



## Myrmecophilous organisms associated with colonies of the ponerine ant *Neoponera villosa* (Hymenoptera: Formicidae) nesting in *Aechmea bracteata* bromeliads: a biodiversity hotspot

Franklin H. ROCHA, Jean-Paul LACHAUD & Gabriela PÉREZ-LACHAUD

### Abstract

Ants and their resources are exploited by a plethora of other organisms, some using remarkable morphological and behavioral adaptations for host deception and social integration. The diversity, abundance, and distribution of myrmecophiles are likely underestimated, particularly regarding Neotropical ants. This study aims to document the diversity of myrmecophiles associated with the colonies of *Neoponera villosa* (FABRICIUS, 1804) in the southern part of the Yucatan Peninsula, Mexico, a region with rapid transformation and high risk of habitat loss and habitat fragmentation. Between January 2016 and January 2019, 82 colonies (or parts of colonies) established in the core of the tank bromeliad *Aechmea bracteata* (Sw.) GRISEB., 1864, were collected. All invertebrates present in the nest chambers were recorded, and ants and their brood were inspected under a stereomicroscope for the presence of any sign of parasitism. Natural history of some of the recorded myrmecophiles and nest associates was determined. Results showed a diverse array of associated taxa, with organisms from six classes distributed in at least 43 different taxa belonging to 16 orders and 24 families. Twelve different taxa belonging to 12 families, eight arthropod orders and one fungus order, were encountered in direct physical association with the ant brood and / or the adults in the central part of the nest: Hymenoptera (Diapriidae, Eucharitidae), Lepidoptera (Riodinidae), Diptera (Syrphidae), Coleoptera (Staphylinidae, Tenebrionidae), Acari Mesostigmata (Laelapidae, Oplitidae), Acari Trombidiformes (Scutacaridae), Acari Sarcoptiformes (Galumnidae), Pseudoscorpiones (Chernetidae), and Hypocreales (Ophiocordycipitaceae). These specialized myrmecophiles showed diverse trophic interactions with the ants, mostly antagonistic (parasites, parasitoids, predators, cleptoparasites). Although their prevalence was low, their combined effect upon the host population was not negligible. Not integrated, facultative, guests included several scavengers and predators found in the refuse pile within the nest or in the periphery of the chambers: springtails, rove beetles, mites, and other small ant species that nested close to *N. villosa*. With the exception of the parasitoid syrphid fly *Hypselosyrphus trigonus* HULL, 1937, which had been previously reported, all associations are new to science. The diverse group of obligate myrmecophiles and facultative guests associated with this highly aggressive ant species confirms arboreal ant colonies as reservoirs of diversity and suggests that ant species with relatively small colony sizes, such as ponerines, can also harbor a high diversity of associated taxa.

**Key words:** Ant parasitoids, cleptoparasitism, predation, colony integration, ant associates, interaction.

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Franklin H. Rocha & Gabriela Pérez-Lachaud (contact author), El Colegio de la Frontera Sur, Conservación de la Biodiversidad, Avenida Centenario km 5.5, Chetumal 77014, Quintana Roo, México. E-mail: igperez@ecosur.mx

Jean-Paul Lachaud, El Colegio de la Frontera Sur, Conservación de la Biodiversidad, Avenida Centenario km 5.5, Chetumal 77014, Quintana Roo, México; Centre de Recherches sur la Cognition Animale (CRCA), Centre de Biologie Intégrative (CBI), Université de Toulouse; CNRS, UPS, Toulouse, France.

### Introduction

The nests and colonies of many ants and other social insects are, in general, stable and long-lived. Favorable physical and environmental conditions are encountered within their nests, and both the ants and their brood represent a valuable resource in terms of biomass (HÖLLDOBLER &

WILSON 1990). This makes ant colonies suitable targets for other organisms, which, in addition, gain enemy-free space (ATSATT 1981). Ants are hosts for many other invertebrates (predominantly other arthropods) that live in their nests, the nest surroundings, or in / on their bodies

(DONISTHORPE 1927, KISTNER 1982, HÖLLDOBLER & WILSON 1990, SCHMID-HEMPEL 1998, LACHAUD & al. 2012b, 2013, QUEVILLON & HUGHES 2018). Organisms that live in or near ant nests and establish a range of symbiotic interactions with their hosts for a considerable part of their life cycle are termed myrmecophiles (DONISTHORPE 1927, KISTNER 1982, HÖLLDOBLER & WILSON 1990, HUGHES & al. 2008, LACHAUD & al. 2012b, 2013, but see PARKER 2016 for a more restricted definition). To date, known ant associated species include at least 39 orders of Arthropoda and more than 50 orders belonging to other phyla and kingdoms, including vertebrates, fungi and bacteria (KISTNER 1982, HÖLLDOBLER & WILSON 1990, SCHMID-HEMPEL 1998, WITTE & al. 2002, RETTENMEYER & al. 2011, POINAR 2012, LACHAUD & PÉREZ-LACHAUD 2012, MARUYAMA & al. 2013, LACHAUD & al. 2016, QUEVILLON & HUGHES 2018, CASTAÑO-MENESES & al. 2019).

Myrmecophiles range from highly integrated species that rely on physiological, morphological and behavioral adaptations that allow them to withstand ant aggressiveness and to be tolerated or even treated as nestmates, to poorly integrated guests that try to elude the hosts and rely on fleetness (WASMANN 1894, HÖLLDOBLER & WILSON 1990, VON BEEREN & al. 2018). Adaptations to myrmecophily include morphological, behavioral, chemical or acoustical mimicry (MCIVER & STONEDAHL 1993, LENOIR & al. 2001, AKINO 2008, BAGNÈRES & LORENZI 2010, DI GIULIO & al. 2015, SCHÖNROGGE & al. 2017). However, many myrmecophiles lacking such adaptations are tolerated by their aggressive hosts, possibly because they exhibit either a neutral odor or are below a specific critical size and are not recognized as a threat by their hosts (CUSHING 1997, PÉREZ-LACHAUD & al. 2019b). Specificity to the host is also very variable and interactions between ants and myrmecophiles range from loose facultative associations to an obligate dependency for one or for both partners. These interactions often appear complex and specialized and, in many cases, are restricted to a single ant host genus or species; however, some myrmecophiles are known to be generalists, interacting with ant species from several families (HÖLLDOBLER & WILSON 1990, PIERCE & al. 2002, PÄIVINEN & al. 2003, PÉREZ-LACHAUD & al. 2006, ELIZALDE & al. 2018, GLASIER & al. 2018).

The dynamics of particular ant symbioses (particularly ant mutualisms) have been the focus of substantive reviews (e.g., WAY 1963, DELABIE 2001, MUELLER & al. 2001, PIERCE & al. 2002, STADLER & DIXON 2005, FIEDLER 2006, 2012, DE FREITAS & ROSSI 2015). However, other types of interactions, and more generally the study of the macro and microorganisms associated with ants, have been somewhat neglected (DE BEKKER & al. 2018), though in the last few years there seems to be a regain in interest in myrmecophiles (e.g., HÄRKÖNEN & SORVARI 2014, WITEK & al. 2014, PARMENTIER & al. 2014, PARKER 2016, DI SALVO & al. 2019). Most studies on the associated fauna of ants have focused on temperate rather than tropical species which have not been studied in detail or have largely been

overlooked (but see LIM & al. 2008, RETTENMEYER & al. 2011, LACHAUD & PÉREZ-LACHAUD 2015).

It has been hypothesized that ant species with exceptionally large mature (populous) colonies, or those regionally widespread, support a higher diverse community of myrmecophiles than species with small colonies. Such a relationship may be due to a high microhabitat diversity within large ant nests and a high capacity to sustain numerous associates over longer time periods (HÖLLDOBLER & WILSON 1990, KRONAUER & PIERCE 2011), and this appears to be supported by empirical evidence, at least for myrmecophilous beetles (PÄIVINEN & al. 2003). Further, because of high genetic diversity and reduced aggressiveness, it is also agreed that polygynous ant species should support more myrmecophile species than monogynous and more aggressive ant species (PÄIVINEN & al. 2003). Notwithstanding, a high diversity of accompanying fauna can be found even for ant species with small colony size or nesting in very specific microhabitats, such as ant species occupying fallen or hanging dry cocoa pods (e.g., CASTAÑO-MENESES & al. 2015a). However, because of their hidden life style, the global distribution and abundance of myrmecophiles is unclear and likely underestimated (DE BEKKER & al. 2018), and the interactions with their hosts are not well understood.

Here, we report on the myrmecophilous organisms closely associated with the Neotropical ponerine ant *Neoponera villosa* (FABRICIUS, 1804) in the southern region of the Yucatan Peninsula in Mexico, with a special focus on macro-invertebrates. This ant is an opportunistic cavity breeder; however, in our study area, *N. villosa* nests almost exclusively in the tank bromeliad *Aechmea bracteata* (Sw.) GRISEB, 1864 (see DEJEAN 1990, DEJEAN & al. 1992, 1995, DEJEAN & OLMSTED 1997), one of the largest epiphytic bromeliads. Many aquatic and terrestrial organisms find shelter in tank bromeliads, including microorganisms and vertebrates (BENZIG 1990, DEJEAN & al. 1995). Each *A. bracteata* plant constitutes a complex ecosystem with unique associated fauna (DEJEAN & OLMSTED 1997). This bromeliad is commonly associated with several ant species, including *N. villosa* (see DEJEAN & al. 1995, DEJEAN & OLMSTED 1997), and the identity of the ant associate has been shown to influence both the aquatic biodiversity and the interaction network within the *A. bracteata* niche (DEJEAN & al. 2018). To date, *N. villosa* has only been reported as reliably associated with an unidentified nematod species (Mermithidae) in Colombia and Venezuela (EMERY 1904, WHEELER 1928), several phoretic mite species in Brazil (LOPES & al. 2015a), and two species of brood parasitoids in southern Mexico, *Hypselosyrphus trigonus* HULL, 1937 (Diptera: Syrphidae) and *Blanchardiscus* sp. (Hymenoptera: Encyrtidae) (PÉREZ-LACHAUD & al. 2014, PÉREZ-LACHAUD & LACHAUD 2017), both associated with colonies nesting in *A. bracteata*. Additionally, three species in the *Apocephalus miricauda*-group (Diptera: Phoridae) have been signaled as potential parasitoids of this ant in Brazil, Colombia and Costa Rica (BROWN 2000) and several springtail species have been reported as inquilines

of colonies nesting in dry cocoa pods (CASTAÑO-MENESES & al. 2015b).

Ants and their colonies have been considered as reservoirs of a yet unknown biodiversity (PÉREZ-LACHAUD & LACHAUD 2014, DE BEKKER & al. 2018). Habitat fragmentation and habitat loss threaten all arboreal ant species and all the organisms that inhabit their colonies and nests which, in some cases, are very host specific and, for the most part, are still unknown (PÉREZ-LACHAUD & LACHAUD 2014). Here, we aimed at unveiling the hidden diversity of myrmecophiles associated with the arboreal nests of *Neoponera villosa* in a region with rapid transformation and high risk of habitat loss and fragmentation.

## Materials and methods

The data presented here were gathered as part of a larger research project assessing the whole community of organisms found in *Aechmea bracteata* bromeliads inhabited by *Neoponera villosa*. This study focuses on myrmecophiles, and more specifically on the macroinvertebrates found in close proximity with the ant brood or the adults within the central part of the bromeliad where larvae and cocoons are grouped and attended.

