El Colegio de la Frontera Sur

# Planta hospedera e invertebrados mirmecófilos asociados a la hormiga *Neoponera villosa* en la Península de Yucatán

Tesis
presentada como requisito parcial para optar al grado de
Doctor en Ciencias en Ecología y Desarrollo Sustentable
Con orientación en Conservación de la Biodiversidad

Por

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# El Colegio de la Frontera Sur

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Planta hospedera e invertebrados mirmecófilos asociados a la hormiga *Neoponera villosa* en la Península de Yucatán

para obtener el grado de **Doctor en Ciencias en Ecología y Desarrollo Sustentable**

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**Humberto Rocha ł**

y

**Ninfa Vela Carrillo**

A mi Hermana y Sobrinos

**Blanca Arlett Rocha Vela,**

**Camila Rocha**

y

**Faryd Rocha**

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## ÍNDICE DE CONTENIDO

# RESUMEN

La hormiga neotropical *Neoponera villosa* (Ponerinae) está ampliamente distribuida en la Península de Yucatán, en donde sus colonias nidifican principalmente en la bromelia-tanque *Aechmea bracteata* (Bromeliceae). Esta especie de hormiga presenta una red compleja de interacciones con otros organismos denominados mirmecófilos. Se ha señalado que la pérdida de hábitat pone en riesgo a las hormigas arborícolas y a los organismos mirmecófilos asociados. Por esta razón, nos centramos en el estudio de la diversidad de organismos asociados a los nidos de *N. villosa* en el sur de la Península de Yucatán, una región de alto riesgo de pérdida de hábitat. El estudio se enfoca en: 1) determinar la diversidad y abundancia de los mirmecófilos asociados a las colonias y nidos de *N. villosa*, 2) contribuir al conocimiento de las interacciones comportamentales entre *N. villosa* y sus organismos mirmecófilos, en particular con moscas de la familia Syrphidae, y 3) corroborar la fidelidad de las colonias de *N. villosa* hacia su planta de nidificación, lo cual podría también determinar la diversidad de organismos que interactúan con las hormigas. Entre enero de 2016 y enero de 2019 se recolectaron 84 colonias (o parte de ellas) de *N. villosa* que nidificaban en *A. bracteata*, así como cinco colonias en oquedades de árboles vivos. Se registraron todos los invertebrados presentes en las cámaras del nido y se inspeccionaron a las hormigas y sus crías bajo un estereomicroscopio para detectar la presencia de signos de parasitismo. Se determinó la historia natural de algunos de los mirmecófilos asociados. En particular, se esclareció a través de observaciones y bioensayos en el laboratorio, cómo las moscas adultas del parasitoide *Hypselosyrphus trigonus* logran escapar ilesas del cocón y nido de un hospedero tan agresivo. Usando hormigas con y sin experiencia previa sobre *A. bracteata*, se realizaron bioensayos de elección pareados para probar la preferencia de *N. villosa* por refugios hechos con hojas de *A. bracteata* sobre otras dos bromelias, *A. bromeliifolia* y *Ananas comosus*. Además, usando plantas enteras, se evaluaron algunos rasgos asociados a la bromelia (tamaño, forma) que podrían influir en la selección del sitio de nidificación. Los resultados de este estudio sistemático, muestran varios taxones asociados a las colonias y nidos de *N. villosa*, con organismos pertenecientes a 43 taxones diferentes, de los cuales al menos 12 especies fueron encontradas en asociación física directa con la cría o con los adultos de la hormiga hospedera, siendo muchas de ellas mirmecófilos obligados. Estos mirmecófilos presentaron diversas interacciones tróficas con *N. villosa*, en su mayoría interacciones antagónicas (parasitoidismo, depredación y cleptoparasitismo). También se registraron otras especies asociadas a las colonias de esta hormiga, pero sin estar íntimamente integradas a la colonia (mirmecófilos facultativos). Los resultados de bioensayos de comportamiento sugieren que para poder escapar del cocón y del nido del hospedero, el parasitoide *H. trigonus* manipula el comportamiento de las obreras posiblemente a través de los hidrocarburos u otras substancias cuticulares que empiezan a producir una vez que se ha formado el pupario. Estos puparios presentaron un perfil de hidrocarburos y otros compuestos cuticulares particulares que dispararon la conducta de apertura del cocón y posterior rechazo del pupario en la hormiga hospedera. En todos los bioensayos, las obreras de *N. villosa* abrieron sólo los cocones parasitados y desecharon los puparios del parasitoide. La cutícula rígida de éstos representó una defensa mecánica exitosa que impide el ataque al ser manipulados por las hormigas. El rechazo de los puparios permitió a la mosca parasitoide completar su desarrollo y emerger en un lugar seguro. Por otro lado, en los bioensayos de elección pareados, se encontró que las obreras sin contacto previo con *A. bracteata*, seleccionaron significativamente esta bromelia sobre otras, lo que sugiere la existencia de una atracción innata hacia la planta. Las obreras discriminaron fácilmente y eligieron entre refugios de especies de bromelias de géneros diferentes, pero no entre refugios de especies del mismo género, lo cual sugiere que los estímulos químicos de la planta hospedera podrían jugar un papel importante. La selección del sitio de nidificación por obreras de *N. villosa* parece depender tanto de las preferencias innatas como de la experiencia temprana con los estímulos de las plantas durante el desarrollo pre-imaginal y neonatal. El grupo de mirmecófilos asociados a las colonias de *N. villosa* que nidifican en *A. bracteata* confirma a las colonias de hormigas arborícolas como reservorios de biodiversidad, por lo que requieren especial atención como componentes de los programas de conservación y manejo.

