

Acromyrmex charruanus: a new inquiline social parasite species of leaf-cutting ants

C. Rabeling¹ · T. R. Schultz² · M. Bacci Jr.³ · M. Bollazzi⁴

Received: 27 September 2014 / Revised: 26 March 2015 / Accepted: 26 March 2015
© International Union for the Study of Social Insects (IUSSI) 2015

Abstract Social parasites exploit the colony resources of social species to secure their own survival and reproduction. Social parasites are frequently studied as models for conflict and cooperation as well as for speciation. The eusocial Hymenoptera harbor a diverse array of socially parasitic species with idiosyncratic life history strategies, but it is probably in the ants where social parasites are most speciose and have evolved the highest degrees of morphological and behavioral specialization. In the fungus-growing ants, a total of five obligate social parasites are known: four species are parasites of leaf-cutting ants and one species parasitizes a primitive fungus-growing ant species in the genus *Mycocetopus*. Here we describe a new species of socially parasitic leaf-cutting ant, *Acromyrmex charruanus* sp. nov., from Uruguay, and we report initial observations on the parasite's life history as well as on the morphological and behavioral adaptations related to the inquiline syndrome. Our observations suggest that *Acromyrmex charruanus* is an obligate inquiline social parasite of the thatch-mound-building, leaf-cutting ant *Acromyrmex heyeri*. *Acromyrmex charruanus* appears to be tolerant of the host, producing sexual offspring in the presence of the *A. heyeri* host queen.

Queens of *A. charruanus* appear to reproduce semelparously and sexual offspring are produced during the austral fall (February), which differs significantly from the mating biology of the host species, which reproduces during the southern-hemisphere spring (October–December). We suggest that the diametrically opposed mating seasons of parasite and host might be adaptive, allowing the parasite to avoid competition for resources with the host sexual brood. The morphological and behavioral adaptations of *A. charruanus* accord with characters observed to arise early during the evolution of other ant inquiline parasite species, and so far we have no evidence for the existence of a worker caste in *A. charruanus*. Further field studies and behavioral experiments need to confirm our first observations and explore *A. charruanus*'s behavioral ecology, evolution, and life history in more detail.

Keywords Leaf-cutter ants · Fungus-growing ants · Attini · Social parasitism · Inquilinism · *Acromyrmex* · *Pseudoatta*

Introduction

Fungus-growing ants live in an obligate mutualism with basidiomycete fungi, which they cultivate for food (Möller 1893; Wheeler 1907; Weber 1972; Mueller et al. 2005; Schultz et al. 2005; Hölldobler and Wilson 2010). In return the ants nourish the fungus, protect it from a diverse array of parasites, and disperse it to new locations (Herre et al. 1999; Caldera et al. 2009; Pagnocca et al. 2012; Yek et al. 2012). The fungus-growing ants inhabit the tropical and subtropical biomes of Central and South America, including most Caribbean Islands, and a single species, *Trachymyrmex septentrionalis* (McCook), extends the geographic

✉ C. Rabeling
crabeling@gmail.com

¹ Department of Biology, University of Rochester, Rochester, NY 14627, USA

² Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013-7012, USA

³ Center for the Study of Social Insects, São Paulo State University, Rio Claro, SP 13506-900, Brazil

⁴ Section of Entomology, Faculty of Agronomy, University of the Republic, 12900 Montevideo, Uruguay

distribution of attine ants as far north as New York state in the northeastern United States (Mayh -Nunes and Jaff  1998; Rabeling et al. 2007a). Currently, a total of 257 species of fungus-growing ants are known from one fossil and 16 extant genera (Kempf 1972; Brand o 1991; Bolton 2014). Only recently, the new genus *Cyatta* Sosa-Calvo et al. (2013) was discovered in the Brazilian Cerrado, adding to the known diversity of this fascinating group of ants and implying that additional species await discovery.

The morphologically and behaviorally specialized leaf-cutting ants of the genera *Atta* Fabricius and *Acromyrmex* Mayr represent an evolutionarily highly specialized clade within the fungus-growing ants (Mayh -Nunes 1995; Schultz and Meier 1995; Schultz and Brady 2008; Della Lucia 2011; Cristiano et al. 2013). Among the leaf-cutting ants, the genus *Acromyrmex* currently contains 32 species and almost the same number of subspecies ($n = 30$) (Emery 1905; Santschi 1925; Gonalves 1961; Bolton 2014). Many *Acromyrmex* species are geographically widespread and a large number of subspecies was erected to recognize morphologically aberrant, geographically disjunct populations. Whether these subspecies represent morphological variation within geographically widespread species or whether these distinct morphs constitute reproductively isolated species is currently under investigation (Rabeling et al. in preparation).

In 1916, Angel Gallardo described the highly specialized inquiline social parasite, *Pseudoatta argentina* Gallardo, which usurps colonies of *Acromyrmex lundii* (Gu rin-M neville), *A. heyeri* (Forel), and possibly *A. balzani* (Emery) (Gallardo 1916; Bollazzi & Rabeling, personal observation). Brown provisionally considered this parasitic satellite genus to be a junior synonym of *Acromyrmex* (Brown 1973), but a formal taxonomic treatment is pending (Rabeling et al. in preparation). In addition to *P. argentina* and its subspecies *P. a. platensis* Santschi, three other obligate social parasite species are recognized in the leaf-cutting ants: *Acromyrmex ameliae* De Souza et al. (2007) exploits colonies of *A. subterraneus* (Forel) in Minas Gerais, Brazil; *Acromyrmex insinuator* Schultz et al. (1998) parasitizes colonies of *A. echinator* (Forel) and rarely of *A. octospinosus* (Reich) in Panama (Jacobus Boomsma, pers. comm.); and a hitherto undescribed parasite species from Bahia in Brazil usurps colonies of *A. rugosus* (Smith) (Delabie et al. 1993; Sumner et al. 2004).

Based on their morphology and behavior, the socially parasitic leaf-cutting ant species represent different parasitic life-history strategies. *Pseudoatta argentina* and the distantly related new species from Bahia have converged on the anatomical parasitic syndrome, or inquiline syndrome, which is characterized by a reduction of body size, mouthparts, and integumental sculpturing, as well as by a loss of the worker caste (Kutter 1969; Wilson 1971, 1984;

H lldobler and Wilson 1990). In contrast, *A. insinuator* strongly resembles its host species, and *A. ameliae* differs from all other parasites in that it still resembles the host while at the same time having experienced a marked reduction in size and an increased abundance of pilosity (Schultz et al. 1998; De Souza et al. 2007). Both *A. insinuator* and *A. ameliae* retain the ability to produce minor and medium-size workers (Sumner et al. 2003a; De Souza et al. 2007). The only known attine inquiline parasite outside the leaf-cutting ants is *Mycocepurus castrator* Rabeling & Bacci, an obligate social parasite of *Mycocepurus goeldii* (Forel), which also exhibits the distinct morphological and behavioral characteristics of the inquiline syndrome (Rabeling and Bacci 2010).

Inquiline social parasites are of general interest to evolutionary biology and behavioral ecology because some species evolved reproductive isolation from their host species in sympatry (Buschinger 1986, 2009; Bourke and Franks 1991; Savolainen and Veps l inen 2003; Jansen et al. 2010; Boomsma and Nash 2014; Rabeling et al. 2014b), and the many species of independently evolved social parasites provide unique opportunities for comparatively studying the convergent evolution of social parasitism. Here we add to the increasing diversity of ant social parasites by describing *Acromyrmex charruanus* Rabeling, Schultz, Bacci & Bollazzi, a new species of social parasite from Uruguay, which exploits colonies of *Acromyrmex heyeri* (Forel). We also report the first observations of the behavioral ecology and natural history of *A. charruanus* and compare the life histories of host and parasite.

Materials and methods

Field site

Colonies of *A. heyeri* and its social parasite *A. charruanus* were studied at Plantaci n Cruz Roja, a *Eucalyptus* tree plantation that is located 6 km southwest of Cerro Colorado in the Department of Florida in Uruguay (GPS coordinates: S33.9042 , W55.59418 , ± 600 m, elevation 224 m above sea level). Fieldwork was conducted during the southern hemisphere fall, between the 25th and the 27th of February 2013, and during the southern spring, between the 1st and the 5th of November 2013.

Due to its geographic position in the southeast of the South American continent, Uruguay occupies a transitional zone between tropical and temperate climates that is characterized by cold winters, hot summers, and the marked absence of a dry season (Cabrera and Willink 1980). The natural vegetation consists of a mosaic of shrublands and forests that are embedded in a grassland matrix. The natural vegetation of these southern *Campos* is dominated by grass

and herb species, with some co-occurring shrub and tree species (Overbeck et al. 2007). In 2013, the type locality of *A. charruanus* was a seven-year-old *Eucalyptus globulus* plantation covering an area of 150 ha that was planted over unmodified natural grassland. The dominant species of herbaceous undergrowth in this *Eucalyptus* plantation belong to the genera *Stipa*, *Aristida*, *Baccharis*, and *Paspalum*. *Acromyrmex heyeri* is the most frequently encountered leaf-cutting ant species in both the *Eucalyptus* plantation and in the surrounding natural grassland.

To detect social parasites and collect samples of host and parasite species, we opened the thatch mounds of *A. heyeri* nests and scooped out portions of fungus gardens with a trowel. The fungus garden was examined for the presence of social parasites and ant samples were preserved in 100 % ethanol. During our field studies we opened 100 *A. heyeri* nests per season (austral spring and fall of 2013), resulting in a total of 200 sampled nests.

Material examined

The specimens examined have been deposited in the institutions listed below:

CPDC	Laboratório de Mirmecologia at the Cocoa Research Center at CEPLAC, Itabuna, Bahia, Brazil
CRC	Christian Rabeling Collection, University of Rochester, Rochester, NY, USA
MBC	Martin Bollazzi Collection, University of the Republic, Montevideo, Uruguay
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA
MZSP	Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil
USNM	United States National Museum of Natural History, Washington, DC, USA

Morphological analysis

Specimens were examined and measured using a Carl Zeiss stereomicroscope fitted with a stage micrometer. Measurements were recorded to the nearest 0.01 mm at 40× magnification. To generate composite images of the specimens we utilized a Leica DFC450 digital camera mounted on a Leica M205C stereomicroscope. Composite images were assembled using the Leica Application Suite (Version 4.5) and the Helicon Focus (Version 6.2.2) software packages. Conventions for morphological terminology, measurements, and indices follow those utilized in recent taxonomic studies of fungus-growing ants (Klingenberg and Brandão 2009; Rabeling et al. 2007a;

Sosa-Calvo et al. 2013). Morphological terminology of male genitalia and internal mouthparts follow Kempf (1956) and Gotwald (1969). Measurements are given in millimeters. Measurements and indices are defined as follows:

Cephalic index (CI): $HW/HL \times 100$.