**The ant host:** *Neoponera villosa* has a wide geographical distribution, from Texas to Argentina (MACKAY & MACKAY 2010), and is widespread both in wet and dry forests (WILD 2002). This opportunist cavity-breeder nests both in live and dead trees, in bromeliads, hollow stems of *Cecropia* (LÖEFLING, 1758) (DEJEAN & al. 1992, 1995; VALENZUELA-GONZÁLEZ & al. 1994, FERNANDES & al. 2013), and is commonly encountered in hanging dry cocoa pods (FOWLER 1993, CASTAÑO-MENESES & al. 2015a). Workers are monomorphic and measure 12 - 13 mm (WHEELER 1908); they are generalized arboreal predators of other arthropods and collect extra-floral nectar (LACHAUD & al. 1984, PÉREZ-BAUTISTA & al. 1985, DEJEAN & CORBARA 1990, VALENZUELA-GONZÁLEZ & al. 1994). Both monogyny and polygyny have been reported

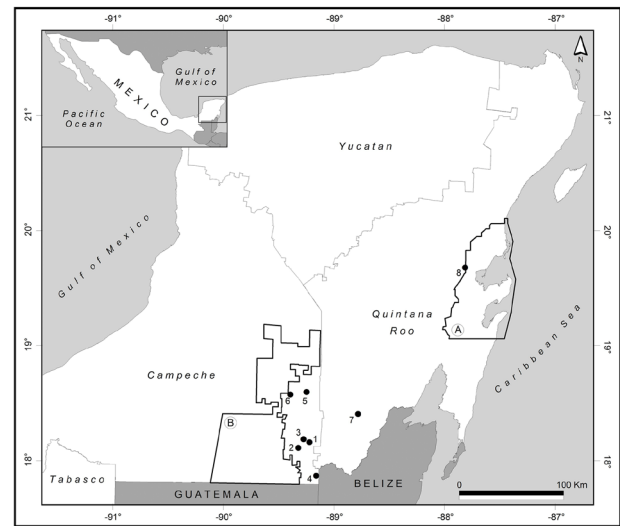


Fig. 1: Localization of *Neoponera villosa* collecting sites in the southern part of the Yucatan Peninsula, Mexico. Collecting sites: 1) Hermenegildo Galeana, 2) Ejido Blasillo, 3) Felipe Ángeles, 4) Pioneros del Río Xnohá, 5) Nuevo Becal, 6) Zoh-Laguna, 7) Kohunlich, 8) Sian Ka'an. Polygons: A) Sian Ka'an Biosphere Reserve, B) Calakmul Biosphere Reserve.

in different populations (PÉREZ-BAUTISTA & al. 1985, TRUNZER & al. 1998). Adults of *N. villosa* are very aggressive and provided with a powerful sting; their venom, composed of 145 proteins, presents hemolytic properties (PESSOA & al. 2016).

**Study sites and sampling method:** Ant nesting behavior in bromeliads in our study area allowed collection of complete colonies and the evaluation of the diversity of nest associates and, in some cases, to determine the nature of interactions with their host. Ant colonies were collected at eight sites in the Yucatan Peninsula (Fig. 1, Tab. 1). The climate across the peninsula is of the type "Aw" according to GARCÍA (1973), warm, sub-humid, with rainfall during

Tab. 1: Collecting sites of *Neoponera villosa* ants nesting in *Aechmea bracteata* bromeliads in the southern region of the Yucatan Peninsula, Mexico, and number of colonies collected. a.m.s.l.: above mean sea level.

Localities	Latitude	Longitude	Altitude (m a.m.s.l.)	Number of colonies collected
<b>Campeche</b>				
Hermenegildo Galeana	18° 10' 36.41" N	89° 14' 24.29" W	190	7
Ejido Blasillo	18° 7' 37.98" N	89° 20' 20.93" W	261	19
Felipe Ángeles	18° 12' 8.94" N	89° 17' 36.54" W	212	1
Pioneros del Río Xnohá	17° 53' 13.99" N	89° 10' 31.94" W	65	1
Nuevo Becal	18° 36' 39.36" N	89° 16' 15.54" W	239	23
Zoh-Laguna	18° 35' 11.61" N	89° 25' 4.67" W	257	2
<b>Quintana Roo</b>				
Kohunlich	18° 25' 31.08" N	88° 48' 9.89" W	143	2
Sian Ka'an	19° 41' 56.17" N	87° 50' 18.31" W	18	27

the summer and the driest season during March and April. All the *Aechmea bracteata* bromeliads found in the sites were examined for the presence of ants and those housing *Neoponera villosa* were sampled; the diameter and height of a sample of bromeliads housing ants was measured ( $n = 42$ ). Colonies of *N. villosa* and their associated organisms are established in the central portion of the epiphyte, which provides shelter and protection. The shoot housing the ants was cut off from the supporting branch, placed in plastic bags, and transported to the laboratory for immediate dissection. Temperature and precipitation data were obtained from the nearest weather stations run by the National Water Commission (CONAGUA). Each epiphyte was dismantled leaf-by-leaf in plastic bins coated with Fluon (Whitford GmbH), and all *N. villosa* ants, their brood and all the organisms contained between the leaves of the plant were secured, recording the localization of the individuals, that is, in the central or in the peripheral part of the bromeliads. For the first 17 colonies sampled this distinction was not made, and data for some myrmecophiles was thus based on a subsample of only 65 colonies. During the rainy season, the phytotelm impounds water between external leaves that shelters an abundant aquatic fauna (BROUARD & al. 2012; DÉZERALD & al. 2013, 2014). However, this was not recorded in this study as the water was drained off by making a small hole with fine forceps before collecting the epiphyte to prevent brood and adults drowning during transport. As previously specified, only the myrmecophiles present in the central part of the host nest, close to or in direct contact with the ants and the brood, were considered and analyzed in the present study.

Ants were counted and their caste, and developmental stage recorded. Eggs were not counted, but their presence was noted. All the material was examined under a stereomicroscope. Adult ants were inspected for any sign of parasitism by scrutiny for the possible presence of external parasites, or nematodes (Nematoda: Mermithidae) within the distended abdomens of mermithized specimens (see PÉREZ-LACHAUD & LACHAUD 2014). Ant larvae were examined both for the possible presence of planidia (the first instar larvae of eucharitid wasps and some other parasitoids) attached to their surfaces and for other external signs of endoparasitism (i.e., scars, visible external changes in color appearance or respiratory funnels). Cocoons from the same colony were kept at room temperature ( $28 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  relative humidity) in glass vials stuffed with cotton at one end to allow air into the vial and prevent emerged parasitoids from escaping. Vials from each colony were kept for a total of 10 days, and checked every day for the presence of adult parasitoids inside the vials. Subsequently, cocoons were dissected under a stereomicroscope; any parasitoid larva or pupa, or any fly puparium were further incubated until adult emergence. Notes on both behavioral interactions between ants and myrmecophiles and life history traits were recorded whenever possible.

Samples of ants and myrmecophiles were preserved in 96% ethanol. *Neoponera villosa* ants were previously identified by J.C.H. Delabie (see PÉREZ-LACHAUD & al. 2014).

Identification was confirmed through DNA extraction and barcoding as part of an ongoing project (J.-P. Lachaud & G. Pérez-Lachaud, unpubl.; GenBank accession numbers MK779595, MK779597, MK779600, MK779602 and MK779604). Associated organisms were identified to the lowest possible taxon. Effort, in terms of cost and time, was focused on the identification of true myrmecophiles which were sent to the specialists of the relevant group and were identified to the genus level. Facultative myrmecophiles were identified with appropriate taxonomic keys to family level. Identification to species level was hindered by the lack of resources for identifying most Neotropical arthropods (many groups have not been thoroughly revised yet and detailed keys are not available). Voucher specimens of ants, parasites, parasitoids and other myrmecophiles were deposited in the Arthropoda and Formicidae collections of El Colegio de la Frontera Sur at Chetumal, Quintana Roo, Mexico (ECO-CH-AR and ECO-CH-F, respectively).

**Data analyses:** Prevalence of parasitism was calculated at the population level, across all samples, as the proportion of parasitized colonies or parasitized cocoons. Parasitism rate was calculated as the proportion of parasitized cocoons in parasitized colonies. The Pearson correlation coefficient was used to test whether or not larger colonies harbored more parasitoids. The same test was used to verify possible correlations between available potential host castes and abundance of predators. The possible effect of the number of potential hosts (number of cocoons or larvae) or that of environmental factors (mean temperature, mean precipitation and season) upon the probability of a nest being parasitized were also explored by conducting a binomial logistic regression analysis with occurrence of parasitism as the dependent variable (parasitized – unparasitized colonies). Since there were very few records of parasitism by eucharitids, this analysis was performed including both eucharitids and syrphids. Alpha was set at 0.05 for all statistical tests. Logistic regressions were computed in R (R CORE TEAM 2017). To estimate species richness and in order to check for the adequacy of our sampling effort, data were organized into a presence / absence matrix for each *Neoponera villosa* colony, and species accumulation curves were created using subsequent collects as a surrogate of sampling effort. Accumulation curves were computed using the statistical package Primer, version 6.1.11 (CLARKE & GORLEY 2006) both for myrmecophiles in direct physical contact with the ant brood or adults and for arthropods not integrated but present in the central part of the host nest.

## Results and discussion

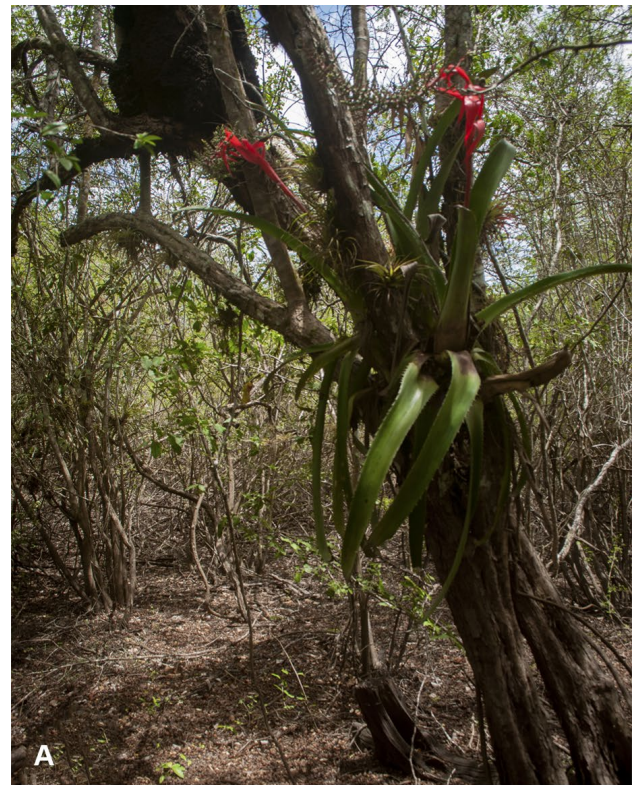
**Ant colony composition and nesting behavior:** A total of 72 queenright and ten queenless colonies (or parts of colonies) of *Neoponera villosa*, almost all containing larvae and pupae, were obtained between January 2016 and January 2019. On average colonies contained:  $3.3 \pm 0.5$  queens (mean  $\pm$  SEM, range: 0 - 20),  $97.8 \pm 7.9$  workers (range: 3 - 322),  $42.2 \pm 4.9$  pupae in cocoons (range: 2 - 261), and  $41.1 \pm 4.8$  larvae (range: 0 - 265).

Twenty-five colonies were producing winged females and in ten colonies adult males were present at the moment of collection (Tab.S1, as digital supplementary material to this article, at the journal's web pages). The colonies were found exclusively in mature *Aechmea bracteata* bromeliads (Fig. 2A), 14.3 ± 0.3 cm in diameter and 85.0 ± 3.4 cm in height, on average (n = 42). Ants established their nest in the watertight, inner central cavity created by several rosetta leaves rolling up and overlapping. Adult ants cut open a hole (approximately 1 cm diameter) through the leaf layers in order to reach the central cavity, that served as the nest entrance. The central cavity of the bromeliad was organized in separate chambers built by the ants and made with thatch, where the queen(s) and brood were found (Fig. 2B, C). A refuse pile was always situated among the external leaves of the bromeliad and contained arthropod remains and other debris not considered here; however, refuse and earth also accumulated at the base of the central cavity in which some scavengers were found.

**Diversity of associated organisms:** A total of 8538 adults of *Neoponera villosa*, 3463 cocoons, and 3368 larvae were examined. Five classes of arthropods and one of fungi, distributed in at least 43 taxa from 16 orders and 24 families were found in the core of the bromeliads inhabited by *N. villosa* and showed a varying degree of interaction with their hosts (Tab. 2, Tab. 3). More specifically, twelve different taxa belonging to 12 families from eight arthropod orders and one fungus order were encountered in direct physical association with the brood or the adults of *N. villosa* within the nest chambers, and were considered as true myrmecophiles (Tab. 2); all of the identified interactions observed in this study were with these specialized associates.