**Palabras clave:** Formicidae, Ponerinae, interacción, parasitoide, *Aechmea bracteata*, diversidad.

# CAPÍTULO I

## 1.1. Introducción General

Las hormigas son uno de los grupos de insectos sociales con mayor diversidad, abundancia y distribución geográfica en casi todos los hábitats terrestres (Hölldobler y Wilson 1990; Davidson y Patrell-Kim 1996). Las hormigas juegan un papel clave en la provisión de servicios ecosistémicos, ya que participan en la dispersión de semillas, reciclan nutrientes y son consideradas como ingenieros del ecosistema por sus actividades de nidificación que tienen impacto en la estructura del suelo y sobre otros organismos (Lobry de Bruyn y Conacher 1990; Wang et al. 1996; Frouz y Jilková 2008; Sanders y van Veen 2011; Del Toro et al. 2012; Griffiths et al. 2017). Además, interactúan en tiempo y espacio con muchos otros organismos de diferentes niveles tróficos. Estas interacciones, que pueden ser negativas o positivas, son la base ecológica de las comunidades (Wilby et al. 2001).

Las colonias y nidos de las hormigas ofrecen un microhábitat con condiciones ambientales estables y recursos alimenticios abundantes (Wheeler 1910; Hölldobler y Wilson 1990; Hughes et al. 2008), que son atractivos para una gran variedad de organismos, incluyendo diversos grupos de otros artrópodos, denominados en general mirmecófilos, es decir, organismos que habitan dentro de los nidos o en las inmediaciones de los mismos y que establecen diferentes tipos de asociaciones con la hormiga hospedera (Donisthorpe 1927; Kronauer y Pierce 2011). Los organismos mirmecófilos pueden diferir en el grado de especificidad con el hospedero: mientras que algunos están restringidos a una sola especie de hospedero (ver Akino et al. 1999), otros se asocian con diferentes taxones de hormigas (Donisthorpe 1927; Päivinen et al. 2002). Las interacciones que establecen los organismos mirmecófilos con sus hormigas hospederas van desde el mutualismo y comensalismo, a la depredación, parasitismo o parasitoidismo (Kistner 1982; Hölldobler y Wilson 1990; Fiedler 1991; Schmid-Hempel 1998; Thomas y Elmes 1998; Feener 2000; Heath y Claassens 2000; Delabie 2001; Pierce et al. 2002; Witte et al. 2008; Barbero et al. 2009; Kaminski y Carvalho-Filho 2012; Lachaud y Pérez-Lachaud 2012; Poinar 2012; Lachaud et al. 2013; Pérez-Lachaud et al. 2014; Dupont et al. 2016).