Eye length (EL): Maximum diameter of the eye from the dorsal to the ventral margin, measured in full-face view.

Eye width (EW): Maximum diameter of the eye between both lateral margins, measured in lateral view.

Frontal lobe distance (FLD): Maximum horizontal distance between the outer borders of the frontal lobes, measured in full-face view.

Gaster length (GL): Maximum length of the gaster, measured from the meeting of the fourth abdominal tergum and sternum at their anterior ends to the posteriormost point of the last segment, measured in lateral view.

Head length (HL): Maximum vertical distance of the head in full-face view, excluding mandibles, measured in a straight line from the midpoint of the anterior clypeal margin to the midpoint of the posterior margin of the head. In species where the posterior margin of the head or the clypeal margin (or both) is concave, the measurement is taken from the midpoint of a transverse line spanning the anteriormost or posteriormost projecting points, respectively.

Head width (HW): Maximum horizontal width of the cephalic capsule, excluding the eyes, measured in full-face view.

Interocular distance (IOD): Maximum horizontal width of the head, including the eyes, measured in full-face view.

Mandible length (ML): Maximum mandible length, measured in a straight line from the mandibular insertion into the head capsule to the distal end of the apical tooth, measured in full-face view.

Mandibular index (MI): $ML/HL \times 100$.

Metafemur length (FL): Maximum length of metafemur, measured in dorsal view.

Petiolar width (PW): Maximum width of petiole, measured in dorsal view.

Petiole length (PL): Maximum length of the petiole, measured in lateral view from the posteriormost margin of the metapleural lobe to the posteriormost margin of the petiole.

Postpetiolar width (PPW): Maximum width of postpetiole, measured in dorsal view.

Postpetiole length (PPL): Maximum length of the postpetiole, measured in lateral view along the margin where postpetiolar tergite and sternite meet.

Pronotal width (PrW): Maximum width of the pronotum, measured in dorsal view.

Scape index (SI): $SL/HW \times 100$.

Scape length (SL): Maximum length of the antennal scape, excluding the condylar bulb.

Total length (TL): Sum of ML, HL, WL, PL, PPL, and GL.

Weber length (WL): Diagonal length of the mesosoma from the point at which the pronotum meets the cervical shield to the posterior base of the metapleuron, measured in lateral view.

Testing for the presence of social parasite workers

Some morphologically highly derived socially parasitic fungus-growing ant species lack a worker caste entirely whereas other social parasites that are morphologically similar to their hosts are known to produce between 9 and 48 % of the workers in infected host colonies (Sumner et al. 2003a; De Souza et al. 2007). The morphological differences between parasite and host workers can be subtle. In the case of the leaf-cutting ant social parasites *A. ameliae* and *A. insinuator*, the ratio of the diameter of the bulla of the metapleural gland to the pronotal width and the distance of the bulla to the spiracle are diagnostic for differentiating host and parasite workers (Sumner et al. 2003a; De Souza et al. 2007). To test whether parasitized *A. heyeri* colonies also contained workers of *A. charruanus*, we complemented our morphological studies by genotyping workers from two parasitized colonies using the mitochondrial “barcoding” region, a ~658 bp fragment of *cytochrome c oxidase I* (*COI*; see below). The obtained barcoding sequences of workers were compared to the *COI* barcodes of host and parasite gynes and males. We genotyped a total of 144 randomly selected workers from two *A. heyeri* host colonies: CR130225-03 ($n = 96$) and CR130227-31 ($n = 48$). To ensure that the subsample of k contains at least one parasite with a probability of greater 0.95 given a parasite frequency of p , we chose k so that $(1 - p)^k$ was no greater than 0.05; i.e., chose k such that $(1 - p)^k \leq 0.05$, or $k \geq \ln(0.05)/\ln(1 - p)$. Therefore, when randomly sampling 96 individuals from a single colony, we can be 95 % confident to have sampled at least one parasite from the host colony if the parasites occurred at a frequency of 3 % or higher. From the second colony, we sampled 48 individuals, and can be 95 % confident to have detected a parasite, if the rate of parasitism surpassed 6 %. The detection level of 3–6 % is biologically meaningful because the proportion of parasite workers found in host colonies parasitized by *A. insinuator* and *A. ameliae* range between 9 and 48 %.

Molecular genetic analysis

Specimens that were previously preserved in 100 % ethanol were selected at random and whole genomic DNA was extracted. Each individual was removed from the ethanol, dried at room temperature, and then transferred to a 1.5 ml Eppendorf tube. DNA was extracted non-destructively using a Qiagen QIAamp DNA Micro Kit following the manufacturer’s instructions for isolation of genomic DNA

from tissues, and DNA was re-suspended in 50 μ l of H₂O. The barcoding region of the mitochondrial gene *cytochrome c oxidase I* (*COI*) was amplified using the primer pairs LCO1490 and HCO2198 (Folmer et al. 1994), resulting in a 658 bp fragment in *Acromyrmex heyeri*, after trimming the primer sequences. 25 μ l PCR reactions contained 1 μ l of DNA extract, 12.5 μ l Omega Taq polymerase Master Mix, 1 μ l of forward and reverse primers at 10 μ M concentration, and 9.5 μ l of ddH₂O. *COI* fragments were amplified under the following conditions: initial denaturation at 94 °C for 2 min; 34 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min, and extension at 70 °C for 2 min; and a final extension at 70 °C for 10 min. Four microliters of the PCR product were run on a 1.5 % agarose gel and visualized with SYBR[®] Safe DNA Gel Stain. The remaining 21 μ l were purified utilizing the enzymatic ExoAp treatment (New England Biolabs: Ipswich, MA) and 1 μ l of the purified PCR product was cycle-sequenced using the ABI BigDye Terminator Kit (version 3.1). Forward and reverse sequences were sequenced on an ABI 3730xl Genetic Analyzer.

Results

Species description

Acromyrmex charruanus Rabeling, Schultz, Bacci & Bollazzi, NEW SPECIES

Figures 1a, c, e, 2a, c, e, 3a, 4a, and c.

Holotype: alate gyne, URUGUAY, Florida Department, 6 km SW of Cerro Colorado, *Eucalyptus globulus* plantation “Plantación Cruz Roja”, GPS coordinates: S33.9042°, W055.59418°, elevation 224 m above sea level, collection date: 25 February 2013, col. Bollazzi & Rabeling; collection code: CR130225-03, ex *Acromyrmex heyeri* nest. The holotype is deposited at the MZSP and carries the unique specimen identifier No. USNMMENT00758784.

Paratypes: same data as holotype, 73 alate gynes and 119 males, USNMMENT00758785-00758977. Same data as holotype, but “27 February 2013, collection code: CR130227-31”, 7 alate gynes and 6 males, USNMMENT00758778-00758990.

Description, holotype gyne: TL 6.92, WL 2.08, HL 1.21, HW 1.33, IOD 1.54, ML 0.92, FLD 0.74, SL 1.28, EL 0.31, EW 0.26, PrW 1.1, FL 2.03, PL 0.51, PW 0.41, PPL 0.41, PPW 0.74, GL 1.79, CI 111, MI 77, SI 96. One of the smallest *Acromyrmex* leaf-cutting ant species known (WL 2.08; TL 6.92), with long mandibles (MI 77) and appendages (FL 2.03; SI 96) relative to body size. Integument with microscopic honey-comb pattern, which becomes visible at 40 \times magnification and higher; sculpturing of head, meso-, and metasoma coarsely granulate with distinct

