Rabeling *et al.*, 2009). Until 3 October, when a *M. goeldii* colony parasitized by *M. castrator* was encountered, the *M. goeldii* workers did not modify the nest mounds for mating flights. Upon excavating the parasitized colony, all individuals from a total of five nest chambers were transferred to artificial nest chambers for behavioural studies.

Parasite mating behaviour. As soon as the uppermost chamber (CR081003-01, Table 1) was opened during excavation, and the ants were transferred to the artificial nest chambers, *M. castrator* males started copulating with *M. castrator* females inside the artificial chamber (*M. goeldii* alates from an adjacent nest, placed into a laboratory nest, were never observed to copulate). During this time of ongoing mating activities, females and males ran erratically in jerky movements, and males mounted females seemingly at random. Observed copulations lasted between 18 and 27 s ($n = 4$). Single males attempted to copulate more than once. It is unknown whether repeated copulations resulted in successful transfer of sperm. Within 3 h after transfer to the artificial nest, three queens shed their wings, and subsequent dissections demonstrated that these females had been inseminated. However, their ovaries were still developing (i.e. ripe oocytes and yellow bodies were absent), indicating recent insemination (note: queens were preserved for dissection 2 weeks after copulation). Postcopulatory females did not tolerate mating attempts of males, and walked faster to outdistance their pursuer. After copulation, and wing shedding,

the recently mated queens gathered and engaged in allogrooming, frequently licked each others meso- and metasomas, and wings for extensive periods of time. The first dead males were found 12 h after the mating event. Alates from other chambers did not copulate after transfer to the artificial nest. Potentially, the individuals in the topmost chamber were anticipating the upcoming nuptial flight and the opening of their nest chamber triggered the mating behaviour.

Host worker–parasite interactions. Host workers and parasite alates frequently antennated and interacted nonaggressively. *Mycocepurus castrator* alates did not require grooming by host workers because individuals cleaned themselves (i.e. licking appendages, cleaning antennae), and females groomed each other. Dealate *M. castrator* queens groomed *M. goeldii* workers, and were groomed by them also. On several occasions, *M. goeldii* workers licked the tip of a *M. castrator* metasoma for several minutes; it is not clear if the workers removed fecal droplets, or *M. castrator* queens laid either fertile or trophic eggs. *Mycocepurus goeldii* workers fed the parasite queens via trophallaxis. To be fed, *M. castrator* females frequently climbed onto the host workers' backs, antennated the host's antennae and head, until it bent its head backwards, regurgitated liquid, which was then consumed by the parasite. In addition to being fed, *M. castrator* males and females actively licked the fungus garden.

Three days after insemination, the host workers aggressively attacked one dealate queen from the topmost chamber (CR081003-01); six to eight workers secured her by the antennae, legs, head and petiole, until she died. Approximately 24 h after her death, three workers continued to carry around her corpse in the nest chamber. Six days after insemination, the host workers had attacked and killed several *M. castrator* queens, and had placed them on the refuse dump. Three dealate queens remained unmolested by hiding together in the fungus garden.

Host queen–parasite interactions. To observe the interactions between the host queen and the dealate *M. castrator* queens ($n = 12$), we placed the queens in a smaller nest chamber, after *M. goeldii* workers had arranged the fungus garden. The *M. castrator* queens were much more agile than the *M. goeldii* queen and initially walked around the nest chamber until they encountered a suitable spot; there they aggregated and started licking each other. When first placed in the chamber, the *M. goeldii* queen crawled under an adjacent piece of fungus garden and remained motionless; a worker then picked her up by the metasoma and moved the queen to a different position. During the carrying, the *M. goeldii* queen remained motionless. After several minutes, one *M. castrator* queen left the aggregation, 'searching' for the *M. goeldii* queen. When the host queen was encountered, she was surrounded by host workers antennating her. Regardless, the parasite climbed on the host queen's back (Fig. 3), and started licking her mesosoma, petiole, postpetiole and metasoma. Shortly afterwards, a second *M. castrator* queen joined the first; the *M. goeldii*

Fig. 3. A queen of the social parasite *Mycocepurus castrator* standing on the host queen's back. The photograph was taken in a laboratory nest, set up at São Paulo State University in Rio Claro, Brazil in October 2008. The photograph was taken by Scott Solomon.