Las hormigas presentan una variedad de canales de comunicación que les permiten regular su organización social y actuar como un superorganismo (Hölldobler y Wilson 1990). El principal canal de comunicación que está involucrado con la mayoría de las actividades de las hormigas es el químico, por el cual las obreras son capaces de discriminar entre individuos que pertenecen a la misma colonia y aquellos ajenos (Hölldobler y Wilson 1990; Lenoir et al. 2001; Breed 2003; Bagnères y Lorenzi 2010; d'Ettorre y Lenoir 2010). Se ha señalado que una mezcla de hidrocarburos cuticulares específica de la colonia permite el reconocimiento y la discriminación de miembros de su propia colonia de otros que no lo son (Vander Meer et al. 1989; Lenoir et al. 2001; Hefetz 2007; d'Ettorre y Lenoir 2010; van Zweden y d'Ettorre 2010); aunque también otros estímulos pueden intervenir (p. ej. táctiles, visuales, acústicos: Le Masne 1953; Nelson y Jackson 2006; Cammaerts 2007; Barbero et al. 2009, 2012; Di Giulio et al. 2015). A pesar de este complejo sistema de comunicación y de las sofisticadas defensas de las hormigas, muchos organismos mirmecófilos han desarrollado diferentes estrategias (comportamentales, morfológicas, acústicas, químicas, etc.) que les permiten quebrantar los sistemas de comunicación y defensa de las hormigas, para ser tolerados y explotar los recursos dentro de los nidos (Hölldobler 1971; Vander Meer y Wojcik 1982; Steidle y Dettner 1993; Fiedler et al. 1996; Akino 2002; Pierce et al. 2002; Barbero et al. 2009; Hojo et al. 2009; Bagnères y Lorenzi 2010; Riva et al. 2016; de Bekker et al. 2018). Por ejemplo, las obreras de la hormiga *Myrmica schencki* Viereck, 1903 (Myrmicinae) transportan al interior de su nido, larvas de la mariposa *Maculinea rebeli* (Hirschke, 1904) (Lycaenidae), que son tratadas como congéneres y son alimentadas por trofalaxis (Barbero et al. 2009). Cuando la colonia de *M. schencki* es perturbada, las obreras rescatan prioritariamente a las larvas de *M. rebeli* (Thomas et al. 1998), e incluso matan a su propia cría para alimentar a este parásito social cuando el alimento es escaso (Elmes et al. 2004). Barbero et al. (2009) encontraron que la integración de las poblaciones de *M. rebeli* se lleva a cabo inicialmente a través del mimetismo químico. Una vez que dicho parásito social se ha integrado al nido y es aceptado como parte de la colonia, imita la acústica de las hormigas adultas (principalmente de las reinas), para así avanzar hacia la posición jerárquica más alta de la colonia y ser alimentada de manera preferencial (Barbero et al. 2009). De igual manera, los escarabajos del género *Paussus* Linnaeus, 1775 (Coleoptera: Carabidae: Paussini) son simbiontes obligados que dependen durante todas sus etapas de desarrollo, de las hormigas (Di Giulio 2008). Los adultos de *Paussus* depredan sobre las larvas del hospedero (Maurizi et al. 2012). Estos escarabajos secretan sustancias químicas a través de tricomas presentes en todo el cuerpo para apaciguar el ataque de las obreras (Di Giulio et al. 2009). *Paussus favieri* Fairmaire, 1851 mimetiza, además, las estridulaciones de las diferentes castas de su hormiga hospedera *Pheidole pallidula* (Nylander, 1849) (Myrmicinae) para ser tratado como congénere dentro del nido (Di Giulio et al. 2015).

A pesar de que las hormigas han sido estudiadas ampliamente, hay una falta de conocimiento sobre la biota asociada con la mayoría de las especies, sobre la naturaleza de sus interacciones con las hormigas y, en general, sobre los mecanismos utilizados para integrarse a la vida de la colonia hospedera (Lachaud et al. 2013). La pérdida de hábitat pone en riesgo a las colonias de hormigas arborícolas y a los organismos mirmecófilos que habitan en sus nidos, que en algunos casos presentan alta especificidad a sus hormigas hospederas (p. ej. parasitoides y/o depredadores) (Pérez-Lachaud y Lachaud 2014). Por ello, los nidos de las hormigas arborícolas han sido catalogados como focos de biodiversidad, mismos que requieren especial atención.