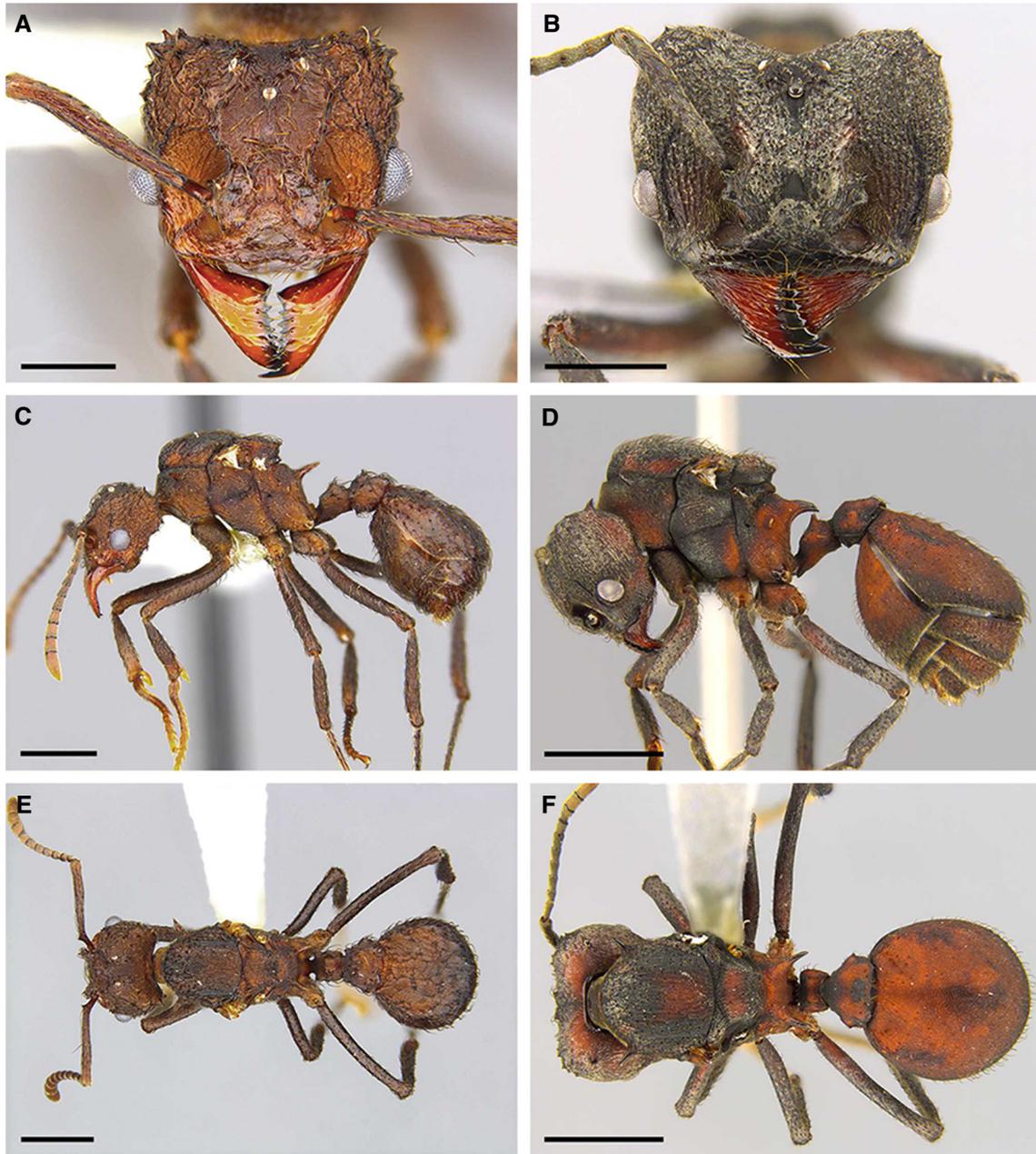


Fig. 1 Gynes of the social parasite *Acromyrmex charruanus* (a, c, e) and its host *A. heyeri* (b, d, f) in full-face (a, b), lateral (c, d), and dorsal (e, f) views. The depicted social parasite gyne is the holotype

with the unique specimen identifier USNMENT00758784. The scale bars represent 0.5 mm in a, 1 mm in b, c and e, and 2 mm in d and f

rugae and tubercles; integument with oily or waxy sheen. Body surface covered with long, appressed, and distinctly coarse setae. Color: shades of brown, variable from yellowish to reddish to dark brown; mandibles distinctly lighter in color, dark orange; ridges of rugae and tips of tubercles black; dorsum of mesosoma dark brown, with black markings. Head: head shape trapezoidal, slightly wider than long (CI 111); sides moderately tapering anteriorly between the eyes and mandibular insertion; head size small relative to

mesosoma. Mandible broadly triangular with a distinct apical and preapical tooth, followed by seven smaller teeth, some of which are interspersed by even smaller denticles; mandible surface smooth and distinctly shiny. Palp formula 4:2, the plesiomorphic condition of fungus-growing ants. Posterior margin of clypeus trapezoidal, broadly inserted between frontal lobes; anterior margin of clypeus shiny and median portion concave. Unpaired median clypeal seta thin and short (0.15), only projecting over the anterior clypeal

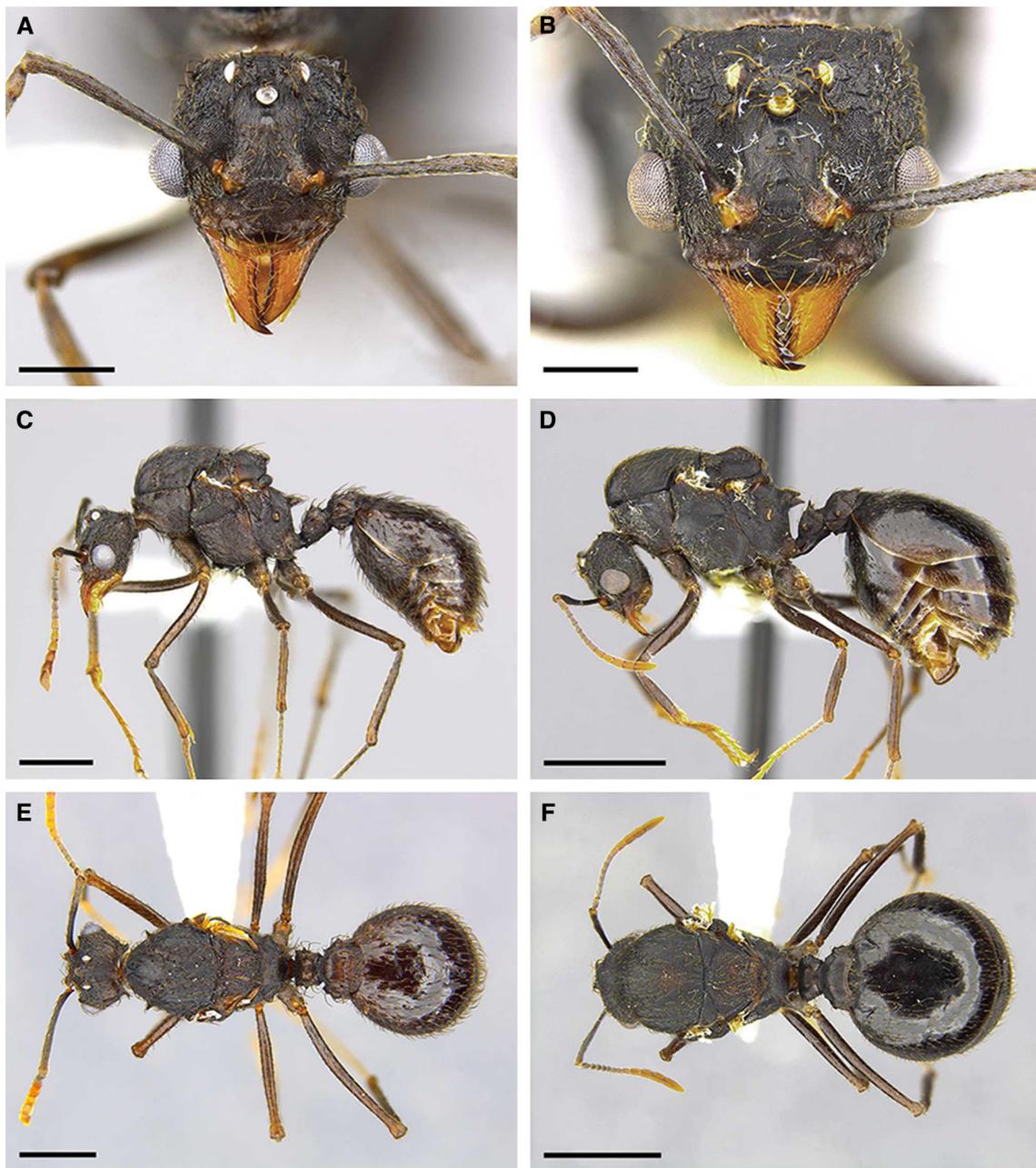


Fig. 2 Males of the social parasite *Acromyrmex charruanus* (**a, c, e**) and its host *A. heyeri* (**b, d, f**) in full-face (**a, b**), lateral (**c, d**), and dorsal (**e, f**) views. The *scale bars* represent 0.5 mm in **a** and **b**, 1 mm in **c** and **e**, and 2 mm in **d** and **f**

margin by half its length. Frontal lobe broadly rounded, fully covering the condylar bulb in full-face view; lateral margin of frontal lobe serrate with two distinct tooth-like projections. Frontal carina extending towards the posterolateral corner of the head. Preocular carina forming a straight line in lateral view and traversing the area of the antennal scrobe by one third of the scrobe's width. Eyes large (EL 0.31, EW 0.26) and strongly convex. In contrast, the three ocelli are small and embedded in the integument. Antennae with 11 segments. Antennal scape long (SL 1.28)

with abundant, appressed setae, surpassing the posterior margin of the head by one third of its length. Mesosoma: Mesosoma slender with caste-specific modifications related to wing bearing. Dorsolateral pronotal spine long, slender, and sharply pointed in dorsal view. Ventrolateral pronotal spine narrowly triangular and sharply pointed, not curved. Dorsum of mesosoma covered with longitudinal and reticulate rugulae. Posterior margin of scutellum concave and bidentate in dorsal view; teeth wide at base, forming a broad, almost 90° angle. Bulla and meatus of metapleural

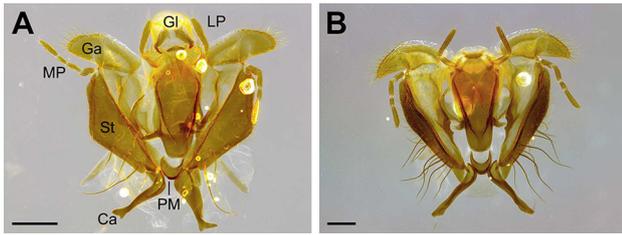


Fig. 3 Comparative anatomy of *Acromyrmex charruanus* (a) and *A. heyeri* (b) female mouthparts, depicting cardo (Ca), galea (Ga), glossa (Gl), labial palp (LP), maxillary palp (MP), postmentum (PM), and stipes (St). The palp formula of both parasite and host is 4:2 (number of segments of maxillary:labial palps), representing the plesiomorphic condition in fungus-growing ants. The scale bars represent 0.2 mm in both figures