queen continued to remain motionless. The remaining *M. castrator* queens eventually joined the grooming cluster, and alternated grooming themselves by pulling their legs and antennae through the tibio-tarsal cleaning apparatus of the foreleg, with grooming the host queen. When the first *M. castrator* queen climbed on the host queen's back, the attending workers left and resumed fungus-gardening activities. They did not react aggressively to the parasite queens and often returned to antennate and to feed the host queen via trophallaxis. Once a worker carried the host queen to a different part of the fungus garden, and a *M. castrator* queen rode on her back during the location, licking her, and was not chased away. Either workers or *M. castrator* queens attended the host queen for most of time. Rarely and then for very short periods of time, she sat by herself. The *M. castrator* queens attempted constantly to climb on the backs of either other *M. castrator* queens, the host queen or host workers.

Introduction of parasite queen into a field colony. Two inseminated *M. castrator* queens from the topmost chamber (CR081003-01) were introduced to a *M. goeldii* colony, which opened its nest mound in preparation for the nuptial flight the previous day. The *M. castrator* queen was placed next to the nest mound. After orienting briefly, she immediately walked towards one of the entrance holes, and within a few seconds she disappeared into one of the entrances. The *M. goeldii* workers, which guarded the entrances, were not seen to attack, catch or struggle with the invading parasite. After 3 h the observation was stopped, and until then, *M. goeldii* workers had not expelled the *M. castrator* queen.

A second parasite queen was placed next to a *M. goeldii* colony, which had closed the supernumerary nest entrances after the nuptial flight. In contrast, the parasite did not start searching for the nest entrance and we repeatedly (five times) placed her on the side of the nest mound before she finally,

perhaps by chance, walked over the nest entrance. When crossing the entrance, *M. goeldii* workers attacked the parasite immediately. We collected the parasite queen and a dissection identified her as recently inseminated with developing ovaries.

Introduction of parasite queen into a laboratory colony. To observe how *M. castrator* queens invade a *M. goeldii* colony, we maintained a nonparasitized *M. goeldii* colony in the laboratory. Upon transfer to the artificial nest, *M. goeldii* workers immediately covered the host queen with mycelial tufts, until she was completely hidden some 5 min later. To introduce the parasite queen, she was placed in a tube, which was connected to the fungus chamber. Quickly, she found her way out, headed directly towards the fungus garden, and immediately encountered the host queen. Then she started running in circles on the piece of fungus garden, under which the host queen was hidden. At that point, the host workers started chasing her, until one worker got hold of her petiole, and a second worker grabbed an antenna. The trio remained motionless for about 10 min, until both workers suddenly released the captive. Immediately, the *M. castrator* queen made a beeline for the host queen's hiding place, where she was captured again. When being captured, the *M. castrator* queen assumed a characteristic position: she tucked her metasoma under the mesosoma, with the first gastric sternite touching the coxae. The combination of smooth body surface and broad, concave first gastric tergite provided little contact surface for attacking host workers, and their mandibles repeatedly slipped, upon which the host workers turned around and left. While being held by workers, the parasite queen was often antennated and licked at the base of the mandibles. After she was 'dumped' on the refuse pile, she promptly aimed for the host queen. This cycle of capture and release was repeated overnight. Meanwhile, the *M. goeldii* worker removed the fungus garden fragments from the host queen, releasing her from her mycelium prison. Unfortunately, the first contact of host and parasite queen was not observed, but early the next morning, the *M. castrator* queen was 'riding' on the *M. goeldii* queen, licking her mesosoma, petiole, postpetiole and metasoma. The *M. goeldii* workers frequently antennated and licked both queens, and the parasite queen was not attacked anymore. Subsequent observations revealed that the *M. castrator* queen was always 'riding' on top of the *M. goeldii* queen, and *M. goeldii* workers seemed to attend both queens equally.