*Neoponera villosa* (Fabricius, 1804) (Ponerinae) es una especie de hormiga que tiene una distribución geográfica bastante amplia, desde Texas hasta Argentina, a alturas no mayores a los 1100 msnm (Forel 1899; Kempf 1972; Pérez-Bautista et al. 1985; Mackay y Mackay 2010) y habita tanto selvas húmedas como secas (Wild 2002). Esta hormiga nidifica de manera oportunista en cavidades de madera muerta y ramas vivas, en las bases de bromelias y en tallos huecos de varias especies mirmecófitas de *Cecropia* Loefling (Urticaceae) (Dejean et al. 1992, 1995; Valenzuela-González et al. 1994; Fernandes et al. 2013). Sus colonias generalmente son fundadas por grupos de reinas (pleometrosis) (Trunzer et al. 1998). En Brasil, las colonias de esta especie son poligínicas (más de una reina) (Trunzer et al. 1998), pero en Chiapas, México, las colonias han sido reportadas como monogínicas (una sola reina) (Pérez-Bautista et al. 1985). Esta hormiga es muy agresiva y con una picadura potente (Pessoa et al. 2016). Las obreras presentan una estrategia de caza de tipo individual, sin reclutamiento (Lachaud et al. 1984). Además, tienen un ritmo de forrajeo crepuscular-nocturno y pueden presentar una fuerte fidelidad a un área de forrajeo en particular (Lachaud et al. 1984). Son principalmente depredadoras generalistas arborícolas de otros artrópodos, aunque también se alimentan de líquidos azucarados colectados de frutos o nectarios extraflorales (Lachaud et al. 1984; Hölldobler 1985; Pérez-Bautista et al. 1985; Dejean y Corbara 1990; Paul y Roces 2003).

Hasta la fecha, *N. villosa* ha sido reportada como hospedera de nemátodos de la familia Mermithidae en Colombia y Venezuela (Emery 1904; Wheeler 1928; Poinar et al. 2006) y de dos especies de parasitoides: *Hypselosyrphus trigonus* Hull, 1937 (Diptera: Syrphidae) y una especie de la familia Encyrtidae (Hymenoptera), probablemente del género *Blanchardiscus* De Santis 1964. Ambos atacan esta hormiga en el sureste de la Península de Yucatán, en colonias que nidifican en *Aechmea bracteata* (Sw.) Griseb (Bromeliaceae) (Pérez-Lachaud et al. 2014; Pérez-Lachaud y Lachaud 2017), lo que sugiere que esta bromelia podría ser un factor importante en la estructura de las interacciones de *N. villosa* con otros organismos. Es probable, que otros organismos mirmecófilos estén también asociados a *N. villosa*, como ocurre con otras hormigas ponerinas (Devries 1991; Janssen et al. 2002; Pérez-Lachaud et al. 2006; Maruyama et al. 2010; Lachaud y Pérez-Lachaud 2012; Castaño-Meneses et al. 2014; Guimaraes et al. 2018). Por ejemplo, se sabe que los fóridos *Apocephalus paraponerae* Borgmeier*,* 1958, *A. crassilatus* Brown, 2000 y *A. globosus* Brown, 2000 (Diptera) son atraídos por obreras lesionadas de *N. villosa* en Costa Rica y Brasil (Brown 2000), aunque la oviposición y desarrollo del parasitoide en esta hormiga no han sido confirmados.

En el sur de la Península de Yucatán, *N. villosa* nidifica principalmente en la bromelia-tanque *A. bracteata* (Dejean 1990; Dejean et al. 1992, 1995; Dejean y Olmsted 1997), a pesar de que otras especies de *Aechmea* con el mismo tipo de crecimiento, están disponibles en la región (Espejo-Serna y López-Ferrari 2004). *Aechmea bracteata* es una bromeliácea de tipo "fitotelmata" que combina la presencia de un depósito periférico capaz de almacenar agua y una cavidad central impermeable delimitada por una hoja en forma de ánfora (Greeney 2001) apta para albergar hormigas (Dejean et al. 1995; Dejean y Olmsted 1997; Beutelspacher 1999; Hénaut et al. 2014).