**Parasitoids and parasitism rate.** None of the larvae or adults of *Neoponera villosa* examined showed signs of parasitism. In contrast, cocoons were attacked by two different larval-pupal ectoparasitoid species: (i) a species of the genus *Kapala* CAMERON, 1884 (Hymenoptera: Eucharitidae), close to *K. izapa* and most probably a new species (J. Heraty, pers. comm.) (Tab. 2, Fig. 3); and (ii) the hoverfly *Hypselosyrphus trigonus* (Fig. 4). Both parasitoid species attack the same ant immature stage and develop within the protection of the cocoon's silky envelope; the host continues to develop and is only killed when the parasitoid completes its larval growth (koinobiont strategy).

Five out of 82 colonies (6.1%) were parasitized by eucharitids with 0.23% of the cocoons parasitized at the population level and a mean parasitism rate of 2.7 ± 0.9% cocoons per parasitized colony (mean ± SEM, n = 5). The life-cycle of *Kapala* sp., with the exception of the place of oviposition, could be ascertained (Fig. 3A - D). Eucharitidae females oviposit away from the host, and the very mobile first instar larva, termed planidium, is responsible for gaining access to the host colony (CLAUSEN 1940). Four females (Fig. 3F), three males (Fig. 3E), and a second instar larva that died as pupa were secured. Species of *Kapala* have already been reported as parasitoids of two other species of *Neoponera* EMERY, 1901, *N. apicalis* (LATREILLE,



2 mm

Fig. 2: The nest-site of *Neoponera villosa*. (A) An *Aechmea bracteata* plant in a seasonally inundated site in the Sian Ka'an Biosphere Reserve. (B) The core of an *A. bracteata* used by *N. villosa* as nest. Note that the ants created a separate chamber. (C) The outer leaf and all adult ants have been previously removed to show the brood.

1802) and *N. verenae* FOREL, 1922 (DE LA MORA & PHILPOTT 2010, LACHAUD & al. 2012a; Tab.S2), and *N. villosa* is the third species of this ant genus recorded as host for *Kapala* parasitoid wasps.

Additionally, 36.6% of the sampled colonies (30 / 72) were parasitized by *Hypselosyrphus trigonus*. A total of 193 cocoons contained ant prepupae / pupae (or their remains) that had been attacked by *H. trigonus* representing

Tab. 2: Organisms found in the core of the nests of *Neoponera villosa*, in direct physical contact with the brood or the adults (true myrmecophiles). The column “Identification” refers to the taxonomist who identified the taxon or to the resources used by the authors. SEM = standard error of the mean.

Class	Order	Family	Taxon	Size (mm) (mean ± SEM)	Number of specimens	Number of occupied colonies	Observed relationship	Identification	References
<b>Animalia, Arthropoda</b>									
Arachnida	Acari Mesostigmata	Laelapidae	<i>Cosmolaelaps</i> sp.	≈ 0.4	Not estimated	74	Cleptoparasitic	H. Klömpen	This study
		Oplitidae	<i>Oplitis</i> sp.	≈ 0.4	Not estimated	9	Phoretic on both adults and larvae	H. Klömpen	This study
	Acari Trombidiformes Prostigmata	Scutacaridae	Unidentified	< 0.3	Not estimated	81	Possibly fungivorous; found on the ventral side of the thorax of adult ants, between the coxae	H. Klömpen	This study
		Galumnidae	Unidentified	0.5 (n = 1)	1	1	Phoretic on larvae	H. Klömpen	This study
	Pseudoscorpiones	Chernetidae	<i>Chelodamus mexicolens</i> CHAMBERLIN, 1925	3.4 ± 0.15 (n = 22)	223	52	Brood predator	M. Harvey	This study
Insecta	Coleoptera	Staphylinidae	<i>Myrmigaster</i> sp. (Aleocharinae)	2.05 ± 0.02 (n = 43)	79	14	Unknown	M. Maruyama	This study
		Tenebrionidae	Unidentified (Alleculinae)	8.0 (n = 1)	1	1	Possible brood predator	TRIPLEHORN & JOHNSON (2005)	This study
	Diptera	Syrphidae	<i>Hypselosyrphus trigonus</i> HULL, 1937 (Microdontinae)	6.2 ± 0.25 (n = 9)	193	30	Solitary ectoparasitoid of prepupae		PÉREZ-LACHAUD & al. (2014); PÉREZ-LACHAUD & LACHAUD (2017); this study
	Hymenoptera	Diapriidae	<i>Trichopria</i> sp. (Diapriinae)	2.3 ± 0.06 (n = 7)	14	4	Unknown; found exploring ant cocoons	L. Masner	This study
		Eucharitidae	<i>Kapala</i> sp. (Eucharitinae)	4.3 ± 0.12 (n = 6)	8	5	Solitary ectoparasitoid, larval-pupal	J. Heraty	This study
	Lepidoptera	Riodinidae	Unidentified	9.0 (n = 1)	1	1	Brood predator	L.A. Kaminski	This study
<b>Fungi, Ascomycota</b>									
Sordariomycetes	Hypocreales	Ophiocordy- cipitaceae	<i>Ophiocordyceps</i> sp.	38.0 (n = 1)	1	1	Parasitic on adults	J. Arau	This study

5.6% of the pupae at the population level, with a mean parasitism rate of  $26.3 \pm 4.2\%$  cocoons per parasitized colony (range: 1.6 - 75%, n = 30). The parasitoid fly develops within the protecting space of the host cocoons, rapidly consuming its host and then pupating (Fig. 4A - F). In six parasitized cocoons containing early developmental stages of the parasitoid, the ant host had already pupated and was in a state of advanced development (Fig. 4C). Both worker and sexual castes of the host were attacked (178 worker, 12 male and 3 gyne pupae were found parasitized). The syrphid fly attacked significantly less gyne pupae than the number expected according to the availability of this caste ( $\chi^2 = 16.18$ , d.f. = 2,  $P < 0.001$ ). Six hosts were superparasitized (parasitized by more than one parasitoid first-instar larva): four hosts had two first instar larvae and two other hosts had three and four fly larvae, respectively; however, in all cases only a single syrphid adult developed per host.

Both parasitoid species co-occur in the same host population. Globally, 41.5% (34 / 82) of the *Neoponera villosa* colonies were parasitized by one or the other parasitoid, with 5.8% (201 / 3463 cocoons) of all the cocoons being parasitized and consequently being lost for the host population. In only a single colony, concurrent parasitism by both larval-pupal parasitoids (the wasp and the fly) was

observed. The probability of a colony being parasitized was influenced by the number of available cocoons (Logistic binomial regression,  $Z = 2.06$ , d.f. = 1,  $P < 0.05$ ) and by temperature ( $Z = -2.10$ ,  $P < 0.05$ ), but not by precipitation ( $Z = 1.21$ ,  $P > 0.05$ ). There was a significant positive correlation between the number of parasitized cocoons and the number of available cocoons in parasitized colonies ( $r = 0.31$ ,  $t = 1.75$ , d.f. = 31,  $P < 0.05$ , Fig. S1A).

**Brood predators.** On a single occasion, a late instar riodinid larva (Lepidoptera) was found within the core of the nest, in direct contact with the brood, appearing to feed on the host larvae (Tab. 2). The specimen did not pupate and soon died. The details concerning this association are presented in a companion paper and are not further discussed.

A second brood predator referred to a species of *Chelodamus* CHAMBERLIN, 1925 (Pseudoscorpiones: Chernetidae). This genus has been previously reported as inhabiting *Aechmea bracteata* (BEUTELSPACHER BAIGTS 1999). In the nests of *Neoponera villosa*, individuals of *Chelodamus mexicolens* CHAMBERLIN, 1925 were observed unequivocally feeding on ant larvae (Fig. 5A, B). This furtive predator was far more common than the riodinid caterpillar as a total of 223 specimens (adults and im-

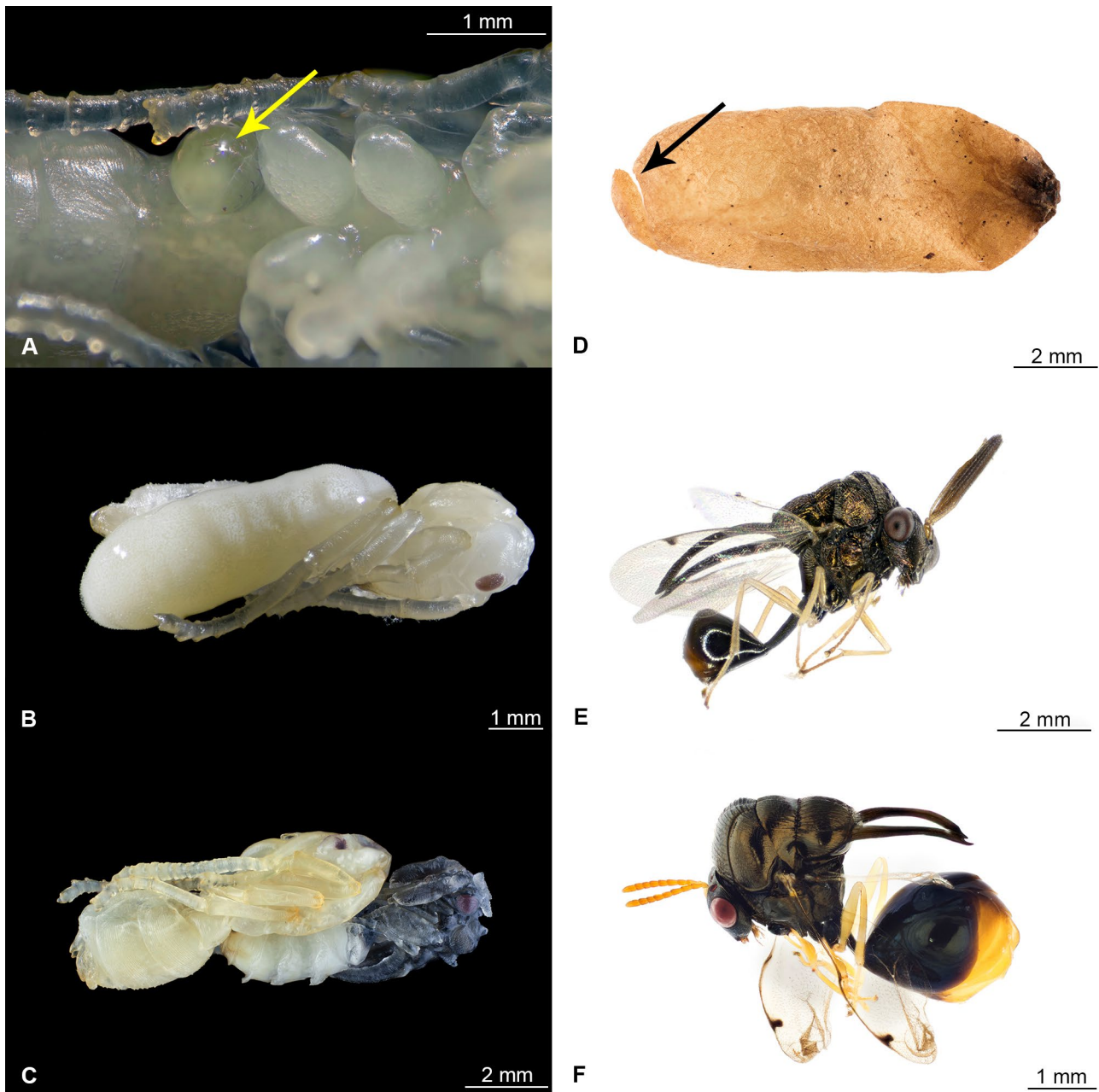


Fig. 3: The ectoparasitoid eucharitid wasp *Kapala* sp. (A) Second larval instar (arrow) under the third coxa of a host pupa. (B) Third larval instar upon a host pupa. (C) A *Kapala* sp. male pupa and the host remains. (D) Operculum made by the wasp (arrow) on emergence from the host cocoon. (E) Male, lateral view. (F) Female, lateral view. (A) - (C): the host cocoon was removed to show the developing parasitoid.

matures, including a female carrying developing embryos) were found in 80% of the colonies in a subsample (52 / 65) inspected for this interaction. On average, colonies with pseudoscorpions contained  $4.3 \pm 0.5$  individuals per nest (range 1 - 16,  $n = 52$ ). The number of pseudoscorpions was positively correlated with the number of available larvae and pupae in occupied colonies ( $r = 0.44$ ,  $t = 3.46$ ,  $d.f. = 50$ ,  $P < 0.05$ ; and  $r = 0.52$ ,  $t = 3.78$ ,  $d.f. = 50$ ,  $P < 0.05$ , respectively; Fig. S1B - C), but no pattern of occupancy was observed according to abiotic parameters or season.