Discussion

Several lines of evidence from natural history, behaviour and morphology identify *M. castrator* as an evolutionarily derived inquiline parasite of *M. goeldii*. *Mycocepurus castrator* is functionally polygynous and host-queen tolerant. It does not seem to produce a worker caste. Parasite queens apparently suppress the production of host sexual offspring, effectively castrating the infested host colony. Mating takes place inside the host nest and mating flights of the parasite have not been observed and probably do not occur. Alates of *M. castrator*, although

fully alate and seemingly capable, do not fly. After mating, we opened artificial laboratory nests to test if queens and/or males disperse via flight and none did. Alates were encouraged to climb objects that could serve as potential launch pads (i.e. pencils), but they plunged down after reaching the tip, without becoming airborne. Thus, dispersing queens must walk to new host nests, severely limiting their dispersal.

Recently inseminated *M. castrator* queens found new colonies by invading host nests and showed elaborate behaviours related to securing adoption by the hosts. Compared with the host, parasite queens and males are reduced in body size and exhibit several morphological specializations known as the inquiline or anatomical parasite syndrome (Kutter, 1969; Wilson, 1971, 1984; Hölldobler & Wilson, 1990). The new parasite appears to have only one host, *M. goeldii*, even though other free-living congeners are present in the type locality. Lastly, even though the host ant is widespread and abundant throughout much of southern South America, *M. castrator* has been collected only twice, both times at the type locality. This suggests that parasite populations are probably few in number, small in size and patchily distributed. Intranidal mating, limited dispersal and small, isolated populations imply minimal gene flow between populations, and high levels of inbreeding within populations, conditions that have been postulated to exist for a number of other inquilines (Kutter, 1969; Wilson, 1971; Zamora-Muñoz *et al.*, 2003; Buschinger, 2009). Inbreeding, however, has not yet been documented genetically in *M. castrator* (Rabeling, in preparation). Alternatively, it might be possible that the queens from a single nest are not necessarily closely related, because *M. castrator* is functionally polygynous, and parasite queens seem to invade host colonies independently.

Mycocepurus castrator shows several additional morphological features related to its parasitic lifestyle (Table 3). *Mycocepurus castrator* is the only inquiline known from the Lower Attini and, interestingly, it shares convergently evolved derived morphological characters with another distantly related attine workerless inquiline, *P. argentina* (Table 3). In both species, males and females exhibit a reduced palp formula of 3,2 (Figs 1G, 2G), whereas the plesiotypic attine palp formula is 4,2 (Figs 1H, 2H) (Gallardo, 1916; Schultz *et al.*, 1998). Elsewhere in the Attini, the reduced palp formula is found only in the free-living genus *Apterostigma* Mayr 1865 (Kusnezov, 1951, 1954; Schultz, 2007), where it evolved independently. In addition, in *M. castrator* the number of antennal segments is reduced from the plesiotypic attine condition of 11 segments in the females and 13 in the males to 11 segments in both sexes. The reduction of segments in *M. castrator* and *P. argentina* suggests that both species are evolutionarily derived inquilines. According to Wilson's (1984) character analysis of nine presumably independently evolved inquilines in the genus *Pheidole* Westwood, the reduction of antennal segmentation occurred secondarily during parasite evolution, preceded by the loss of the worker caste and other morphological reductions, like the reduction in size and body sculpture.

The wing morphology of *M. castrator* provides indirect support for the functional wing morphology hypothesis of the Paleoattini (sensu Kusnezov, 1963; Schultz & Meier, 1995) (Fernández-Marín *et al.*, 2005). Emery (1913) first noted that the forewings of queens of *Myrmicocrypta* F. Smith bear a small clear spot, or fenestra, which is not covered with fine setae and lacks pigmentation. This fenestra is also present in other paleoattines (Fernández-Marín *et al.*, 2005; Schultz, 2007). During colony founding, queens of Paleoattini inoculate their fungus garden on their shed forewings, and fix the forewing under the ceiling of the new nest chamber to grow the fungus cultivar (Fernández-Marín *et al.*, 2004). The fenestra was hypothesized to serve some unspecified function during nest founding (Fernández-Marín *et al.*, 2005). Interestingly, the clear spot is absent in *M. castrator* wings, whereas it is present in all *Mycocepurus* species. The loss of the fenestra in obligate social parasites, which do not found colonies independently, suggests that it indeed has an adaptive function during independent nest founding, because otherwise the fenestra would be maintained by natural selection in obligate inquilines. Potentially, the clear area is easier to clean before inoculation with a novel cultivar, or it may provide a tactile or even visual cue to the queen where to place the inoculum. It would be interesting to test if parasite queens contribute a fungal inoculum to the new host colony [suggested by Schultz *et al.* (1998)], and if the infrabuccal cavity of socially parasitic attines experienced morphological modifications or reductions.