Las hormigas arborícolas son particularmente propensas a mover sus colonias de un sitio a otro (McGlynn 2012) como respuesta a numerosos factores bióticos y abióticos (p. ej. Möglich 1978; Smallwood y Culver 1979; Droual 1984; Gordon 1992; Gordon et al. 2001; McGlynn et al. 2004, 2010; Heller y Gordon 2006; McGlynn 2006, 2012; Dahbi et al. 2008; Heller et al. 2008; Tay y Lee 2015). La Península de Yucatán ha sido identificada como una región afectada por huracanes y sequías (Whigham et al. 2003; Mardero et al. 2015), los cuales pueden ocasionar que las bromelias caigan y generen la consecuente necesidad de reubicación de las colonias de las hormigas. Por ello, la selección rápida del sitio de nidificación es ecológicamente crítica para la colonia (Visscher 2007; Paul et al. 2016).

Se ha demostrado que el reconocimiento de la planta hospedera en algunas hormigas arborícolas se basa principalmente en dos factores: una atracción innata, determinada genéticamente, y la influencia del medio ambiente a través del contacto de los individuos con la planta hospedera durante la etapa larvaria y los primeros días de la etapa adulta (aprendizaje temprano), que puede incluso suplantar a la atracción determinada genéticamente (Jaisson 1980; Dejean 1990; Djieto-Lordon y Dejean 1999a, 1999b). Por ejemplo, Djieto-Lordon y Dejean (1999a) encontraron que las hormigas arborícolas *Tetramorium aculeatum* (Mayr, 1866) (Myrmicinae) y *Oecophylla longinoda* (Latreille, 1802) (Formicinae) presentan un proceso de familiarización que puede reemplazar a la atracción innata en ambas especies; este aprendizaje, únicamente se desarrolla en la etapa neonatal, período sensible, después del cual, la influencia del medio ambiente cesa (ver Jaisson 1980). Dejean (1990) demostró en condiciones de laboratorio, que la atracción de las fundadoras y obreras jóvenes (nurses) de *N. villosa* hacia *A. bracteata*, parece deberse al aprendizaje durante la etapa larvaria y en los primeros días de la etapa adulta (imago) de esta hormiga. Sin embargo, la selección del sitio de nidificación en *N. villosa* no ha sido estudiada en el contexto de la reubicación del nido, es decir, un proceso de toma de decisiones, que es realizado por varias exploradoras (forrajeras) que localizan sitios potenciales de nidificación, con movimiento masivo de la colonia hacia el nuevo sitio (Visscher 2007).

Considerando lo anterior, en el Capítulo II de este estudio se contribuyó a la determinación de la fauna asociada a la hormiga *N. villosa* que nidifica en la bromelia-tanque *A. bracteata* en el dosel, una parte de la biodiversidad aún desconocida pero amenazada por la fragmentación y pérdida de hábitat en la Península de Yucatán. Asimismo, se exploraron las interacciones comportamentales entre mirmecófilos y hormigas; en particular, cómo las moscas adultas *H. trigonus* logran escapar ilesas del cocón y nido de un hospedero tan agresivo, como *N. villosa* (Capítulo III).

Finalmente, y dada la importancia potencial de esta bromelia en el establecimiento de interacciones de *N. villosa* con otros organismos, se realizaron diferentes experimentos de elección (Capítulo IV), con el objetivo de corroborar la fidelidad de esta especie de hormiga hacia *A. bracteata* y determinar los factores que influyen en la selección del sitio de nidificación en las obreras de *N. villosa* durante la reubicación de sus nidos.

## 1.2. Objetivos

### General

Determinar la diversidad y abundancia de organismos mirmecófilos de colonias de *N. villosa* que nidifican en *A. bracteata*, así como contribuir al conocimiento de las interacciones comportamentales con sus parasitoides potenciales y corroborar la fidelidad de esta hormiga hacia *A. bracteata* como sitio de nidificación en la Península de Yucatán.

### Particulares

1. Determinar la diversidad y abundancia de organismos mirmecófilos asociados a los nidos y colonias de *N. villosa* en la Península de Yucatán.

2. Esclarecer las interacciones comportamentales entre *N. villosa* y sus organismos mirmecófilos. En particular, determinar el/los mecanismo(s) utilizado(s) por *Hypselosyrphus trigonus* para escapar del nido de la hormiga hospedera.

3. Corroborar la fidelidad de *N. villosa* hacia la planta de nidificación (*A. bracteata*) e identificar algunos de los factores involucrados en esta selección.