Finally, a single beetle pupa was found among the cocoons and could be reared up to the adult stage and de-

termined as belonging to the Tenebrionidae (Alleculinae) (Fig. 5C, D). The location of the pupation site suggests a predatory diet upon *Neoponera villosa* larvae for the larva of this tenebrionid, although its actual behavior could not be observed directly.

**Cleptoparasites.** Mites of a species of the genus *Cosmolaelaps* BERLESE, 1903 (Mesostigmata: Laelapidae) (Fig. 6A) were found solitarily or in groups upon the ventral side of ant larvae, apparently feeding on the proteinaceous food remains provided by workers. This abundant cleptoparasitic mite was found in 91.3% of the colonies containing larvae (73 / 80), with mites present in all larvae in some



Fig. 4: The ectoparasitoid syrphid fly *Hypselosyrphus trigonus*. (A) First-instar larva. (B) First-instar larva (arrow) upon a *N. villosa* larva. (C) First-instar larva (arrow) upon a *N. villosa* pupa. Note the advanced developmental stage of the host pupa. (D) Puparium inside the host cocoon. (E) Puparium. (F) Female, dorsal view. (A) - (C), (E): the host cocoon was removed to show the developing parasitoid.

colonies and sporadically on eggs, in spite of the hygienic and defensive behaviors of the host workers; however, their prevalence among host larvae and the number of mites per larva were not quantified. As all other ponerine ants, workers of *Neoponera villosa* permanently supply their nests with invertebrate prey which are allocated to brood development (LACHAUD & al. 1984; PÉREZ-BAUTISTA & al. 1985; DEJEAN & CORBARA 1990). Adults and immatures of *Cosmolaelaps* were always found close to the host mouthparts, where workers deposited prey. By stealing food collected by foragers outside the nest, cleptoparasites affect the food provisioning rate, which is increased, and

probably impose high costs on ant colonies (FRANKS & al. 1991). It is worth noting, however, that several species of *Cosmolaelaps* reported as associated with ponerine ants in Brazil, including *N. villosa* (LOPES & al. 2015a, b), have been suspected to be phoretic on brood, and the cleptobiotic behavior of the species found in our nests might not be their sole interaction with *N. villosa*.

Scavenger and phoretic mites. Mites from three Acari orders were phoretic on ants. A species of Galumnidae (Sarcoptiformes: Oribatida) was found attached to a larva on a single occasion (Fig. 6B), and individuals of a species of *Oplitis* BERLESE, 1884 (Mesostigmata:



Oplitidae) were found attached to workers in 11% of the colonies (9 / 82) and occasionally to larvae in four of these colonies (Fig. 6C). Finally, a very common species belonging to the Scutacaridae family (Trombidiformes: Prostigmata) was found attached to the ventral side of adult ants (both workers and sexual forms) between the coxae, a place where mites cannot be easily dislodged by ants during allogrooming (Fig. 6D). These mites were present in almost all of the colonies sampled (98.8%, n = 82). In a subsample of 19 colonies examined in detail, mites were present in 95% of the colonies and in 60% of the adults (984 / 1641) including workers, queens and males. The mean colony infestation rate was 51.6% (proportion of individuals infested by scutacarid mites per infested colony; range: 0 - 83.3%) with an average of  $3.53 \pm 0.11$  mites per individual adult ant (range: 0 - 19 mites). According to KHAUSTOV (2008), probably all species of this family are scavengers, feeding on fungi, but whether they provide any benefice or harm ants in anyway is unknown and mere phoresy cannot be excluded.

**Pathogenic fungi.** A dead worker attacked by a fungus of the genus *Ophiocordyceps* PETCH, 1931 (Ophiocordycipitaceae) (Fig. 6E) was found in the inner refuse pile of a queenless colony in bad condition, with very few workers still present. No other case of such an attack was ever observed.

**Unknown myrmecophilous interactions.** Two insect myrmecophiles were found exploring the cocoons, though the exact nature of their relationship with their host could not be revealed. In four colonies, female wasps of a species of *Trichopria* ASHMEAD, 1893 (Hymenoptera: Diapriidae: Diapriinae) (Fig. 6F, Fig. S2) were observed wandering on the cocoons. Their antennae were in direct contact with the cocoon surface in a definite searching behavior (9, 2, 1, and 2 females observed, respectively), although egg laying was not observed.

Similarly, a new species (M. Maruyama, pers. comm.) of myrmecophilous beetle, belonging to the genus *Myrmigaster* SHARP, 1876 (Coleoptera: Staphylinidae) (Fig. 6G, H, Fig. S2) was found exploring the cocoons in 17.1% of the colonies (14 / 82). In some instances, they were found in large numbers (mean number of beetles per infested colony:  $5.6 \pm 2.5$ ; range: 1 - 37, n = 14). Both the wasps and the beetles persisted in their searching behavior over the cocoons when the latter were manipulated and isolated in petri dishes (Video S1). Most Dapriinae are endoparasitoids of Diptera or more rarely of Coleoptera (MASNER & GARCÍA 2002), and a few species parasitize ant larvae (LACHAUD & PASSERA 1982, LOIÁCONO & al. 2013). Although oviposition and development of the wasps was not observed, this species might parasitize other ant parasitoids within the host cocoons, most probably the syrphid fly *Hypselosyrphus trigonus* which was also present in two out of the four colonies where the diapriid wasp occurred. Further research is needed to elucidate the feeding ecology of *Trichopria* sp. and *Myrmigaster* sp.

**Non-integrated associates.** Several other invertebrates were found in association with *Neoponera*

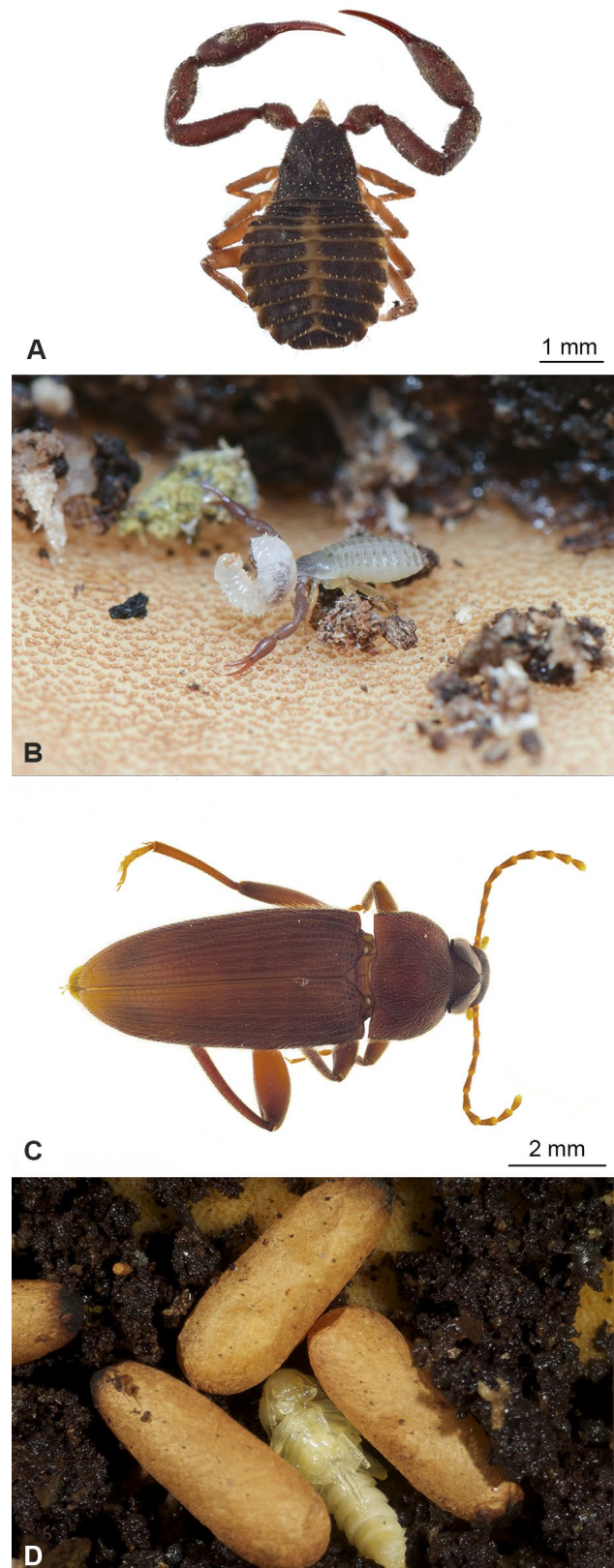


Fig. 5: Predatory myrmecophiles. (A) *Chelodamus mexicolens* (Pseudoscorpiones: Chernetidae). (B) Same species feeding on a *Neoponera villosa* larva. (C) An unidentified species of Tenebrionidae (Alleculinae). (D) Same species; pupa among ant cocoons.



Fig. 6: *Neoponera villosa* myrmecophiles. (A) *Cosmolaelaps* sp. (Acari: Laelapidae). (B) Unidentified species of a galumnid mite on a *N. villosa* larva. (C) *Oplitis* sp. phoretic on adults. (D) Unidentified species of a scutacarid mite. (E) Host worker parasitized by a species of *Ophiocordyceps* fungus. (F) *Trichopria* sp. (Hymenoptera: Diapriidae). (G) *Myrmigaster* sp. (Coleoptera: Staphylinidae). (H) *Myrmigaster* sp. exploring a cocoon. Arrows point at the myrmecophiles.

Tab. 3: Arthropods found within the nests of *Neoponera villosa*, at the periphery of the central brood chambers or in the inner refuse pile, but not closely integrated with the brood or the adults. Size and number of specimens of each taxon and number of colonies occupied are provided. When known, the type of possible interaction with their host or their trophic level is reported; biological studies or works that provide summaries of the known biology are listed: <sup>1</sup> JAŁOSZYŃSKI & OLSZANOWSKI (2015); <sup>2</sup> WOJCIK & NAVES (1992); <sup>3</sup> ISHII & YAMAOKA (1982); <sup>4</sup> CASTAÑO-MENESES & al. (2015b); <sup>5</sup> BELL & al. (2007); <sup>6</sup> KISTNER (1982); <sup>7</sup> HÖLDOBLER & KWAPICH (2017); <sup>8</sup> ASHE & KISTNER (2005); <sup>9</sup> IRMLER (2010); <sup>10</sup> BETZ & al. (2018); <sup>11</sup> BEUTELSPACHER BAIGTS (1999); <sup>12</sup> PARK (1942); <sup>13</sup> PARK (1947); <sup>14</sup> MOLLEMAN & WALTER (2001); <sup>15</sup> ROTHERAY & al. (2007); <sup>16</sup> ROSS (2000); <sup>17</sup> MARIÑO P. (1994); <sup>18</sup> MACKAY & MACKAY (2002); <sup>19</sup> ROEDER & al. (2018); <sup>20</sup> MURAKAMI & HIGASHI (1997); <sup>21</sup> BLÜTHGEN & al. (2000); <sup>22</sup> SARNAT & al. (2015); <sup>23</sup> DAROCHA & al. (2015); <sup>24</sup> LONGINO & FERNÁNDEZ (2007); <sup>25</sup> MOLERO-BALTANÁS & al. (2017); <sup>26</sup> ARAUJO & al. (1996). The column “Identification” refers to the taxonomist who identified the taxon or to the resources used by the authors. SEM = standard error of the mean.