Comparing *M. castrator* with other attine inquilines is instructive and provides insight into the life history evolution of social parasites (Table 3). Like *M. castrator*, *P. argentina* appears to be a phylogenetically derived, workerless inquiline and shares some morphological and life history traits with *M. castrator* (see Table 3). *Acromyrmex ameliae* and *A. insinuator*, however, are much different. Like *M. castrator* and *P. argentina*, the *Acromyrmex* inquilines are each associated with a single host species. However, they show much less morphological and life history specialization (Schultz *et al.*, 1998; de Souza *et al.*, 2007). For example, *A. ameliae* and *A. insinuator* lack the palpal and antennal segment reductions and other morphological features defining the inquiline syndrome (Table 3). Both species have mating flights, sometimes synchronously with host alates. Both species produce a worker caste, and parasitize host colonies at higher frequencies than most inquilines for which we have data (Bekkevold & Boomsma, 2000; de Souza *et al.*, 2007). Because mating flights are retained, dispersal is probably much less restricted, and inbreeding may be slight or nonexistent, as was demonstrated for *A. insinuator* (Sumner *et al.*, 2004a). These traits and other aspects of their biology (summarized in Table 3) strongly suggest that these species are recently evolved inquiline social parasites that have yet to develop the morphological inquiline syndrome and full workerless parasitism.

It should be stressed that the attine inquilines are not closely related to each other (Sumner *et al.*, 2004b; Schultz & Brady, 2008) and what is known of their life histories provides additional evidence of separate and idiosyncratic evolutionary trajectories. For instance, limited evidence suggests that the derived inquiline *P. argentina* may be host-queen intolerant (Bruch, 1928). In addition, there is some indication that

the fungus garden in *P. argentina* colonies breaks down after a parasite brood has been reared by the host workers (Bruch, 1928). Thus, *Pseudoatta* colonies may be quite short lived, perhaps even semelparous. Curiously, evidence has accumulated that the recently evolved inquiline *A. insinuator* may also be semelparous (Bekkevold & Boomsma, 2000), even though parasitized colonies retain a fertile host queen and could theoretically last as long as she lives and lays eggs. Nonetheless, Bekkevold & Boomsma (2000) provided evidence that the colony's fungus garden breaks down after a generation of parasite sexuals is produced. In contrast, in *M. castrator* colonies the host queen is retained and the fungus garden appears to remain healthy after the production of parasite sexuals. Thus, the colonies may last for several parasite reproductive cycles. Although one might expect that colony longevity should be favoured by natural selection under a broad range of conditions, such counterintuitive examples remind us that we are far from fully understanding the complex and diverse life history phenomena grouped under the term 'inquiline social parasitism'.

In conclusion, obligate social parasites are prime examples for the study of convergent evolution. Over 80 inquiline species are known currently (Hölldobler & Wilson, 1990; Huang & Dornhaus, 2008), and mapping parasite lineages onto recent phylogenies (Brady *et al.*, 2006; Moreau *et al.*, 2006; Rabeling *et al.*, 2008) illustrates that inquilines evolved at least 30 times convergently. A higher taxonomic resolution of speciose groups will probably increase the number of independent parasite origins. In fungus-gardening ants, two groups of inquilines evolved: incipient and evolutionarily derived social parasites. *Mycocepurus castrator* is a derived parasite, showing multiple morphological and life history traits of the inquiline syndrome (Kutter, 1969; Wilson, 1971, 1984). *Mycocepurus castrator* is the first social parasite of the Lower Attini and an additional social parasite from a subtropical habitat, a geographical region from which social parasites are little known. A phylogenetic and population genetic study is underway and will answer additional questions about the evolutionary origin of *M. castrator* (Rabeling, in preparation).

Acknowledgements

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