## 1.3. Metodología General

La metodología específica para los diferentes estudios que conforman esta tesis se presentan en los capítulos correspondientes, por lo que en este apartado sólo se detallarán los sitios y métodos de colecta.

### Sitios de estudio y método de muestreo

Se recolectaron manualmente 84 colonias (o partes de colonias) de *N. villosa* que nidificaban en la bromelia-tanque *A. bracteata*, así como 5 colonias (o partes de colonias) en oquedades de árboles vivos (Tabla 1) (permiso de colecta: FAUT-0277 para artrópodos). Los clones de *A. bracteata* que albergaban a las colonias de *N. villosa* y sus organismos asociados, fueron cortados de la rama de soporte y colocados en bolsas de plástico; se prosiguió a transportar al laboratorio para su inmediata disección. Cada epífita fue desmantelada hoja por hoja en contenedores de plástico recubiertos de Fluon (Whitford GmbH). Se colectaron y contabilizaron todos los organismos contenidos entre las hojas de la bromelia. Asimismo, se registró la casta y el estado de desarrollo de las hormigas. No se contaron los huevos, pero se registró su presencia.

Tabla 1. Sitios de muestreo, número y coordenadas geográficas de las colonias de *N. villosa* en *A. bracteata* y tronco de árboles en la Península de Yucatán, México.

| Localidades | Latitud N | Longitud O | Altitud (m) | Número de colonias | Sitio de nidificación |
|---|---|---|---|---|---|
| **Campeche** | | | | | |
| Hermenegildo Galeana | 18° 10' 36.41" | 89° 14' 24.29" | 190 | 7 | Bromelia |
| Ejido Blasillo | 18° 7' 37.98" | 89° 20' 20.93" | 261 | 19 | Bromelia |
| Felipe Ángeles | 18° 12' 8.94" | 89° 17' 36.54" | 212 | 1 | Bromelia |
| Zoh-Laguna | 18° 35' 11.61" | 89° 25' 4.67" | 257 | 2 | Bromelia |
| Nuevo Becal | 18° 36' 39.36" | 89° 16' 15.54" | 239 | 23 | Bromelia |
| Pioneros del Río Xnohá | 17° 53' 13.99" | 89° 10' 31.94" | 65 | 1 | Bromelia |
| **Quintana Roo** | | | | | |
| Kohunlich | 18° 25' 31.08" | 88° 48' 9.89" | 143 | 2 | Bromelia |
| Sian Ka`an | 19° 41' 56.17" | 87° 50' 18.31" | 18 | 27 | Bromelia |
| Chetumal | 18° 32' 37.90" | 88° 15' 46.38" | 10 | 2 | Cavidad en árbol |
| **Yucatán** | | | | | |
| Cuxtal | 20° 51' 46.58" | 89° 36' 40.68" | 16 | 3 | Cavidad en árbol |

Los muestreos de bromelias se realizaron en ocho sitios (Figura 1 y Tabla 1), en las temporadas de secas (febrero–mayo), lluvias (junio-septiembre) y nortes (octubre–enero). Para cada nido, se registró la especie de árbol y la altura a la que se colectó, así como la temperatura y humedad. *Aechmea bracteata* no requiere de permiso de colecta, sin embargo, se cuidó de tomar solo un individuo (clon) por epífita muestreada. Por otro lado, para los bioensayos del Capítulo IV, se recolectaron hormigas que nidificaban en oquedades de árboles vivos (principalmente *Lysiloma latisiliquum* (L.) Benth., *Caesalpinia gaumeri* (Britton & Rose) Greenm. y *Leucaena leucocephala* (Lam.) de Wit (Fabaceae), y *Bursera simaruba* (L.) Sarg. (Burseraceae)) en dos sitios (Figura 1 y Tabla 1). Todos los muestreos se llevaron a cabo del 20 de enero de 2016 al 16 de enero de 2019.