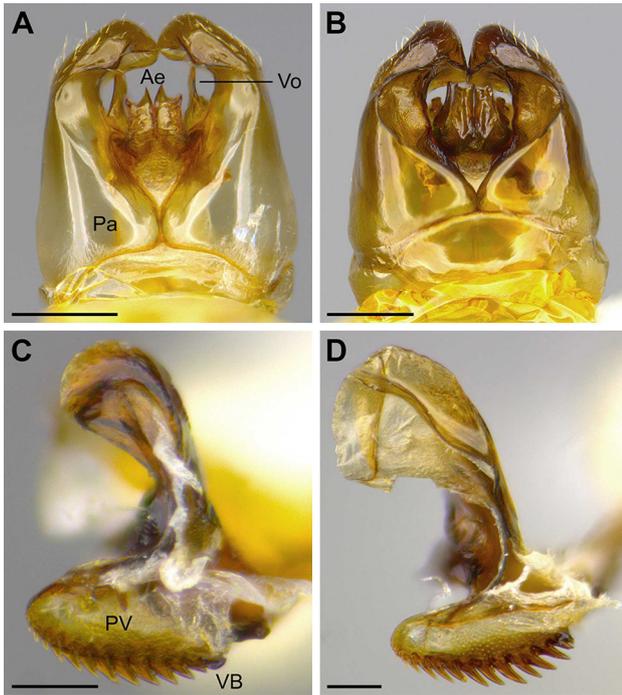


Fig. 4 Comparative anatomy of *Acromyrmex charruanus* (a, c) and *A. heyeri* (b, d) male genitalia. Male genitalia in toto in dorsal view (a, b) and aedeagus in lateral view (c, d), depicting aedeagus (Ae), paramere (Pa), penis valve (PV), ventral border of aedeagus with row of spines (VB), and volsella (Vo). The scale bars represent 0.25 mm in a, 0.5 mm in b, and 0.1 mm in c and d

gland not notably modified from the condition in the host species. Propodeal spines straight, long, slender, and sharply pointed, projecting away from the propodeum at a 90° angle in lateral view. Metasoma: anterior peduncle of petiole short, about one fourth the length of the petiolar node. Dorsum of petiolar node with a pair of short teeth, almost as wide at their bases as they are high. Postpetiole wider than long in dorsal view (PPW 0.74; PPL 0.41), posterior margin straight. Gaster short (GL 1.79). First gastric tergite notably tuberculate, covered with abundant

recurved setae. Except for the smaller size, forewing and hindwing resembling the wings of *A. heyeri*. Measurements; paratype gynes ($n = 10$): TL 6.64–7.23, WL 2.03–2.15, HL 1.15–1.28, HW 1.28–1.36, IOD 1.46–1.59, ML 0.9–0.95, FLD 0.74–0.77, SL 1.26–1.31, EL 0.28–0.31, EW 0.23–0.28, PrW 1.1–1.21, FL 1.92–2.08, PL 0.46–0.54, PW 0.41–0.46, PPL 0.38–0.46, PPW 0.72–0.77, GL 1.67–1.97, CI 106–113, MI 72–80, SI 94–100.

Description, paratype males ($n = 10$): TL 6.64–7.23, WL 2.03–2.15, HL 1.15–1.28, HW 1.28–1.36, IOD 1.46–1.59, ML 0.9–0.95, FLD 0.74–0.77, SL 1.26–1.31, EL 0.28–0.31, EW 0.23–0.28, PrW 1.1–1.21, FL 1.92–2.08, PL 0.46–0.54, PW 0.41–0.46, PPL 0.38–0.46, PPW 0.72–0.77, GL 1.67–1.97, CI 106–113, MI 72–80, SI 94–100. A small male (WL 2.03–2.28; TL 6.54–7.03), smaller than any other *Acromyrmex* male. Integument with microscopic honeycomb pattern; sculpturing of head and mesosoma finely granulate; posterior half of the head with fine rugulae; metasoma smooth and shiny. Body surface covered with long, dark, both semi-erect and appressed, distinctly coarse setae. Color: head and mesosoma black; appendages and metasoma dark brown; anterior half of antennal flagellum and mandibles light brown. Head: approximately as wide as long (CI 97–106); sides subparallel behind the level of the eye, tapering anteriorly towards the mandibular insertion; head size small relative to mesosoma. Mandible narrowly triangular, slender, with a distinct apical and preapical tooth, followed by two to three smaller teeth, which are interspersed by tiny denticles; mandible surface smooth, shiny. Palp formula 4:2. Clypeus and unpaired clypeal seta as in gyne. Frontal lobe narrow, leaving the anterior half of the condylar bulb exposed in full-face view. Preocular carina indistinct, traversing the area of the antennal scrobe by half the scrobe's width. Eyes large (EL 0.33–0.38, EW 0.31–0.33) and strongly convex. Ocelli large and raised above the surface of the head. Antennae with 13 segments. Antennal scape long (SL 1.13–1.26) with appressed setae, surpassing the posterior margin of the head by half its length. Mesosoma: Mesosoma relatively slender with sex-specific modifications related to wing bearing. Dorsolateral pronotal spine short, triangular, and sharply pointed in dorsal view. Ventrolateral pronotal spine broadly triangular, with broad tip, not curved. Dorsum of mesosoma with fine longitudinal rugulae. Scutellum as in gyne. Bulla and meatus of metapleural gland small, not notably modified from the condition in *A. heyeri*. Propodeal spines narrowly triangular. Orifice of propodeal spiracle round in lateral view, not slit-shaped. Orifice of metapleural gland oval, pointing dorso-ventrally; shape not notably modified from metapleural gland opening in *A. heyeri*. Metasoma: petiole and postpetiole as in gyne. Gaster slender: first gastric tergite smooth, shiny, and covered with abundant semi-erect setae. Genitalia: in toto, excluding the basal ring, parameres

slightly longer (0.9) than wide (0.85); apical lobe of paramere evenly rounded with less than 10 long, erect setae. In lateral view, aedeagus small (0.23), ventral border of penis valve bearing 12 recurved teeth, the anterior three of which are small and weakly sclerotized, whereas the posterior nine are distinctly larger and heavily sclerotized, as notable by the darker brown coloration.

Worker caste

To test whether *A. charruanus* produces a worker caste, we examined workers morphologically and genotyped host and parasite gynes and males to compare them to a total of 144 workers genotypes. We sampled 96 minor and medium-sized workers from colony CR130225-03 and 48 workers from colony CR130227-31. All genotyped individuals had mitochondrial DNA sequences that are identical to winged reproductives of *A. heyeri*. By sampling 96 and 48 individuals from a given colony we can be 95 % confident that parasite workers would have been sampled successfully if they occurred at rates equal to or greater than 3 and 6 %, respectively. Morphological differences observed in comparative studies of host and parasite minor workers of other *Acromyrmex* social parasites, such as the distance between the metapleural gland bulla to the propodeal spiracle, could not be detected in workers from parasitized *A. heyeri* colonies. Therefore, we have no evidence of a worker caste in *A. charruanus*. Because we only found two parasitized colonies at Plantación Cruz Roja, we cannot be certain whether (1) the worker caste is completely absent from *A. charruanus*, whether (2) workers were only absent from the two colonies we studied, or whether (3) workers were not sampled from the parasitized colonies by chance because they occurred at frequencies below 3 % or 6 % in the respective colonies.

Differential diagnosis

Acromyrmex charruanus is a social parasite of the leaf-cutting ant *Acromyrmex heyeri* and does not exhibit the morphological adaptations and/or reductions that are observed in *Pseudoatta argentina*, *Mycocepurus castrator*, and a hitherto undescribed species of *Acromyrmex* social parasite from Bahia in Brazil (Figs. 1, 2, 3, 4; Table 1). Notwithstanding, morphologically the gyne of *A. charruanus* can easily be distinguished from the host gyne by its smaller size, darker color, and abundance of recurved, coarse setae (Fig. 1). Relative to its smaller body size in comparison to the host, *A. charruanus* is also characterized by longer appendages, mandibles, and antennal scapes, as well as a shorter gaster (Fig. 1; Table 1). In the field, *A. charruanus* can be distinguished from its host by the significantly smaller size and the presence of alate females and males in the nest during the austral fall (February), whereas *A. heyeri*'s mating season typically occurs during the austral spring (October–December; see below).

The male of *A. charruanus* is not gynandromorphic and can easily be distinguished from the host male by its smaller size and the presence of dark, long, distinctly coarse, semi-erect setae, instead of the short, yellow, appressed setae found in the host males (Fig. 2; Table 1). The head of the parasite male is quadrate, not wider than long (as in *A. heyeri*), and the parasite's mandibles are slender, bearing a variable number of teeth and indistinct denticles, in contrast to the broadly triangular mandibles of the host (Fig. 2; Table 1). The parasite's genitalia are smaller than the host's genitalia (paramere length: *A. heyeri* = 1.5 mm, *A. charruanus* = 0.9 mm; aedeagus length: *A. heyeri* = 0.33 mm, *A. charruanus* = 0.23 mm) and the ventral border of the aedeagus bears 12 teeth instead of 14, as in *A. heyeri* (Fig. 4).

Table 1 Mean values, standard deviations, and ranges (in parentheses) of morphometric measurements and indices of *A. charruanus* and *A. heyeri* diagnostic for species identification

	Gynes		Males	
	<i>A. charruanus</i> (n = 11)	<i>A. heyeri</i> (n = 10)	<i>A. charruanus</i> (n = 10)	<i>A. heyeri</i> (n = 10)
Weber length	2.09 ± 0.05 (2.03–2.15)	3.21 ± 0.05 (3.15–3.27)	2.14 ± 0.09 (2.03–2.28)	2.99 ± 0.07 (2.84–3.08)
Head length	1.22 ± 0.03 (1.15–1.28)	2.04 ± 0.03 (2.00–2.08)	0.99 ± 0.04 (0.92–1.05)	1.30 ± 0.05 (1.23–1.39)
Head width	1.33 ± 0.02 (1.28–1.36)	2.55 ± 0.05 (2.46–2.62)	0.99 ± 0.03 (0.95–1.03)	1.47 ± 0.04 (1.42–1.54)
Scape length	1.28 ± 0.02 (1.26–1.31)	1.71 ± 0.03 (1.65–1.77)	1.19 ± 0.04 (1.13–1.26)	1.32 ± 0.05 (1.27–1.39)
Scape index	94–100	65–69	115–126	89–95
Mandible length	0.93 ± 0.01 (0.90–0.95)	1.32 ± 0.03 (1.27–1.35)	0.67 ± 0.02 (0.64–0.69)	0.78 ± 0.05 (0.69–0.85)
Mandible index	72–80	61–67	65–69	53–66
Pronotal width	1.15 ± 0.03 (1.10–1.21)	1.90 ± 0.05 (1.85–2.00)	1.21 ± 0.06 (1.13–1.31)	1.87 ± 0.04 (1.81–1.92)
Metafemur length	2.00 ± 0.05 (1.92–2.08)	2.74 ± 0.07 (2.63–2.85)	2.27 ± 0.10 (2.13–2.44)	2.54 ± 0.07 (2.42–2.65)

Measurements are given in mm. Definitions of morphometric measurements and indices are provided in the “Materials and methods” section

Interestingly, both gynes and males of *A. charruanus* are morphologically similar to a potentially distantly related *Acromyrmex* species, the social parasite *A. ameliae* from Brazil, which presumably evolved convergently due to similar selective pressures related to the parasitic lifestyle. Gynes of *A. charruanus* can be distinguished from those of *A. ameliae* by their slightly smaller size, and, notably, by their long setae, which are recurved and appressed, instead of being erect or semi-erect, as is the case in *A. ameliae*. In addition, *A. ameliae* gynes are darker in color and the rugoreticulations on the head, meso-, and metasoma are more strongly developed than they are in *A. charruanus*. Males of *A. charruanus* are significantly smaller than *A. ameliae* males and are darker in color, the spines and ridges on the petiole and postpetiole are less pronounced, the integumental sculpturing is less developed, and the propodeal spines are shorter. Geographic distribution is also indicative of species identity, unless the discovery of additional populations markedly alters the distribution of either species. So far, *A. charruanus* is only known from central Uruguay and *A. ameliae* is only known from the Brazilian state of Minas Gerais.