Class	Order	Family	Subfamily	Taxon	Size (mm) (mean ± SEM)	Number of specimens	Number of colonies	Known relationships	Identification	
Arachnida	Acari (Oribatida and Mesostigmata)	Unidentified	Unidentified	Unidentified (at least 3 spp.)	< 0.5	Not estimated	57	Soil mites, potential prey for Seydmaniae <sup>1</sup>	WALTER & PROCTOR (2013)	
Diplopoda	Polydesmida	Aphelidesmidae	Unidentified	Unidentified	3.7 ± 0.21 (n = 7)	9	2	Often collected in ant nests as potential commensals or scavengers <sup>2</sup> , including arboreal ant nests <sup>3</sup>	BUENO-VILLEGAS & al. (2004)	
Entognatha	Collembola	Unidentified	Unidentified	Unidentified (at least 2 spp.)	1.0 - 2.0	Not estimated	57	Detritivorous <sup>4</sup>	TRIPLEHORN & JOHNSON (2005)	
Insecta	Archaeognatha	Unidentified	Unidentified	Unidentified	3.2 (n = 1)	1	1	Detritivorous	TRIPLEHORN & JOHNSON (2005)	
	Blattodea	Unidentified	Unidentified	Unidentified	4.5 ± 0.1 (n = 2)	4	4	Omnivorous or scavengers. Obligate myrmecophile species are apterous <sup>5</sup>	TRIPLEHORN & JOHNSON (2005)	
	Coleoptera	Histeridae	Unidentified	Unidentified	Unidentified	1.0 ± 0.02 (n = 35)	98	23	Predaceous; some species feed on ant prey or beg for food (trophallaxis) <sup>6</sup>	TRIPLEHORN & JOHNSON (2005)
						1.4 ± 0.02 (n = 8)	8	6	Scavengers or fungivorous. <i>Amphotis marginata</i> is a cleptoparasite of <i>Lastus fuliginosus</i> <sup>7</sup> (LATREILLE, 1798)	TRIPLEHORN & JOHNSON (2005)
		Ptiliidae	Unidentified	Unidentified	< 1.0	3	2	Fungivorous; found in refuse piles in ant nests <sup>6</sup>	TRIPLEHORN & JOHNSON (2005)	
		Staphylinidae	Aleocharinae	<i>Myrmigaster</i> sp. 2	3.7 ± 0.04 (n = 38)	85	23	Generalist predator <sup>8</sup>	M. Maruyama	
	Osoriinae		<i>Thoracophorus</i> sp.	2.4 (n = 1)	2	2	Fungivorous; at least two neotropical species associated with termites. Association with canopy ants is suspected <sup>9</sup>	M. Maruyama		
	Paederinae		Unidentified sp. 1	2.9 ± 0.05 (n = 23)	30	13	Generalist predators in decaying plant material; common in damp habitats <sup>10</sup>			
			Unidentified sp. 2	3.5 (n = 1)	1	1				
			Unidentified sp. 3	5.2 (n = 1)	1	1		M. Maruyama		
Pselaphinae	<i>Oxarthrus</i> sp. (Batisini)		2.0 ± 0.04 (n = 15)	15	12	Litter dwelling, predaceous; occurs in <i>A. bracteata</i> bromeliads <sup>11</sup> ; <i>O. attaphilus</i> BRUCH, 1933 associated with <i>Atta sexdens</i> <sup>12</sup> (LINNAEUS, 1758)	ASENJO & al. (2018)			
	<i>Rhytus</i> sp. (Arhytodini)	1.7 (n = 1)	1	1	Generalist predators <sup>13</sup> ; <i>R. myrmecophilus</i> is associated with <i>W. auropunctata</i> <sup>12</sup>	M. Maruyama				
<i>Tyropsis</i> sp. (Tyrini)	2.5 ± 0.08 (n = 21)	32	20	Generalist predators; litter dwelling <sup>12</sup>	M. Maruyama					

Class	Order	Family	Subfamily	Taxon	Size (mm) (mean ± SEM)	Number of specimens	Number of colonies	Known relationships	Identification
Insecta	Coleoptera	Staphylinidae	Pselaphinae	Unidentified	1.2 - 1.3	2	2		
			Seydmaeninae	<i>Euconnus</i> sp.	1.2 ± 0.05 (n = 10)	15	7	Mite and springtail predators; scavenger on dead ants <sup>14</sup>	FERRO & al. (2015)
	Diptera	Syrphidae	Eristalinae	<i>Copestylum</i> sp.	9.9 ± 0.21 (n = 5)	5 (larvae)	2	Larvae develop upon decomposing plant material <sup>15</sup>	G. Rotheray
			Unidentified	Unidentified			2	2	? (poorly preserved)
	Embioptera	Unidentified	Unidentified	Unidentified	4 - 4.9	2	1	Nymphs and adult females are herbivorous, feeding on leaf litter, moss, bark and lichen <sup>16</sup> . Some species have been found in bromeliads ( <i>Tillandsia</i> ) <sup>17</sup>	TRIPLEHORN & JOHNSON (2005)
	Hymenoptera	Formicidae	Dolichoderinae	<i>Forelius pruinosus</i> (ROGER, 1863)	1.8 ± 0.06 (n = 9)	17 adults + brood	1	Omnivorous <sup>18</sup> ; a termophilic species <sup>19</sup>	J.-P. Lachaud
			Myrmicinae	<i>Cyphomyrmex rimosus</i> (SPINOLA, 1851)	2.0 ± 0.03 (n = 6)	6 adults	1	Feed on the yeast fungus cultivated in their gardens and on the nectar and sap of plants <sup>20</sup> ; found nesting in bromeliads <sup>21</sup>	J.-P. Lachaud
				<i>Pheidole flavens</i> ROGER, 1863	minors: 1.2 ± 0.02 (n = 12) majors: 2.0 ± 0.09 (n = 3)	133 adults + brood	3	Predators or scavengers on other arthropods; nests in the soil <sup>22</sup> and also in bromeliads in the canopy <sup>23</sup>	J.-P. Lachaud
				<i>Wasmannia auropunctata</i> (ROGER, 1863)	1.4 ± 0.02 (n = 15)	277 adults + brood	3	Omnivorous scavengers and predators; nests are almost anywhere, including epiphytes <sup>24</sup>	J.-P. Lachaud
			Ponerinae	<i>Hypoponera opactor</i> (FOREL, 1893)	3.8 (n = 1)	1 queen	1	Predator; nests under stones but also in bromeliads <sup>23</sup>	J.-P. Lachaud
Zygentoma	Nicoletiidae	Unidentified	Unidentified	4.5 ± 0.26 (n = 8)	15	11	Some species are ant cleptoparasites/commensals <sup>25</sup>	TRIPLEHORN & JOHNSON (2005)	
Malacostraca	Isopoda (Oniscidea)	Unidentified	Unidentified	Unidentified	4 - 4.5 mm	4	3	Detritivorous; leaf litter decomposers; some species found in bromeliads <sup>26</sup>	TRIPLEHORN & JOHNSON (2005)

*villosa* within the central part of the nest, but without direct contact with the host and occupying the base or periphery of the nest chambers where some debris accumulated. These associates were not really integrated into the colony and included several very small scavengers and refuse dwellers (springtails, silverfishes, some other mite species) and predators (clown beetles, rove beetles). For most of these species little is known about the type of interaction with the ants, if any. As a consequence, and because specimens could not be identified to the species level, we only provide a list of records and some information according to the taxonomic group to which they belong (Tab. 3).

Coleoptera (mainly rove beetles, Figs. 7, 8) and Hymenoptera (other ant species, Fig. 9) were the orders more

represented in our samples in terms of number of morphospecies, with Staphylinidae (eleven taxa) representing 73.3% of beetles. Staphylinids are also the most common beetle family associated with nests of temperate ant species (e.g., PÄIVINEN & al. 2002, 2003, 2004, ROBINSON & ROBINSON 2013). In our samples, most rove beetles belong to genera known to be generalist predators but at least one species is fungivorous and an unidentified species of *Euconnus* belongs to a group known to prey on mites and springtails (present in most of the colonies), or to scavenge on dead ants (Tab. 3). Their occurrence in the core of *Neoponera villosa* nests could be related to the waste found at the base of the nest chambers and the presence of potential prey associated with these refuse piles. How-



Fig. 7: Not integrated guests. Diversity of rove beetles encountered in the refuse pile within the nest. (A) *Myrmigaster* sp. 2 (Aleocharinae). (B) *Thoracophorus* sp. (Osoriinae). (C) - (D) Unidentified Paederinae.

ever, predation on adult ants and brood by staphylinids has been reported on various occasions (DONISTHORPE 1927, RETTENMEYER & al. 2011, MATHIS & TSUTSUI 2016, PARMENTIER & al. 2016a, PARKER 2016), and such an interaction cannot be discarded here. Direct feeding tests and stable isotope analyses demonstrated numerous trophic interactions among myrmecophiles associated with *Formica* wood ants (intraguild predation), and confirmed that most staphylinids prey on ant brood, at least facultatively (PARMENTIER & al. 2016a). As far as Formicidae are concerned, large groups of workers along with numerous brood of *Wasmannia auropunctata* (ROGER, 1863), *Forelius pruinosus* (ROGER, 1863) and *Pheidole flavens* ROGER, 1863, all species with a small body size range (Fig. 9), nested in the same chambers as *N. villosa*, apparently without aggressive interactions. This seems to be a common pattern as small ants may take advantage of different nesting resources (bark interstices) while larger ants like *N. villosa* use the main cavity of cocoa pods, for example (CASTAÑO-MENESES & al. 2019). Moreover, as shown for several myrmecophilous beetles (PARMENTIER & al. 2014, 2016b) and the isopod *Platyarthrus hoffmannseggii* BRANDT, 1833 (PARMENTIER & al. 2017), small size and slow movement seemingly contribute to the evasion of ant aggression: Myrmecophiles much smaller than their host are mainly being ignored while those matching the host size are attacked. Apart from adaptations to the host colony

life, such as those of some very specialized and integrated myrmecophiles (chemical, morphological, acoustical or behavioral mimicry), there is some support for traits such as small body size range, morphological / anatomical defenses (heavily sclerotized integument, convex or limuloid body form), or behavioral responses that permit rapid escape, as factors allowing poorly integrated myrmecophiles to intrude within ant colonies and withstand ant attack (PARKER 2016, VON BEEREN & al. 2018, PÉREZ-LACHAUD & al. 2019 a,b), at least temporarily. Most myrmecophiles in our study, as well as some less integrated, peripheral nest associates, including mites, ants nesting in the same chambers as *N. villosa*, *Myrmigaster* sp., *Thrichopria* sp., and most rove beetles, are small sized arthropods, less than 4 mm length. Other myrmecophiles interact with ants only for a short time, and rely on furtive behavior (pseudoscorpions) or spend most of their development inside the host cocoons (pupal parasitoids), all strategies against which the host ant defenses seem ineffective.

#### Species richness of associated invertebrates:

The species accumulation curve for specialized myrmecophiles attained an asymptote according to Chao2 but not according to the other estimators (Fig. 10A). Likewise, the global species accumulation curve including all the species found in the central part of the nest (myrmecophiles and not integrated associates; Fig. 10B), did not reveal a tendency to reach an asymptote. The Chao2 estimator gave a



Fig. 8: Not integrated guests. Diversity of rove beetles encountered in the refuse pile within the nest (continued). (A) Unidentified Paederinae. (B) *Rhytus* sp. (formerly *Arhytodes*) (Pselaphinae). (C) *Tyropsis* sp. (Pselaphinae). (D) *Oxarthrius* sp. (Pselaphinae). (E) Unidentified Pselaphinae. (F) *Euconnus* sp. (Scydmaeninae).

total estimated richness of 58 species, while the observed richness was 36 species. This suggests that our systematic sampling, though performed over a period of three years, was insufficient and that additional sampling would yield more associated species. For example, we failed to recover the encyrtid wasp *Blanchardiscus* sp., a specific gregarious endoparasitoid of *Neoponera villosa* male pupae, previously collected in Zoh-Laguna in 1999 (PÉREZ-LACHAUD & LACHAUD 2017), one of our study sites. Though *N. villosa* male pupae were available in 28% of the colonies sampled (23 / 82), no evidence was found about new cases of this association. The caste specificity of this parasitoid and

the very low abundance of the target caste (mean number of male pupae in colonies producing this caste:  $8.6 \pm 2.1$ , range: 1 - 43,  $n = 23$ ) might have been decisive in failing to recover this parasitoid species.

**Conclusions:** Contrary to the expectation of low myrmecophile diversity in aggressive ants with relatively small sized colonies such as those of ponerine ants, our results demonstrated that the colonies of *Neoponera villosa* nesting in *Aechmea bracteata* bromeliads host a very diverse assemblage of myrmecophiles, some of them being highly specific and showing remarkable adaptations to the life of the colony. With the exception of the parasitoid syrphid



Fig. 9: Relative size of ant species found as complete (or partial) colonies at the periphery of the nest chambers of *Neoponera villosa*. (A) The host, *N. villosa*. The guests: (B) *Forelius pruinosus*, (C) *Pheidole flavens*, (D) *Wasmannia auropunctata*. Note the small size of the ant guests by comparison with their host.

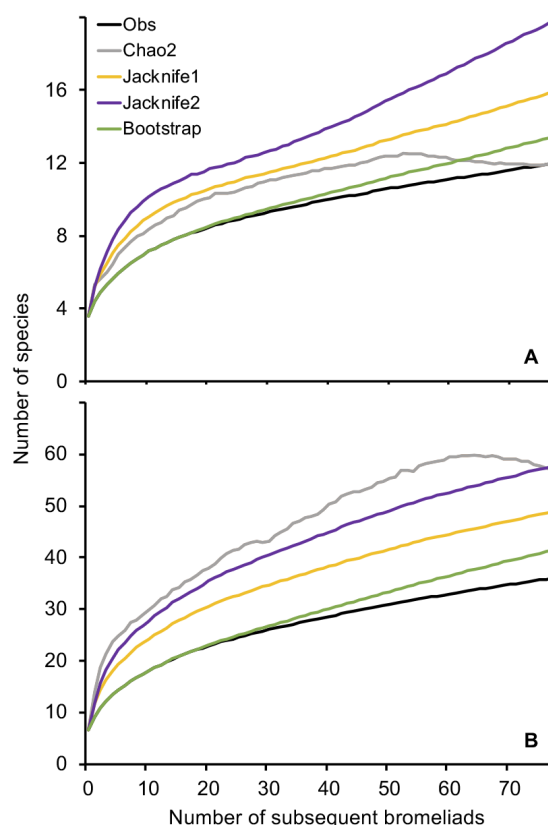


Fig. 10: Species accumulation curves showing number of taxa associated with *Neoponera villosa* ants. (A) True myrmecophiles. (B) Curves for all taxa (both myrmecophiles and not integrated associates) found inside the nest chambers. Graphs show observed species richness and estimated species richness calculated with several estimators.

fly *Hypselosyrphus trigonus*, an association previously reported (PÉREZ-LACHAUD & al. 2014, PÉREZ-LACHAUD & LACHAUD 2017), all of the other associations reported here are new to science, including new species awaiting description (*Kapala* sp., *Myrmigaster* sp.), the first association of a riodinid larva (possibly a brood predator) with ponerine ants, and the presence of furtive pseudoscorpion predators in the core of the nest. These associates are involved in a complex web of interactions.

It is commonly assumed that the greatest diversities of myrmecophiles are found in those ant species that form exceptionally large mature colonies such as army ants (HÖLDOBLER & WILSON 1990, RETTENMEYER & al. 2011, KRONAUER & PIERCE 2011). However, we here contend that small ponerine ant societies can also host a wide variety of symbionts as previously shown for ectaheteromorph ants of the genus *Ectatomma* which also have small colonies (e.g., LACHAUD & PÉREZ-LACHAUD 2015). Most interactions occurring in the core of the nest were with antagonists (parasitoids, predators, parasites, cleptoparasites) which were found in very low abundance, as some of the taxa were recorded as single specimens or only from a single nest. According to HUGHES & al. (2008), specialized parasites of long-lived insect societies will tend to be less damaging

than those associated with non-social hosts, because the enemy-free, homeostatic colony life will tend to reduce virulence, turning parasites into chronic symbionts inflicting only moderate damage. Habitat fragmentation and habitat loss threaten all arboreal ants (GUÉNARD & al. 2012, PÉREZ-LACHAUD & LACHAUD 2014), although globally no tropical ant has been classified as vulnerable or endangered. The low abundance and the high specificity of some of the myrmecophiles found in this study render them yet more vulnerable to these threats.

The genus *Neoponera* has a Neotropical distribution with 54 species recognized (SCHMIDT & SHATTUCK 2014), of which eleven are reported in Mexico (VÁZQUEZ-BOLAÑOS 2015). As with all other poneromorphs, *Neoponera* ants have been little studied with respect to the biota associated with their colonies (but see CASTAÑO-MENESES & al. 2019). Most of the species of this genus are arboreal ants (SCHMIDT & SHATTUCK 2014) and remain poorly documented, probably due to the difficulty in accessing nests in the forest canopy. A review of published literature resulted in a very low number of myrmecophiles known for only 7 of the 54 species in this genus (Tab.S2); most available records consist of incidental observations. To our knowledge, our study is the first attempt to provide a detailed list of invertebrates associated with a ponerine ant based on a thorough search of associated organisms and recording of their interactions. *Neoponera villosa* and *Aechmea bracteata* have almost the same biogeographic distribution, sharing a long evolutionary history seemingly reflected in the innate attraction of the workers to this plant (DEJEAN 1990; F.H. Rocha, J.-P. Lachaud, Y. Hénaut, C. Pozo & G. Pérez-Lachaud, unpubl.). This shared evolutionary history may have provided the grounds for the emergence of a network of *N. villosa* associates exclusive to the microcosm of the tank bromeliad.

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### References

- AKINO, T. 2008: Chemical strategies to deal with ants: a review of mimicry, camouflage, propaganda, and phytomimesis by ants (Hymenoptera: Formicidae) and other arthropods. – *Myrmecological News* 11: 173-181.
- ARAUJO, P.B., BUCKUP, L. & BOND-BUCKUP, G. 1996: Isópodos terrestres (Crustacea, Oniscidea) de Santa Catarina e Rio Grande do Sul, Brasil. – *Iheringia, Série Zoologia, Porto Alegre* 81: 111-138.
- ASENJO, A., ZAMPAULO, R.A. & LOPES FERREIRA, R. 2018: Two new troglitic species of *Oxarthrius* REITTER (Coleoptera, Staphylinidae, Pselaphinae) from Brazil. – *Zootaxa* 4462: 404-414.
- ASHE, J.S. & KISTNER, D.H. 2005: Revision of the myrmecophilous genus *Myrmigaster*, with new synonymy and descriptions of new species (Coleoptera: Staphylinidae: Aleocharinae). – *Sociobiology* 45: 221-254.
- ATSATT, P.R. 1981: Lycaenid butterflies and ants: selection for enemy-free space. – *The American Naturalist* 118: 638-654.
- BAGNÈRES, A.-G. & LORENZI, M.C. 2010: Chemical deception/mimicry using cuticular hydrocarbons. In: BLOMQUIST, G.J. & BAGNÈRES, A.-G. (Eds.): *Insect hydrocarbons: biology, biochemistry, and chemical ecology*. – Cambridge University Press, New York, NY, pp. 282-324.
- BELL, W.J., ROTH, L.M. & NALEPA, C.A. 2007: Cockroaches: ecology, behavior, and natural history. – The Johns Hopkins University Press, Baltimore, MD, 230 pp.
- BENZIG, D.H. 1990: Vascular epiphytes. General biology and related biota. – Cambridge University Press, Cambridge, UK, XVII + 354 pp.
- BETZ, O., IRMLER, U. & KLIMASZEWSKI, J. (Eds.) 2018: *Biology of rove beetles (Staphylinidae): life history, evolution, ecology and distribution*. – Springer, Cham, Switzerland, 351 pp.
- BEUTELSPACHER BAIGTS, C.R. 1999: Bromeliáceas como ecossistemas: con especial referencia a *Aechmea bracteata* (SWARTZ) GRISEB. – Plaza y Valdés, México D.F., México, 123 pp.
- BLÜTHGEN, N., VERHAAGH, M., GOITÍA, W. & BLÜTHGEN, N. 2000: Ant nests in tank bromeliads – an example of non-specific interaction. – *Insectes Sociaux* 47: 313-316.
- BROUARD, O., CÉRÉGHINO, R., CORBARA, B., LEROY, C., PELOZUELO, L., DEJEAN, A. & CARRIAS, J.-F. 2012: Understorey environments influence functional diversity in tank-bromeliad ecosystems. – *Freshwater Biology* 57: 815-823.
- BROWN, B.V. 2000: Revision of the "*Apocephalus miricauda*-group" of ant-parasitizing flies (Diptera: Phoridae). – *Contributions in Science* 482: 1-62.
- BUENO-VILLEGAS, J., SIERWALD, P. & BOND, J.E. 2004: Diplopoda. In: LLORENTE, B.J., MORRONE, J.J., YÁÑEZ, O. & VARGAS, I. (Eds.): *Biodiversidad, taxonomía y biogeografía de artrópodos de México: hacia una síntesis de su conocimiento*, Vol. IV. – Facultad de Ciencias UNAM, México D.F., México, pp. 569-599.
- CASTAÑO-MENESES, G., MARIANO, C.S.F., ROCHA, P., MELO, T., TAVARES, B., ALMEIDA, E., DA SILVA, L., PEREIRA, T.P.L. & DELABIE, J.H.C. 2015a: The ant community and their