Etymology

The species epithet “*charruanus*” refers to “Charrúa”, the name given to the indigenous people of Uruguay by Europeans, who arrived in the sixteenth century. Colloquially, the Uruguayan people also refer to themselves as “charrúas”. Therefore, and in allusion to the etymology of the enigmatic social parasite *Pseudoatta argentina*, the species name refers to the geographic region where this social parasite was discovered.

Host species

Acromyrmex charruanus was found inside the nests of *Acromyrmex heyeri*. Given that *A. ameliae* parasitizes more than one subspecies of *A. subterraneus*, that *A. insinator* parasitizes *A. echinator* and rarely *A. octospinosus*, and that *Pseudoatta argentina* usurps colonies of *A. lundii*, *A. heyeri*, and potentially of *A. balzani*, it is possible that *A. charruanus* parasitizes leaf-cutting ant species other than *A. heyeri*. However, despite opening nests of other sympatric thatch-mound-building leaf-cutting ant species, such as *A. lundii* and *A. lobicornis* (Emery), we found *A. charruanus* only in nests of *A. heyeri*.

Notes on life history, ecology, and biology

Our conclusions about the life history and biology of *A. charruanus* are based on observations made on two parasitized *A. heyeri* colonies. Further attempts to find this social

parasite have so far failed. Therefore, our observations should be regarded as initial insights into the parasite’s biology and life history. Other aspects of its behavioral ecology remain unknown and further field studies and laboratory experiments are required.

Diametrically opposed mating seasons of host and parasite: the chronologies of the mating seasons of host and parasite were observed to be diametrically opposed to one another. Winged gynes and males of *A. charruanus* were present in their host colonies during the southern hemisphere fall (i.e., February) and at this time no sexual offspring of the host species, *A. heyeri*, were observed. The mating flights of *A. heyeri*, and of all other *Acromyrmex* species, occur during the spring months, October to December, in Uruguay, southern Brazil, and northern Argentina (Bonetto 1959; Zolessi and Abenante 1974; Zolessi and González 1974, 1979; Diehl-Fleig 1993). Therefore, sexual offspring of host and parasite are separated by a time window of at least 2 months.

Nuptial flights and mating locality: we did not observe fully developed mass nuptial flights of *A. charruanus* in the field. However, upon opening the thatch mound of one parasitized *A. heyeri* nest, alate reproductives of *A. charruanus* immediately started to fly. This behavior was observed repeatedly in laboratory colonies of *A. charruanus* in which reproductives attempted to fly as soon as the nest boxes were opened. In contrast, the attine inquiline social parasites *Pseudoatta argentina* and *Mycocepurus castrator* were never observed to fly and may in fact be incapable of doing so (Bruch 1928; Gallardo 1929; Rabeling and Bacci 2010). In addition, *A. charruanus* reproductives were never observed to copulate inside lab colonies, which suggests that *A. charruanus* performs regular mating flights and mates in midair or on the ground, as has been observed in non-parasitic leaf-cutting ant species (Diehl-Fleig 1993; Johnson and Rissing 1993). In contrast, both *P. argentina* and *M. castrator* are adelphogamous and mate inside the host colony (Bruch 1928; Gallardo 1929; Rabeling and Bacci 2010).

Reproductive strategy: initial observations suggest that *A. charruanus* reproduces semelparously. We marked the nests of two parasitized *A. heyeri* colonies at Plantación Cruz Roja in February and upon revisiting the same nests in May of the same year, both nests were empty and the thatch mounds had collapsed, suggesting that the colonies had died. We also observed that the fungus gardens of parasitized *A. heyeri* colonies looked tattered while parasite alates were present in the nest and that the amount of fungus garden was reduced in comparison to unparasitized *A. heyeri* colonies; the fungal biomass was not quantified, however. Therefore, it seems possible that the host colony is incapable of recovering from the mass rearing of the parasitic brood and dies after the mass exodus of parasite alates.

Alternatively, *A. charruanus* queens could preferentially exploit old host colonies that have lost the host queen.

Parasitism rate: to estimate the rate at which colonies of *A. heyeri* were parasitized by *A. charruanus* we opened 100 nests of *A. heyeri* during our fall survey (February), when alates of the parasite were present, and found only two *A. heyeri* colonies containing *A. charruanus* alates. During the spring survey (November) we opened 100 nests of the same population and did not find any parasites. Therefore, parasites are probably best detected prior to their mass nuptial flights and likely escaped detection when only the parasite queen(s), and not the alate parasite reproductive offspring, were present. To calculate the approximate rate of parasitism we used only the colony counts from the fall survey, suggesting that in this Central Uruguayan population *A. charruanus* parasitizes *A. heyeri* colonies at a rate of approximately 2%. It is possible that parasites escaped detection in some of the colonies we examined, and therefore our estimated rate of parasitism should be regarded as a minimum estimate. The parasitism rates of the hitherto undescribed social parasite from Bahia, of *A. insinator*, and of *A. ameliae* were estimated to be 8, 40, and 70%, respectively (Delabie et al. 1993; Bekkevold and Boomsma 2000; De Souza et al. 2007).

Host nest density: in Uruguay, northern Argentina, and southern Brazil *A. heyeri* is known to build large dome-shaped thatch-mound nests (Bollazzi et al. 2008). In each season, we opened 100 *A. heyeri* thatch-mound nests distributed over an area of roughly 30 hectares. Accordingly, the nest density of *A. heyeri* was approximately 3–4 colonies per hectare. We did not open every colony we detected at our field site and almost certainly missed some small, incipient colonies in wooded areas. Therefore, this estimate of 3–4 colonies per hectare should be regarded as a minimum estimate of the actual nest density of *A. heyeri* in this habitat.

Male to female sex ratio: upon encountering *A. charruanus* alates in *A. heyeri* nests for the first time, we were uncertain whether these alates represented an unknown social parasite or whether we had encountered microgynes and micraners of *A. heyeri*, because dwarf reproductives have been reported for *Atta cephalotes* (Linnaeus), *A. colombica* Guérin-Méneville, *A. sexdens rubropilosa* Forel, and *A. texana* Buckley (Jutsum and Cherrett 1977; Bueno et al. 2002; Dijkstra and Boomsma 2006). To observe these unusual individuals in the laboratory, we took two samples of about 300 ml of fungus garden and ants per colony, resulting in a total of 600 ml per colony. The first colony (CR130225-03) contained 74 winged *A. charruanus* gynes and 119 males, whereas the second colony (CR130227-31) contained only 7 gynes and 6 males. The female to male sex ratio can be approximated with 0.62 and 1.17 for the first and second colony, respectively. The sampled volume of

fungus garden represents a random sample of each colony and detailed sex ratio estimates for the parasite need to be obtained from comprehensive future surveys. Estimates of *A. heyeri* sex ratios indicated a slight but significant bias towards males (Diehl-Fleig 1993).

Discussion

The social parasite syndrome of *Acromyrmex charruanus*

The morphological, behavioral, and natural history data indicate that *Acromyrmex charruanus* is a permanent and obligate inquiline social parasite of the thatch-mound-building, leaf-cutting ant *Acromyrmex heyeri*. We never encountered free-living colonies of *A. charruanus* and the parasite seems to rely on the host colony for successful reproduction. The presence of female host brood (i.e., worker brood) in the parasitized *A. heyeri* colonies suggests that *A. charruanus* is a queen-tolerant inquiline parasite (Buschinger 1986, 2009; Hölldobler and Wilson 1990; Bourke and Franks 1991). The observation that parasitized host colonies had collapsed 3 months after *A. charruanus* reproductives were found in them could potentially indicate that *A. charruanus* is intolerant of the host queen; however, given the presence of female *A. heyeri* brood in the host colonies, we believe the death of the host colonies is related to the parasite's reproductive strategy (see below) rather than host-queen intolerance. The majority of obligate ant social parasites are host-queen tolerant, whereas the killing of the host queen is usually associated with temporary social parasitism. Some obligate social parasites specialize on invading queenless host colonies, such as *Anergates atratulus* (Schenk) (Kutter 1969), or actively kill the host queen to raise their own sexual brood, such as *Leptothorax goesswaldi* Kutter (Buschinger and Klump 1988). Among the five known species of socially parasitic fungus-growing ants (not including *A. charruanus*), only *P. argentina* seems to be host-queen intolerant, which is supported by the observations that *A. lundii* host colonies (1) collapsed after the parasite's mass release of sexual offspring (Bruch 1928), and (2) did not contain female host brood, implying the absence of the host queen (Bollazzi & Rabeling, personal observation). All other socially parasitic fungus-growing ants (i.e., *A. ameliae*, *A. insinator*, *M. castrator*, and the undescribed parasite species from Bahia) are queen-tolerant inquilines (Delabie et al. 1993; Schultz et al. 1998; Bekkevold and Boomsma 2000; De Souza et al. 2007; Nash and Boomsma 2008; Rabeling and Bacci 2010). Alternatively, *A. charruanus* could preferentially parasitize old host colonies that had lost their host queen. Intraspecific social

parasites of the fire ant *Solenopsis invicta* Buren and microgyne parasites of *Myrmica rubra* (Linnaeus) seek out mature, orphaned colonies, which they enter in order to exploit the existing worker force to raise their own brood (Elmes 1973; Seppä 1994; Tschinkel 1996; DeHeer and Tschinkel 1998; Schär and Nash 2014).