- accompanying arthropods in cacao dry pods: an unexplored diverse habitat. – *Dugesiana* 22: 29-35.
- CASTAÑO-MENESES, G., PALACIOS-VARGAS, J.G. & CARMO, A.F.R. 2015b: Colêmbolos e outros inquilinos de formigueiros de poneromorfas. In: DELABIE, J.H.C., FEITOSA, R.M., SERRÃO, J.E., MARIANO, C.S.F. & MAJER, J.D. (Eds.): *As formigas poneromorfas do Brasil*. – Editus, Ilhéus, Brasil, pp. 389-401.
- CASTAÑO-MENESES, G., SANTOS, R.J., DOS SANTOS, J.R.M., DELABIE, J.H.C., LOPES, L.L. & MARIANO, C.S.F. 2019: Invertebrates associated to Ponerine ants nests in two cocoa farming systems in the southeast of the state of Bahia, Brazil. – *Tropical Ecology* 60: 52-61.
- CLARKE, K.R. & GORLEY, R.N. 2006: PRIMER v6: user manual/tutorial. – PRIMER-E Ltd, Plymouth, Devon, UK, 190 pp.
- CLAUSEN, C.P. 1940: The oviposition habits of the Eucharidae (Hymenoptera). – *Journal of the Washington Academy of Sciences* 30: 504-516.
- CUSHING, P.E. 1997: Myrmecomorphy and myrmecophily in spiders: a review. – *Florida Entomologist* 80: 165-193.
- DAROCHA, W.D., RIBEIRO S.P., NEVES, F.S., FERNANDES, G.W., LEPONCE, M. & DELABIE, J.H.C. 2015: How does bromeliad distribution structure the arboreal ant assemblage (Hymenoptera: Formicidae) on a single tree in a Brazilian Atlantic forest agroecosystem? – *Myrmecological News* 21: 83-92.
- DE BEKKER, C., WILL, I., DAS, B. & ADAMS, R.M.M. 2018: The ants (Hymenoptera: Formicidae) and their parasites: effects of parasitic manipulations and host responses on ant behavioral ecology. – *Myrmecological News* 28: 1-24.
- DE FREITAS, J.D. & ROSSI, M.N. 2015: Interaction between trophobiont insects and ants: the effect of mutualism on the associated arthropod community. – *Journal of Insect Conservation* 19: 627-638.
- DE LA MORA, A. & PHILPOTT, S.M. 2010: Wood-nesting ants and their parasites in forests and coffee agroecosystems. – *Environmental Entomology* 39: 1473-1481.
- DEJEAN, A. 1990: Influence de l'environnement pré-imaginal et précoce dans le choix du site de nidification de *Pachycondyla* (= *Neoponera*) *villosa* (FABR.) (Formicidae, Ponerinae). – *Behavioural Processes* 21: 107-125.
- DEJEAN, A., COMPIN, A., LEPONCE, M., AZÉMAR, F., BONHOMME, C., TALAGA, S., PELOZUELO, L., HÉNAUT, Y. & CORBARA, B. 2018: Ants impact the composition of the aquatic macroinvertebrate communities of a myrmecophytic tank bromeliad. – *Comptes Rendus Biologies* 341: 200-207.
- DEJEAN, A. & CORBARA, B. 1990: Predatory behavior of a Neotropical arboricolous ant: *Pachycondyla villosa* (Formicidae: Ponerinae). – *Sociobiology* 17: 271-286.
- DEJEAN, A. & OLMSTED, I. 1997: Ecological studies on *Aechmea bracteata* (SWARTZ) (Bromeliaceae). – *Journal of Natural History* 31: 1313-1334.
- DEJEAN, A., OLMSTED, I. & CAMAL, J.F. 1992: Interaction between *Atta cephalotes* and arboreal ants in the biosphere reserve Sian Ka'an (Quintana Roo, Mexico): efficient protection of the trees (Hymenoptera: Formicidae). – *Sociobiology* 20: 57-76.
- DEJEAN, A., OLMSTED, I. & SNELLING, R.R. 1995: Tree-epiphyte-ant relationships in the low inundated forest of Sian Ka'an biosphere reserve, Quintana Roo, Mexico. – *Biotropica* 27: 57-70.
- DELABIE, J.H.C. 2001: Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an overview. – *Neotropical Entomology* 30: 501-516.
- DÉZERALD, O., LEROY, C., CORBARA, B., CARRIAS, J.-F., PÉLOZUELO, L., DEJEAN, A. & CÉRÉGHINO, R. 2013: Food-web structure in relation to environmental gradients and predator-prey ratios in tank-bromeliad ecosystems. – *Public Library of Science One* 8: art. e71735.
- DÉZERALD, O., TALAGA, S., LEROY, C., CARRIAS, J.-F., CORBARA, B., DEJEAN, A. & CÉRÉGHINO, R. 2014: Environmental determinants of macroinvertebrate diversity in small water bodies: insights from tank-bromeliads. – *Hydrobiologia* 723: 77-86.
- DI GIULIO, A., MAURIZI, E., BARBERO, F., SALA, M., FATTORINI, S., BALLETO, E. & BONELLI, S. 2015: The pied piper: a parasitic beetle's melodies modulate ant behaviours. – *Public Library of Science One* 10: art. e0130541.
- DI SALVO, M., CALCAGNILE, M., TALÀ, A., TREDICI, S.M., MAFFEI, M.E., SCHÖNRÖGGE, K., BARBERO, F. & ALIFANO, P. 2019: The microbiome of the *Maculinea-Myrmica* host-parasite interaction. – *Scientific Reports* 9: art. 8048.
- DONISTHORPE, H.St.J.K. 1927: The guests of British ants: their habits and life-histories. – George Routledge and Sons, London, UK, XXIII + 244 pp.
- ELIZALDE, L., PATROCK, R.J.W., DISNEY, R.H.L. & FOLGARAIT, P.J. 2018: Spatial and temporal variation in host-parasitoid interactions: leafcutter ant hosts and their phorid parasitoids. – *Ecological Entomology* 43: 114-125.
- EMERY, C. 1904: Zur Kenntniss des Polymorphismus der Ameisen. – *Zoologische Jahrbücher, Supplementheft* 7: 587-610.
- FERNANDES, I.O., DE OLIVEIRA, M.L. & DELABIE, J.H.C. 2013: Notes on the biology of Brazilian ant populations of the *Pachycondyla foetida* species complex (Formicidae: Ponerinae). – *Sociobiology* 60: 380-386.
- FERRO, M.L., THAYER, M.K., NEWTON JR., A.F. & PARK, J.S. 2015: Key to North American Staphylinidae subfamilies. – <<http://keys.lucidcentral.org/keys/v3/nastaphylinidae>>, retrieved on 7 July 2019.
- FIEDLER, K. 2006: Ant-associates of Palaearctic lycaenid butterfly larvae (Hymenoptera: Formicidae; Lepidoptera: Lycaenidae) – a review. – *Myrmecologische Nachrichten* 9: 77-87.
- FIEDLER, K. 2012: The host genera of ant-parasitic Lycaenidae butterflies: a review. – *Psyche* 2012: art. 153975.
- FOWLER, H.G. 1993: Use of fallen cocoa pods by ants (Hymenoptera: Formicidae) in southeastern Brazil. – *Journal of the Kansas Entomological Society* 66: 444-446.
- FRANKS, N.R., HEALEY, K.J. & BYROM, L. 1991: Studies on the relationship between the ant ectoparasite *Antennophorus grandis* (Acarina: Antennophoridae) and its host *Lasius flavus* (Hymenoptera: Formicidae). – *Journal of Zoology, London* 225: 59-70.
- GARCÍA, E. 1973: Modificaciones al sistema de clasificación climática de Köppen, 2<sup>nd</sup> ed. – Instituto de Geografía, Universidad Nacional Autónoma de México, México D.F., México, 246 pp.
- GLASIER, J.R.N., POORE, A.G.B. & ELDRIDGE, D.J. 2018: Do mutualistic associations have broader host ranges than neutral or antagonistic associations? A test using myrmecophiles as model organisms. – *Insectes Sociaux* 65: 639-648.
- GUÉNARD, B., WEISER, M.D. & DUNN, R.R. 2012: Global models of ant diversity suggest regions where new discoveries are most likely are under disproportionate deforestation threat. – *Proceedings of the National Academy of Sciences of the United States of America* 109: 7368-7373.
- HÄRKÖNEN, S.K. & SORVARI, J. 2014: Species richness of associates of ants in the nests of red wood ant *Formica polyctena* (Hymenoptera: Formicidae). – *Insect Conservation and Diversity* 7: 485-495.
- HÖLLDOBLER, B. & KWAPICH, C.L. 2017: *Amphotis marginata* (Coleoptera: Nitidulidae) a highwayman of the ant *Lasius fuliginosus*. – *Public Library of Science One* 12: art. e0180847.

- HÖLLDOBLER, B. & WILSON, E.O. 1990: The ants. – Harvard University Press, Cambridge, MA, 732 pp.
- HUGHES, D.P., PIERCE, N.E. & BOOMSMA, J.J. 2008: Social insect symbionts: evolution in homeostatic fortresses. – *Trends in Ecology & Evolution* 23: 672-677.
- IRMLER, U. 2010: Two new species of the genus *Thoracophorus* MOTSCHULSKY, 1837 (Coleoptera: Staphylinidae, Osoriinae) with remarks on ecology of the genus in the Neotropical region. – *Psyche* 2010: art. 138518.
- ISHII, K. & YAMAOKA, H. 1982: The species and number of symbiotic penicillate millipeds in arboreal ant nests. – *The Canadian Entomologist* 114: 767-768.
- JAŁOSZYŃSKI, P. & OLSZANOWSKI, Z. 2015: Feeding of *Scydmaenus rufus* (Coleoptera: Staphylinidae: Scydmaeninae) on oribatid and uropodine mites: prey preferences and hunting behavior. – *European Journal of Entomology* 112: 151-164.
- KHAUSTOV, A.A. 2008: Mites of the family Scutacaridae of Eastern Palearctic. – *Akademperiodyka*, Kiev, 290 pp.
- KISTNER, D.H. 1982: The social insects' bestiary. In: HERMANN, H.R. (Ed.): *Social insects*. – Academic Press, New York, NY, pp. 1-244.
- KRONAUER, D.J.C. & PIERCE, N.E. 2011: Myrmecophiles. – *Current Biology* 21: R208-R209.
- LACHAUD, J.-P., CERDAN, P. & PÉREZ-LACHAUD, G. 2012a: Poneromorph ants associated with parasitoid wasps of the genus *Kapala* CAMERON (Hymenoptera: Eucharitidae) in French Guiana. – *Psyche* 2012: art. 393486.
- LACHAUD, J.-P., FRESNEAU, D. & GARCÍA-PÉREZ, J. 1984: Étude des stratégies d'approvisionnement chez 3 espèces de fourmis ponérines (Hymenoptera: Formicidae). – *Folia Entomologica Mexicana* 61: 159-177.
- LACHAUD, J.-P., KLÖMPEN, H. & PÉREZ-LACHAUD, G. 2016: *Macrodinychus* mites as parasitoids of invasive ants: an overlooked parasitic association. – *Scientific Reports* 6: art. 29995.
- LACHAUD, J.-P., LENOIR, A. & HUGHES, D.P. (Eds.) 2013: *Ants and their parasites 2013*. – *Psyche Special Issue*. Hindawi Publishing Corporation, New York, NY, 148 pp.
- LACHAUD, J.-P., LENOIR, A. & WITTE, V. (Eds.) 2012b: *Ants and their parasites 2012*. – *Psyche Special Issue*. Hindawi Publishing Corporation, New York, NY, 282 pp.
- LACHAUD, J.-P. & PASSERA, L. 1982: Données sur la biologie de trois Diapriidae myrmécophiles: *Plagiopria passerai* MASNER, *Solenopsia imitatrix* WASMANN et *Lepidopria pedestris* KIEFFER. – *Insectes Sociaux* 29: 561-567.
- LACHAUD, J.-P. & PÉREZ-LACHAUD, G. 2012: Diversity of species and behavior of hymenopteran parasitoids of ants: a review. – *Psyche* 2012: art. 134746.
- LACHAUD, J.-P. & PÉREZ-LACHAUD, G. 2015: Ectaheteromorph ants also host highly diverse parasitic communities: a review of parasitoids of the Neotropical genus *Ectatomma*. – *Insectes Sociaux* 62: 121-132.
- LENOIR, A., D'ETTORRE, P., ERRARD, C. & HEFETZ, A. 2001: Chemical ecology and social parasitism in ants. – *Annual Review of Entomology* 46: 573-599.
- LIM, G.T., KIRTON, L.G., SALOM, S.M., KOK, L.T., FELL, R.D. & PFEIFFER, D.G. 2008: Host plants and associated trophobionts of the weaver ants *Oecophylla* spp. (Hymenoptera: Formicidae). – *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 3: 1-19.
- LOIÁCONO, M.S., MARGARÍA, C.B. & AQUINO, D.A. 2013: Diapriinae wasps (Hymenoptera: Diaprioidea: Diapriidae) associated with ants (Hymenoptera: Formicidae) in Argentina. – *Psyche* 2013: art. 320590.
- LONGINO, J.T. & FERNÁNDEZ, F. 2007: Taxonomic review of the genus *Wasmannia*. In: SNELLING, R.R., FISHER, B.L. & WARD, P.S. (Eds.): *Advances in ant systematics (Hymenoptera: Formicidae): homage to E.O. Wilson – 50 years of contributions*. – *Memoirs of the American Entomological Institute* 80: 271-289.
- LOPES, J.M.S., OLIVEIRA, A.R. & DELABIE, J.H.C. 2015a: Interações formigas/ácaros, com ênfase em ácaros foréticos associados a poneromorfas. In: DELABIE, J.H.C., FEITOSA, R.M., SERRÃO, J.E., MARIANO, C.S.F. & MAJER, J.D. (Eds.): *As formigas poneromorfas do Brasil*. – Editus, Ilhéus, Brasil, pp. 375-387.
- LOPES, J.M.S., OLIVEIRA, A.R., DELABIE, J.H.C. & KLÖMPEN, H. 2015b: A new species of myrmecophile mite of the genus *Oplitis* (Acari: Mesostigmata: Oplitidae) from Brazil. – *International Journal of Acarology* 41: 676-680.
- MACKAY, W.P. & MACKAY, E.E. 2002: *The ants of New Mexico (Hymenoptera: Formicidae)*. – The Edwin Mellen Press, Lewiston, New York, NY, 408 pp.
- MACKAY, W.P. & MACKAY, E.E. 2010: *The systematics and biology of the New World ants of the genus Pachycondyla (Hymenoptera: Formicidae)*. – The Edwin Mellen Press, Lewiston, New York, NY, 642 pp.
- MARIÑO P.E. 1994: Embiópteros de México. V. Especie nueva de *Mesembia* (Embioptera: Anisembiidae). – *Anales del Instituto de Biología, UNAM, Serie Zoología* 65: 233-239.
- MARUYAMA, M., KOMATSU, T., KUDO, T., SHIMADA, T. & KINOMURA, K. 2013: *The guests of Japanese ants*. – Tokai University Press, Minamiyama, 240 pp.
- MASNER, L. & GARCÍA, J.L. 2002: The genera of Diapriinae (Hymenoptera: Diapriidae) in the New World. – *Bulletin of the American Museum of Natural History* 268: 1-138.
- MATHIS, K.A. & TSUTSUI, N.D. 2016: Dead ant walking: A myrmecophilous beetle predator uses parasitoid host location cues to selectively prey on parasitized ants. – *Proceedings of the Royal Society B-Biological Sciences* 283: art. 20161281.
- MCIVER, J.D. & STONEDAHL, G. 1993: Myrmecomorphy: Morphological and behavioral mimicry of ants. – *Annual Review of Entomology* 38: 351-379.
- MOLERO-BALTANÁS, R., BACH DE ROCA, C., TINAUT, A., DIZ PÉREZ, J. & GAJU-RICART, M. 2017: Symbiotic relationships between silverfish (*Zygentoma*: Lepismatidae, Nicoletiidae) and ants (Hymenoptera: Formicidae) in the Western Palearctic. A quantitative analysis of data from Spain. – *Myrmecological News* 24: 107-122.
- MOLLEMAN, F. & WALTER, D.E. 2001: Niche segregation and can-openers: scydmaenid beetles as predators of armoured mites in Australia. In: HALLIDAY, R.B., WALTER, D.E., PROCOR, H.C., NORTON, R.A. & COLLOFF, M.J. (Eds.): *Acarology: Proceedings of the 10<sup>th</sup> International Congress*. – CSIRO Publishing, Melbourne, pp. 283-288.
- MUELLER, U.G., SCHULTZ, T.R., CURRIE, C.R., ADAMS, R.M.M. & MALLOCH, D. 2001: The origin of the attine ant-fungus mutualism. – *The Quarterly Review of Biology* 76: 169-197.
- MURAKAMI, T. & HIGASHI, S. 1997: Social organization in two primitive attine ants, *Cyphomyrmex rimosus* and *Myrmicocrypta ednaella*, with reference to their fungus substrates and food sources. – *Journal of Ethology* 15: 17-25.
- PÄIVINEN, J., AHLROTH, P. & KAITALA, V. 2002: Ant-associated beetles of Fennoscandia and Denmark. – *Entomologica Fennica* 13: 20-40.
- PÄIVINEN, J., AHLROTH, P., KAITALA, V., KOTIAHO, J.S., SUHONEN, J. & VIROLA, T. 2003: Species richness and regional distribution of myrmecophilous beetles. – *Oecologia* 134: 587-595.

- PÄIVINEN, J., AHLROTH, P., KAITALA, V. & SUHONEN, J. 2004: Species richness, abundance and distribution of myrmecophilous beetles in nests of *Formica aquilonia* ants. – *Annales Zoologici Fennici* 41: 447-454.
- PARK, O. 1942: A study in Neotropical Pselaphidae. – Northwestern University, Evanston and Chicago, 403 pp + XXI plates.
- PARK, O. 1947: The pselaphid at home and abroad. – *The Scientific Monthly* 65: 27-42.
- PARKER, J. 2016: Myrmecophily in beetles (Coleoptera): evolutionary patterns and biological mechanisms. – *Myrmecological News* 22: 65-108.
- PARMENTIER, T., BOUILLON, S., DEKONINCK, W. & WENSELEERS, T. 2016a: Trophic interactions in an ant nest microcosm: a combined experimental and stable isotope ( $\delta^{13}\text{C}/\delta^{15}\text{N}$ ) approach. – *Oikos* 125: 1182-1192.
- PARMENTIER, T., DEKONINCK, W. & WENSELEERS, T. 2014: A highly diverse microcosm in a hostile world: a review on the associates of red wood ants (*Formica rufa* group). – *Insectes Sociaux* 61: 229-237.
- PARMENTIER, T., DEKONINCK, W. & WENSELEERS, T. 2016b: Survival of persecuted myrmecophiles in laboratory nests of different ant species can explain patterns of host use in the field (Hymenoptera: Formicidae). – *Myrmecological News* 23: 71-79.
- PARMENTIER, T., VANDERHEYDEN, A., DEKONINCK, W. & WENSELEERS, T. 2017: Body size in the ant-associated isopod *Platyarthrus hoffmannseggii* is host-dependent. – *Biological Journal of the Linnean Society* 121: 305-311.
- PÉREZ-BAUTISTA, M., LACHAUD, J.-P. & FRESNEAU, D. 1985: La división del trabajo en la hormiga primitiva *Neoponera villosa* (Hymenoptera: Formicidae). – *Folia Entomológica Mexicana* 65: 119-130.
- PÉREZ-LACHAUD, G., HERATY, J.M., CARMICHAEL, A. & LACHAUD, J.-P. 2006: Biology and behavior of *Kapala* (Hymenoptera: Eucharitidae) attacking *Ectatomma*, *Gnamptogenys* and *Pachycondyla* (Formicidae: Ectatomminae and Ponerinae) in Chiapas, Mexico. – *Annals of the Entomological Society of America* 99: 567-576.
- PÉREZ-LACHAUD, G., JERVIS, M.A., REEMER, M. & LACHAUD, J.-P. 2014: An unusual, but not unexpected, evolutionary step taken by syrphid flies: the first record of true primary parasitoidism of ants by Microdontinae. – *Biological Journal of the Linnean Society* 111: 462-472.
- PÉREZ-LACHAUD, G., KLÖMPEN, H., POTEAUX, C., SANTAMARÍA, C., ARMBRECHT, I., BEUGNON, G. & LACHAUD, J.-P. 2019a: Context dependent life-history shift in *Macrodimychus sellnicki* mites attacking a native ant host in Colombia. – *Scientific Reports* 9: art. 8394.
- PÉREZ-LACHAUD, G. & LACHAUD, J.-P. 2014: Arboreal ant colonies as “hot-points” of cryptic diversity for myrmecophiles: the weaver ant *Camponotus* sp. aff. *textor* and its interaction network with its associates. – *Public Library of Science One* 9: art. e100155.
- PÉREZ-LACHAUD, G. & LACHAUD, J.-P. 2017: Hidden biodiversity in entomological collections: the overlooked co-occurrence of dipteran and hymenopteran ant parasitoids in stored biological material. – *Public Library of Science One* 12: art. e0184614.
- PÉREZ-LACHAUD, G., ROCHA, F.H., VALLE-MORA, J., HÉNAUT, Y. & LACHAUD, J.-P. 2019b: Fine-tuned intruder discrimination favors ant parasitoidism. – *Public Library of Science One* 14: art. e0210739.
- PESSOA, W.F.B., SILVA, L.C.C., DIAS, L.O., DELABIE, J.H.C., COSTA, H. & ROMANO, C.C. 2016: Analysis of protein composition and bioactivity of *Neoponera villosa* venom (Hymenoptera: Formicidae). – *International Journal of Molecular Sciences* 17: 513.
- PIERCE, N.E., BRABY, M.F., HEATH, A., LOHMAN, D.J., MATHEW, J., RAND, D.B. & TRAVASSOS, M.A. 2002: The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). – *Annual Review of Entomology* 47: 733-771.
- POINAR, G. Jr. 2012: Nematode parasites and associates of ants: past and present. – *Psyche* 2012: art. ID192017.
- QUEVILLON, L.E. & HUGHES, D.P. 2018: Pathogens, parasites, and parasitoids of ants: a synthesis of parasite biodiversity and epidemiological traits. – *bioRxiv*: art. 384495.
- R CORE TEAM. 2017: R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria; <<http://www.R-project.org/>>, retrieved on 10 July 2019.
- RETTENMEYER, C.W., RETTENMEYER, M.E., JOSEPH, J. & BERGHOFF, S.M. 2011: The largest animal association centered on one species: the army ant *Eciton burchellii* and its more than 300 associates. – *Insectes Sociaux* 58: 281-292.
- ROBINSON, N.A. & ROBINSON, E.J.H. 2013: Myrmecophiles and other invertebrate nest associates of the red wood ant *Formica rufa* (Hymenoptera: Formicidae) in north-west England. – *British Journal of Entomology and Natural History* 26: 67-89.
- ROEDER, K.A., ROEDER, D.V. & KASPARI, M. 2018: The role of temperature in competition and persistence of an invaded ant assemblage. – *Ecological Entomology* 43: 774-781.
- ROSS, E.S. 2000: EMBIA Contributions to the biosystematics of the insect order Embiidina. Part 2. A review of the biology of Embiidina. – *Occasional Papers of the California Academy of Sciences* 149: 1-36.
- ROTHERAY, G.E., HANCOCK, E.G. & MARCOS-GARCÍA, M.A. 2007: Neotropical *Copestylum* (Diptera, Syrphidae) breeding in bromeliads (Bromeliaceae) including 22 new species. – *Zoological Journal of the Linnean Society* 150: 267-317.
- SARNAT, E.M., FISCHER, G., GUÉNARD, B. & ECONOMO, E.P. 2015: Introduced *Pheidole* of the world: taxonomy, biology and distribution. – *ZooKeys* 543: 1-109.
- SCHMID-HEMPEL, P. 1998: Parasites in social insects. – Princeton University Press, Princeton, NJ, 409 pp.
- SCHMIDT, C.A. & SHATTUCK, S.O. 2014: The higher classification of the ant subfamily Ponerinae (Hymenoptera: Formicidae), with a review of ponerine ecology and behavior. – *Zootaxa* 3817: 1-242.
- SCHÖNRÖGGE, K., BARBERO, F., CASACCI, L.P., SETTELE, J. & THOMAS, J.A. 2017: Acoustic communication within ant societies and its mimicry by mutualistic and socially parasitic myrmecophiles. – *Animal Behaviour* 134: 249-256.
- STADLER, B. & DIXON, A.F.G. 2005: Ecology and evolution of aphid-ant interactions. – *Annual Review of Ecology, Evolution, and Systematics* 36: 345-372.
- TRIPLEHORN, C.A. & JOHNSON, N.F. 2005: Borror and DeLong's introduction to the study of insects. – Thomson Brooks/Cole, Belmont, CA, 864 pp.
- TRUNZER, B., HEINZE, J. & HÖLLDOBLER, B. 1998: Cooperative colony founding and experimental primary polygyny in the ponerine ant *Pachycondyla villosa*. – *Insectes Sociaux* 45: 267-276.
- VALENZUELA-GONZÁLEZ, J., LÓPEZ-MÉNDEZ, A. & GARCÍA-BALLINAS, A. 1994: Ciclo de actividad y aprovisionamiento de *Pachycondyla villosa* (Hymenoptera: Formicidae) en agroecosistemas cacaoteros del Soconusco, Chiapas, México. – *Folia Entomológica Mexicana* 91: 9-21.
- VÁSQUEZ-BOLAÑOS, M. 2015: Taxonomía de Formicidae (Hymenoptera) para México. – *Métodos en Ecología y Sistemática* 10: 1-53.

- VON BEEREN, C., BRÜCKNER, A., MARUYAMA, M., BURKE, G., WIESCHOLLEK, J. & KRONAUER, D.J.C. 2018: Chemical and behavioral integration of army ant-associated rove beetles – a comparison between specialists and generalists. – *Frontiers in Zoology* 15: art. 8.
- WALTER, D.E. & PROCTOR, H.C. 2013: *Mites: ecology, evolution & behaviour. Life at a microscale.* – Springer, New York, NY, 494 pp.
- WASMANN, E. 1894: Kritisches Verzeichnis der myrmekophilen und termitophilen Arthropoden mit Angabe der Lebensweise und mit Beschreibung neuer Arten. – Felix L. Dames, Berlin, 231 pp.
- WAY, M.J. 1963: Mutualism between ants and honeydew-producing Homoptera. – *Annual Review of Entomology* 8: 307-344.
- WHEELER, W.M. 1908: The ants of Texas, New Mexico and Arizona. Part I. – *Bulletin of the American Museum of Natural History* 24: 399-487.
- WHEELER, W.M. 1928: *Mermis* parasitism and intercastes among ants. – *Journal of Experimental Zoology* 50: 165-237.
- WILD, A.L. 2002: The genus *Pachycondyla* (Hymenoptera: Formicidae) in Paraguay. – *Boletín del Museo Nacional de Historia Natural del Paraguay* 14: 1-18.
- WITEK, M., BARBERO, F. & MARKÓ, B. 2014: *Myrmica* ants host highly diverse parasitic communities: from social parasites to microbes. – *Insectes Sociaux* 61: 307-323.
- WITTE, V., JANSSEN R., EPPENSTEIN A. & MASCHWITZ, U. 2002: *Allopeas myrmekophilos* (Gastropoda, Pulmonata), the first myrmecophilous mollusc living in colonies of the ponerine army ant *Leptogenys distinguenda* (Formicidae, Ponerinae). – *Insectes Sociaux* 49: 301-305.
- WOJCIK, D.P. & NAVES, M.A. 1992: Biology of *Calyptodesmus sanctus* (Diplopoda: Pyrgodesmidae) a facultative myrmecophile introduced into the United States. – *Sociobiology* 20: 77-87.