In comparison to *A. heyeri*, gynes and males of *A. charruanus* have markedly reduced body sizes and an increased abundance of coarse, recurved setae on their integuments (Figs. 1, 2). In contrast to the highly derived attine social parasites that convergently evolved on the inquiline syndrome, *A. charruanus* retains 11 and 13 antennal segments for females and males, respectively, and four maxillary and two labial palps, representing the plesiomorphic conditions for the fungus-growing ants (Fig. 3) (Kusnezov 1954; Schultz 2007). The male genitalia of *A. charruanus* are reduced in size but the ventral aedeagal margin retains the full complement of teeth (Fig. 4). Aedeagal teeth serve to hook the male copulatory organ into the female reproductive pouch (Baer and Boomsma 2006) and are reduced in number in some morphologically derived social parasites such as *Mycocetopus castrator* (Rabeling et al. 2014b). Therefore, *A. charruanus* possesses some of the morphological adaptations associated with the social parasite syndrome, but lacks the more extreme modifications and reductions observed in the more highly derived socially parasitic species of fungus-growing and other myrmicine ants (Gallardo 1916, 1929; Bruch 1928; Kutter 1950, 1969; Wilson 1971, 1984; Hölldobler and Wilson 1990; Delabie et al. 1993; Buschinger 2009; Rabeling and Bacci 2010).

In his study of socially parasitic *Pheidole*, Wilson (1984) proposed that reduced body size and the loss of the worker caste are among the first modifications to evolve during the early stages of social parasite evolution, whereas reductions of antennal segments and mouthparts evolve secondarily. In addition, convergently evolved social parasite species often represent a mosaic of different morphological and behavioral specializations characteristic of the inquiline syndrome (Kutter 1969; Wilson 1971), and these adaptations do not necessarily evolve in concert in independently evolved social parasite species (Wilson 1984; Buschinger 2009). Based on morphological evidence, *A. charruanus* seems to occupy a relatively early stage of the social parasite syndrome and ongoing molecular phylogenetic studies will need to test whether different degrees of morphological and behavioral adaptation to the parasitic lifestyle are correlated with the divergence times separating parasitic lineages from their closest non-parasitic relatives, or whether different parasite morphologies represent differentially adapted life-history strategies that are uncorrelated with divergence time.

Absence or non-detection of the worker caste in *A. charruanus*?

Acromyrmex charruanus appears to be a workerless social parasite. We inspected the morphology of workers from two parasitized *A. heyeri* colonies and obtained nucleotide sequences of the DNA-barcoding fragment of the mitochondrial gene *cytochrome c oxidase I* for 144 workers. Neither approach yielded evidence for a worker caste in *A. charruanus*. The apparent absence of the worker caste is noteworthy because other social parasite species that show a similar degree of morphological specialization to social parasitism, such as *A. ameliae* and *A. insinator*, still produce minor and medium-sized workers (Bekkevold and Boomsma 2000; De Souza et al. 2007). Studies of laboratory colonies showed that *A. insinator* workers were present in the host colonies at frequencies between 40 and 48 % (Sumner et al. 2003a, b), and field studies of *A. ameliae* confirmed the presence of parasite workers at a frequency of approximately 9 % (De Souza et al. 2007). In contrast, only morphologically and behaviorally derived inquilines of fungus-growing ants have been found to be workerless (Gallardo 1916; Delabie et al. 1993; Rabeling and Bacci 2010).

The retention of the worker caste in a social parasite species is thought to be a transitional stage in the evolution of social parasitism, eventually leading to the loss of the worker caste. The “selfish larvae” hypothesis proposes that miniaturization of the social parasite is evolutionarily favored because smaller body size reduces the developmental “caste threshold” of the parasitic species, allowing the parasite’s brood to develop into reproductive queens instead of sterile workers as a result of being fed the same amount that, when fed to the host brood, would result in the development of workers (Nonacs and Tobin 1992). Consequently, the hypothesis predicts that the degree of worker caste loss should be correlated with the degree of miniaturization in a social parasite species. Based on the dry weight of *A. charruanus* alate queens (mean 2.2 mg; range 2–2.4 mg), the parasite gynes are lighter than both host alate queens (mean 9.4 mg; range 4.7–15.5 mg) and host major workers (mean 4.8 mg; range 3.7–5.5 mg). The low dry weight of *A. charruanus* gynes suggests that the degree of miniaturization is relatively advanced, and that, considering that the social parasite gynes are lighter than the host major workers, the selfish larvae hypothesis would predict the absence of a worker caste in *A. charruanus*. In contrast, in the Panamanian social parasite *A. insinator*, which still retains a worker caste, parasite queens are approximately half the dry weight of their host queens and weigh about 40 % more than their host major workers, suggesting that

the evolution of miniaturization is still at an early stage (Bekkevold and Boomsma 2000).

However, given that we were able to study only two parasitized colonies in the field, the results presented here should be interpreted cautiously, and further studies should test whether the worker caste of *A. charruanus* is completely absent or whether social parasite workers remained undetected because they occur at frequencies below 3–6 %.

Mating behavior and reproductive strategy

Dissecting the reproductive behavior of host and parasite is essential for understanding the parasite's life history and ecology, as well as for developing hypotheses regarding the parasite's evolution. The life cycles of many parasites are synchronized with the hosts' life cycles to maximize the availability of exploitable hosts (Wcislo 1987; Calero-Torralbo and Valera 2008; Schmid-Hempel 2011). In contrast, the chronologies of the mating seasons of *A. charruanus* and its host *A. heyeri* were diametrically opposed, with the parasite producing sexual offspring during the austral fall, when host alates were absent from the population, whereas the host reproduced during the austral spring. Importantly, the presence of parasite and host sexual brood during the fall and spring seasons also coincided with the maximum availability of colony resources (i.e., fungal biomass) in *A. heyeri* nests. During the course of 1 year, central Uruguayan *A. heyeri* colonies experience two growth peaks, one in the austral spring and a second in the austral fall, as indicated by the amount of fungal biomass and ant brood present in the nests (Bollazzi, personal observation). In contrast, during the winter, leaf-cutting ant colonies hibernate and during the summer their grass-cutting activity is reduced (Weber 1957; Bollazzi and Roces 2010a, b). As a consequence, *A. heyeri* colony size and fungal biomass is decreased during the summer and during the winter. It therefore seems plausible that the parasite's shift in mating season to the fall could be adaptive, because it (1) would allow the parasite to avoid competing for resources with the host's sexual brood during the spring and (2) would synchronize the parasite's reproductive output with the increase in fungal biomass that occurs during the fall colony growth peak. In addition, the chronologically opposed mating seasons of host and parasite could have contributed to the evolution of reproductive isolation between host and parasite if this behavior was present at the time when *A. charruanus* speciated from its original host.

In contrast to these possible benefits of diametrically opposed mating seasons of host and parasite, the non-overlapping mating flights might also cause challenges for *A. charruanus* queens with regard to accessing new host colonies. It is believed that during synchronized mating flights of hosts and social parasites, invading parasite

queens take advantage of the temporarily relaxed nestmate recognition system of the host colony (Lambardi et al. 2007; Nash and Boomsma 2008). Currently it is unclear whether *A. charruanus* faces additional challenges when entering a host colony outside the host's nuptial flight period, and which behavioral and chemical strategies are utilized by the parasite to successfully invade and become established in a new host colony.

The mating behavior of socially parasitic fungus-growing ants that represent early stages of the inquiline syndrome (i.e., *A. insinuator*, *A. ameliae*) is variable. Laboratory colonies of the Panamanian *A. insinuator* release their alates either slightly before or after the hosts' mating flights, whereas colonies observed in the field produced host and parasite alates simultaneously (Bekkevold and Boomsma 2000). The Brazilian *A. ameliae* was observed to produce alates in two seasons per year, but host and parasite alates did not seem to co-occur, leading the authors to suggest that the biannual release of parasite reproductives increases the parasite's chance of successfully invading new host colonies (De Souza et al. 2007). In contrast, morphologically and behaviorally derived inquilines are known to reproduce in synchrony with unparasitized colonies of the host species in the same population, but, simultaneously, inquiline queens are known to suppress host reproduction in their own host colony, preventing co-occurrence of host and parasite alates in the same colony (Bruch 1928; Gallardo 1929; Rabeling and Bacci 2010). This so-called "host-castration" is a well-known strategy of parasites that directs the hosts' resources to colony growth instead of host reproduction (Baudoin 1975).

The observation that both *A. heyeri* nests observed to be parasitized by *A. charruanus* in February were empty in May suggests that the host colonies had died following parasite reproduction, which indirectly supports the hypotheses that *A. charruanus* reproduces semelparously. Semelparous reproduction, which maximizes reproduction in a single reproductive bout, was observed in *A. insinuator* and was suggested for *P. argentina*, whereas iteroparity has been suggested for *M. castrator* (Bruch 1928; Gallardo 1929; Bekkevold and Boomsma 2000; Rabeling and Bacci 2010). Alternatively, *A. charruanus* could specialize on parasitizing old host colonies that have lost their queens, as has been observed in the microgyne social parasite of *Myrmica rubra* and in orphaned colonies of *Solenopsis invicta* (DeHeer and Tschinkel 1998; Schär and Nash 2014), or the parasitized *A. heyeri* colonies could have moved to new nest sites. Such moves of fully grown fungus-growing ant colonies have been observed in unparasitized *A. heyeri* colonies (Nickele et al. 2012) and in colonies of other *Atta* and *Acromyrmex* species (Fowler 1981).

The evolutionary origins of social parasites are tightly linked to differential mating times and/or localities of hosts

and parasites and to the coexistence of multiple reproductive queens (i.e., polygyny) in the host colony (Bourke and Franks 1991, 1995; Buschinger 1986, 2009; Savolainen and Vepsäläinen 2003; Boomsma et al. 2014; Rabeling et al. 2014b). Polygyny, in particular, presents an opportunity for one queen to reproductively exploit the other queen(s) and can lead to the evolution of intraspecific parasitism (Buschinger 1986; Bourke and Franks 1991; Seifert 2010; Boomsma et al. 2014). Interestingly, every host and parasite species of fungus-growing ants that has been investigated so far is at least facultatively polygynous (Della Lucia and Vilela 1986; Delabie 1989; Bekkevold et al. 1999; Bekkevold and Boomsma 2000; Diehl et al. 2001; De Souza et al. 2004; Rabeling et al. 2007b; Rabeling and Bacci 2010), which supports the hypothesis that the evolutionary origin of social parasitism is contingent on the presence of polygyny in a given species. A population survey of *A. charruanus*'s host species, *A. heyeri*, in southern Brazil revealed that at least 17 % of the colonies were polygynous and polyandrous and that 68 % of the colonies for which monogyny was inferred had to be descendants of a necessarily heterozygous mother, suggesting that the frequency of polygyny in this population was likely higher (Diehl et al. 2001). The sexual strategies of the Uruguayan *A. heyeri* host colonies and of *A. charruanus* are currently unknown, and we are currently employing recently developed genetic markers to infer the mating strategies of this host-parasite pair (Rabeling et al. 2014a).

Conclusions

To conclude, we report a new inquiline social parasite species that usurps colonies of the thatch-mound-building, leaf-cutting ant *A. heyeri* from Uruguay. Morphological and behavioral modifications of *A. charruanus*, such as size reduction and loss of the worker caste, represent early stages of the social parasite syndrome, whereas more derived morphological reductions are absent. *A. charruanus* is morphologically similar to *A. ameliae*, a social parasite of *A. subterraneus*, from Brazil. However, we suspect that the morphological similarities evolved convergently due to comparable selective pressures related to the parasitic habits. Initial behavioral observations suggest that *A. charruanus* is tolerant of the host queen, performs mating flights during the austral fall, and reproduces semelparously. Future field and laboratory studies of this social parasite are needed to confirm our initial observations and will reveal additional life history and behavioral traits that will allow us to understand the evolutionary origins and the complex socially parasitic life history of *A. charruanus* in more detail.

Acknowledgments We gratefully acknowledge the Dirección General de Recursos Naturales Renovables for permission to conduct fieldwork in Uruguay. We thank Naomi Pierce for permission to conduct parts of this study in her laboratory at Harvard University. Sarah Callan and Amelia Harvey kindly assisted in the laboratory. Christian Rabeling was supported by the National Science Foundation (DEB-1456964), the Harvard Society of Fellows, and by the William F. Milton Fund from the Harvard Medical School. Ted Schultz was supported by the National Science Foundation (DEB-0949689, DEB-1456964) and the Smithsonian Competitive Grants Program for Science. Maurício Bacci Jr. acknowledges support from FAPSP (2011/50226-0) and CNPq (311562/2012-4 and 487639/2012-0). Martin Bollazzi is grateful to Daniel Ramírez from Cambium Forestal for facilitating access to field research sites.

References

- Baer B, Boomsma JJ (2006) Mating biology of the leaf-cutting ants *Atta colombica* and *A. cephalotes*. *J Morphol* 267:1165–1171
- Baudoin M (1975) Host castration as a parasitic strategy. *Evolution* 29:335–352
- Bekkevold D, Boomsma JJ (2000) Evolutionary transition to a semelparous life history in the socially parasitic ant *Acromyrmex insinuator*. *J Evol Biol* 13:615–623
- Bekkevold D, Frydenberg J, Boomsma JJ (1999) Multiple mating and facultative polygyny in the Panamanian leafcutter ant *Acromyrmex echinator*. *Behav Ecol Sociobiol* 46:103–109
- Bollazzi M, Roces F (2010a) Leaf-cutting ant workers (*Acromyrmex heyeri*) trade off nest thermoregulation for humidity control. *J Ethol* 28:399–403
- Bollazzi M, Roces F (2010b) The thermoregulatory function of thatched nests in the South American grass-cutting ant, *Acromyrmex heyeri*. *J Insect Sci* 10(137):1–17
- Bollazzi M, Kronenbitter J, Roces F (2008) Soil temperature, digging behaviour, and the adaptive value of nest depth in South American species of *Acromyrmex* leaf-cutting ants. *Oecologia* 158:165–175
- Bolton B (2014) An online catalog of the ants of the world. <http://antcat.org>. Accessed 26 Sept 2014
- Bonetto AA (1959) Las hormigas “cortadoras” de la Provincia de Santa Fé (generos: *Atta* y *Acromyrmex*). Ministerio de Agricultura y Ganadería (Dirección General de Recursos Naturales), Santa Fé, Argentina, p 79
- Boomsma JJ, Nash D (2014) Evolution: sympatric speciation the eusocial way. *Curr Biol* 24:R798–R800
- Boomsma JJ, Huszár DB, Pedersen JS (2014) The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. *Anim Behav* 92:241–252
- Bourke AFG, Franks NR (1991) Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants. *Biol J Linn Soc* 43:157–178
- Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton
- Brandão CRF (1991) Adendos ao catálogo abreviado das formigas da região Neotropical (Hymenoptera: Formicidae). *Rev Brasil Entomol* 35:319–412
- Brown WL Jr (1973) A comparison of the Hylean and Congo-West African rain forest ant faunas. In: Meggers BJ, Ayensu ES, Duckworth WD (eds) Tropical forest ecosystems in Africa and South America: a comparative review. Smithsonian Institution Press, Washington DC, pp 161–185
- Bruch C (1928) Estudios mirmecológicos. *Anales del Museo Nacional de Historia Natural Buenos Aires* 34:341–360
- Bueno OC, Hebling MJ, Schneider MO, Pagnocca FC, Bacci JRM (2002) Occurrence of winged forms of *Atta sexdens rubropilosa*

- Forel (Hymenoptera: Formicidae) in laboratory colonies. *Neotrop Entomol* 31:469–473
- Buschinger A (1986) Evolution of social parasitism in ants. *Trends Ecol Evol* 1:155–160
- Buschinger A (2009) Social parasitism among ants: a review (Hymenoptera: Formicidae). *Myrmecol News* 12:219–235
- Buschinger A, Klump B (1988) Novel strategy of host-colony exploitation in a permanently parasitic ant, *Doronomyrmex goesswaldi*. *Naturwissenschaften* 75:577–578
- Cabrera AL, Willink A (1980) Biogeografía de América Latina. Segunda edición. Monografía 13, serie biología. Programa Regional de Desarrollo Científico y Tecnológico, Organización de los Estados Americanos, p 122
- Caldera E, Poulsen M, Suen G, Currie CR (2009) Insect symbioses—a case study of past, present, and future fungus-growing ant research. *Environ Entomol* 38:78–92
- Calero-Torralbo M, Valera F (2008) Synchronization of host-parasite cycles by means of diapause: host influence and parasite response to involuntary host shifting. *Parasitology* 135:1343–1352
- Cristiano MP, Cardoso DC, Fernandes-Salomão TM (2013) Cytogenetic and molecular analyses reveal a divergence between *Acromyrmex striatus* (Roger, 1863) and other congeneric species: taxonomic implications. *PLoS One* 8:e59784
- De Souza DJ, Lino Neto J, Della Lucia TMC, Peternelli EF (2004) Occurrence of polygyny in *Acromyrmex subterraneus molestans* Santschi 1925 (Hymenoptera: Formicidae). *Ciência Rural* 34:1611–1613
- De Souza DJ, Soares I, Della Lucia TMC (2007) *Acromyrmex ameliae* sp. n. (Hymenoptera: Formicidae): a new social parasite of leaf-cutting ants in Brazil. *Insect Sci* 14:251–257
- DeHeer CJ, Tschinkel WR (1998) The success of alternative reproductive tactics in monogyne populations of the ant *Solenopsis invicta*: significance for transitions in social organization. *Behav Ecol* 9:130–135
- Delabie JHC (1989) Observações sobre a ocorrência de poliginia em colônias de *Acromyrmex subterraneus brunneus* Forel 1893, em cacauais (Formicidae, Myrmicinae, Attini). *Anais da Sociedade Entomologica do Brasil* 18:193–197
- Delabie JHC, Fowler HG, Schindwein MN (1993) Ocorrência do parasita social *Pseudoatta* sp. nova em ninhos de *Acromyrmex rugosus* em Ilhéus, Bahia: primeiro registro para os trópicos. Paper presented at the IV International Symposium on Pest Ants and XI Encontro de Mirmecologia, Belo Horizonte, Minas Gerais, Brazil
- Della Lucia TMC (2011) Formigas Cortadeiras: da Bioecologia ao Manejo. Editora da UFV, Viçosa
- Della Lucia TMC, Vilela EF (1986) Ocorrência de poliginia em *Acromyrmex subterraneus* Forel, 1893 (Hymenoptera: Formicidae). *Anais da Sociedade Entomologica do Brasil* 15:377–378
- Diehl E, de Araujo AM, Cavalli-Molina S (2001) Genetic variability and social structure of colonies in *Acromyrmex heyeri* and *A. striatus* (Hymenoptera: Formicidae). *Brazil J Biol* 61:667–678
- Diehl-Fleig E (1993) Sex ratio and nuptial flight pattern of the leaf-cutting ants *Acromyrmex heyeri* and *A. striatus* (Hymenoptera: Formicidae). *Insect Soc* 40:111–113
- Dijkstra MB, Boomsma JJ (2006) Are workers of *Atta* leafcutter ants capable of reproduction? *Insect Soc* 53:136–140
- Elmes GW (1973) Observations on the density of queens in natural colonies of *Myrmica rubra* L. (Hymenoptera: Formicidae). *J Anim Ecol* 42:761–771
- Emery C (1905) Revisione delle specie del genere *Atta* appartenenti ai sottogeneri *Moellerius* e *Acromyrmex*. *Memorie della R. Accademia delle Scienze dell'Istituto di Bologna* 2:1–18
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial *cytochrome c oxidase subunit I* from diverse metazoan invertebrates. *Mol Mar Biol Biotech* 3:294–299
- Fowler HG (1981) On the emigration of leaf-cutting ant colonies. *Biotropica* 13:316
- Gallardo A (1916) Notes systématiques et éthologiques sur les fourmis attines de la République Argentine. *Anales del Museo Nacional de Historia Natural Buenos Aires* 28:317–344
- Gallardo A (1929) Note sur les moeurs de la fourmi *Pseudoatta argentina*. *Revista de la Sociedad Entomológica Argentina* 2:197–202
- Gonçalves CR (1961) O gênero *Acromyrmex* no Brasil (Hym. Formicidae). *Studia Entomologica* 4:113–180
- Gotwald WH (1969) Comparative morphological studies of the ants, with particular reference to the mouthparts (Hymenoptera: Formicidae). *Memoirs of the Cornell University Agricultural Experiment Station* 408:1–150
- Herre EA, Knowlton N, Mueller UG, Rehner SA (1999) The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol Evol* 14:49–53
- Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge
- Hölldobler B, Wilson EO (2010) The leafcutter ants—civilization by instinct. W. W. Norton & Company, New York
- Jansen G, Savolainen R, Vepsäläinen K (2010) Phylogeny, divergence-time estimation, biogeography and social parasite–host relationships of the Holarctic ant genus *Myrmica* (Hymenoptera: Formicidae). *Mol Phylog Evol* 56:294–304
- Johnson RA, Rissing SW (1993) Breeding biology of the desert leaf-cutter ant *Acromyrmex versicolor* (Pergande) (Hymenoptera: Formicidae). *J Kansas Entomol Soc* 66:127–128
- Jutsum AR, Cherrett JM (1977) Sexualls and a microgyne of *Atta cephalotes* (L.) (Hymenoptera, Formicidae) from laboratory cultures. *Entomol Mon Mag* 113:97–98
- Kempf W (1956) A morphological study on the male genitalia of *Paracryptocerus* (P.) *pusillus* (Hymenoptera: Formicidae). *Rev Brasil Entomol* 5:101–110
- Kempf WW (1972) Catálogo abreviado das formigas da região Neotropical. *Studia Entomologica* 15:3–344
- Klingenberg C, Brandão CRF (2009) Revision of the fungus-growing ant genera *Mycetophylax* Emery and *Paramycetophylax* Kusnezov rev. stat., and description of *Kalathomyrmex* n. gen. (Formicidae: Myrmicinae: Attini). *Zootaxa* 2052:1–31
- Kusnezov N (1954) Phyletische Bedeutung der Maxillar- und Labialtaster der Ameisen. *Zool Anzeig* 153:28–38
- Kutter H (1950) Über eine neue, extrem parasitische Ameise. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 23:81–94
- Kutter H (1969) Die sozialparasitischen Ameisen der Schweiz. *Neujahrsblatt der Naturforschenden Gesellschaft in Zürich* 171:1–62
- Lambardi D, Dani FR, Turillazzi S, Boomsma JJ (2007) Chemical mimicry in an incipient leaf-cutting ant social parasite. *Behav Ecol Sociobiol* 61:843–851
- Mayhé-Nunes AJ (1995) Filogenia de los Attini (Hym., Formicidae): un aporte al conocimiento de las hormigas fungívoras. *Universidad Simón Bolívar, Caracas, Venezuela*, p 274
- Mayhé-Nunes AJ, Jaffé K (1998) On the biogeography of Attini (Hymenoptera: Formicidae). *Ecotropicos* 11:45–54
- Möller A (1893) Die Pilzgärten einiger Südamerikanischer Ameisen. *Botanische Mittheilungen aus den Tropen* 6:1–142
- Mueller UG, Gerardo NM, Aanen DK, Six DL, Schultz TR (2005) The evolution of agriculture in insects. *Ann Rev Ecol Evol Syst* 36:563–595
- Nash DR, Boomsma JJ (2008) Communication between hosts and social parasites. In: Hughes DP, D’Etorre P (eds) *Sociobiology of*

- communication: an interdisciplinary perspective. Oxford University Press, Oxford, pp 55–79
- Nickele MA, Pie MR, Reis Filho W (2012) Emigration of a colony of the leaf-cutting ant *Acromyrmex heyeri* Forel (Hymenoptera, Formicidae). *Rev Brasil Entomol* 56:385–386
- Nonacs P, Tobin JE (1992) Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera. *Evolution* 46:1605–1620
- Overbeck GE, Müller SC, Fidelis A, Pfadenhauer J, Pillar VD, Blanco CC, Boldrini II, Both R, Forneck ED (2007) Brazil's neglected biome: the South Brazilian *Campos*. *Perspect Plant Ecol Evol Syst* 9:101–116
- Pagnocca FC, Masiulionis VE, Rodrigues A (2012) Specialized fungal parasites and opportunistic fungi in gardens of attine ants. *Psyche J Entomol*. doi:10.1155/2012/905109 Article ID 905109, p 9
- Rabeling C, Bacci M (2010) A new workerless inquiline in the Lower Attini (Hymenoptera: Formicidae), with a discussion of social parasitism in fungus-growing ants. *Syst Entomol* 35:379–392
- Rabeling C, Cover SP, Johnson RA, Mueller UG (2007a) A review of the North American species of the fungus-gardening ant genus *Trachymyrmex* (Hymenoptera: Formicidae). *Zootaxa* 1664:1–53
- Rabeling C, Verhaagh M, Engels W (2007b) Comparative study of nest architecture and colony structure of the fungus-growing ants, *Mycocepurus goeldii* and *M. smithii*. *J Insect Sci* 7(40):1–13
- Rabeling C, Bollazzi M, Bacci M Jr, Beasley RR, Lance SL, Jones KL, Pierce NE (2014a) Development and characterization of twenty-two polymorphic microsatellite markers for the leafcutter ant, *Acromyrmex lundii*, utilizing Illumina sequencing. *Con Gen Res* 6:319–322
- Rabeling C, Schultz TR, Pierce NE, Bacci M Jr (2014b) A social parasite evolved reproductive isolation from its fungus-growing ant host in sympatry. *Curr Biol* 24:2047–2052
- Santschi F (1925) Revision du genre *Acromyrmex* Mayr. *Rev Suisse Zool* 31:355–398
- Savolainen R, Vepsäläinen K (2003) Sympatric speciation through intraspecific social parasitism. *Proc Natl Acad Sci USA* 100:7169–7174
- Schär S, Nash DR (2014) Evidence that microgynes of *Myrmica rubra* ants are social parasites that attack old host colonies. *J Evol Biol* 27:2396–2407
- Schmid-Hempel P (2011) *Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics*. Oxford University Press, New York
- Schultz TR (2007) The fungus-growing ant genus *Apterostigma* in Dominican amber. *Mem Am Entomol Inst* 80:425–436
- Schultz TR, Brady SG (2008) Major evolutionary transitions in ant agriculture. *Proc Natl Acad Sci USA* 105:5435–5440
- Schultz TR, Meier R (1995) A phylogenetic analysis of the fungus-growing ants (Hymenoptera: Formicidae: Attini) based on morphological characters of the larvae. *Syst Entomol* 20:337–370
- Schultz TR, Bekkevold D, Boomsma JJ (1998) *Acromyrmex insinuator* new species: an incipient social parasite of fungus-growing ants. *Insect Soc* 45:457–471
- Schultz TR, Mueller UG, Currie CR, Rehner SA (2005) Reciprocal illumination: a comparison of agriculture in humans and ants. In: Vega F, Blackwell M (eds) *Ecological and evolutionary advances in insect-fungal associations*. Oxford University Press, New York, pp 149–190
- Seifert B (2010) Intranidal mating, gyne polymorphism, polygyny, and supercoloniality as factors for sympatric and parapatric speciation in ants. *Ecol Entomol* 35:33–40
- Seppä P (1994) Sociogenetic organization of the ants *Myrmica ruginodis* and *Myrmica lobicornis*: number, relatedness and longevity of reproducing individuals. *J Evol Biol* 7:71–95
- Sosa-Calvo J, Schultz TR, Brandão CR, Klingenberg C, Feitosa RM, Rabeling C, Bacci M Jr, Lopes CT, Vasconcelos HL (2013) *Cyatta abscondita*: taxonomy, evolution, and natural history of a new fungus-farming ant genus from Brazil. *PLoS One* 8(11):e80498
- Sumner S, Hughes WOH, Boomsma JJ (2003a) Evidence for differential selection and potential adaptive evolution in the worker caste of an inquiline social parasite. *Behav Ecol Sociobiol* 54:256–263
- Sumner S, Nash DR, Boomsma JJ (2003b) The adaptive significance of inquiline parasite workers. *Proc R Soc Lond Ser B Biol Sci* 270:1315–1322
- Sumner S, Aanen DK, Delabie J, Boomsma JJ (2004) The evolution of social parasitism in *Acromyrmex* leaf-cutting ants: a test of Emery's rule. *Insect Soc* 51:37–42
- Tschinkel WR (1996) A newly-discovered mode of colony founding among fire ants. *Insect Soc* 43:267–276
- Wcislo WT (1987) The roles of seasonality, host synchrony, and behaviour in the evolutions and distributions of nest parasites in Hymenoptera (Insecta), with special reference to bees (Apoidea). *Biol Rev* 62:515–542
- Weber N (1957) Dry season adaptations of fungus-growing ants and their fungi. *Anat Rec* 128:638
- Weber NA (1972) *Gardening ants: the Attines*. Memoirs of the American Philosophical Society, Philadelphia
- Wheeler WM (1907) The fungus-growing ants of North America. *Bull Am Mus Nat Hist* 23:669–807
- Wilson EO (1971) *The Insect Societies*. Harvard University Press, Cambridge
- Wilson EO (1984) Tropical social parasites in the ant genus *Pheidole*, with an analysis of the anatomical parasitic syndrome (Hymenoptera: Formicidae). *Insect Soc* 31:316–334
- Yek SH, Boomsma JJ, Poulsen M (2012) Towards a better understanding of the evolution of specialized parasites of fungus-growing ant crops. *Psyche*. doi:10.1155/2012/239392 Article ID 239392, p 10
- Zolessi LC, Abenante YP (1974) Nidificación y mesoetología de *Acromyrmex* en el Uruguay III. *Acromyrmex* (A.) *hispidus* Santschi, 1925 (Hymenoptera: Formicidae). *Revista de Biología del Uruguay* 1:151–165
- Zolessi LC, González LA (1974) Nidificación y mesoetología de *Acromyrmex* en el Uruguay, II. *Acromyrmex* (*Acromyrmex*) *lobicornis* (Emery, 1887) (Hymenoptera: Formicidae). *Revista de Biología del Uruguay* 2:37–57
- Zolessi LC, González LA (1979) Observaciones sobre el género *Acromyrmex* en el Uruguay IV. A. (*Acromyrmex*) *lundii* (Guérin, 1838) (Hymenoptera: Formicidae). *Revista de la Facultad de Humanidades y Ciencias Serie Ciencias Biológicas* 1:9–28