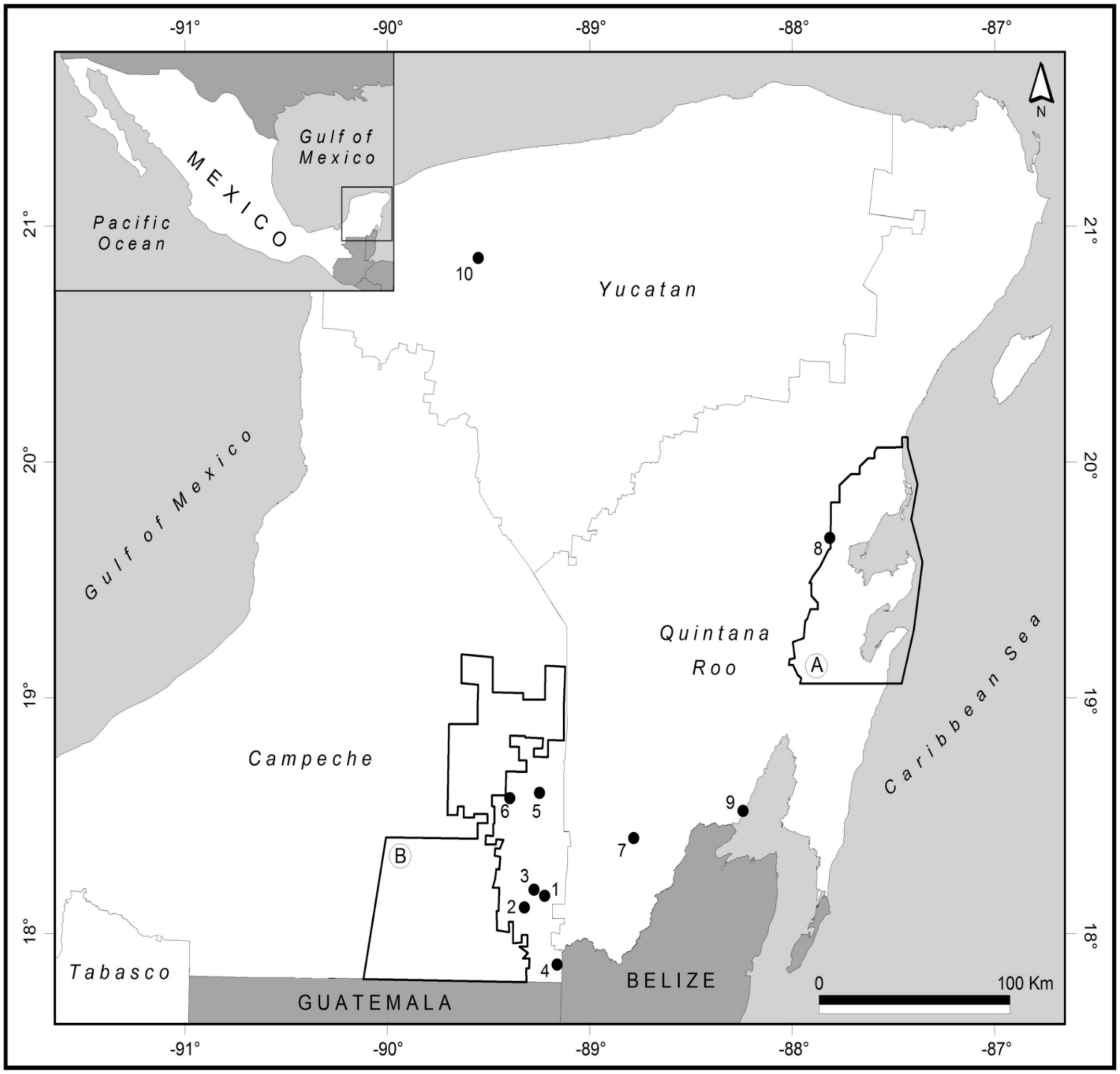

Figura 1. Ubicación geográfica de las localidades de muestreo de colonias de *N. villosa* en Campeche (1 = Hermenegildo Galeana, 2 = Ejido Blasillo, 3 = Felipe Ángeles, 4 = Pioneros del Río Xnohá, 5 = Nuevo Becal, 6 = Zoh-Laguna), Quintana Roo (7 = Kohunlich, 8 = Sian Ka'an, 9 = Chetumal) y Yucatán (10 = Cuxtal), en la Península de Yucatán, México. De igual manera, se señalan en polígonos, las Reservas de la Biosfera de Sian Ka'an (A) y Calakmul (B).

# CAPÍTULO II

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

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

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## 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 part 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 of the other 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

## 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 (WASSMAN 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 generalist, 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 (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* (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 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 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, i.e. 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.

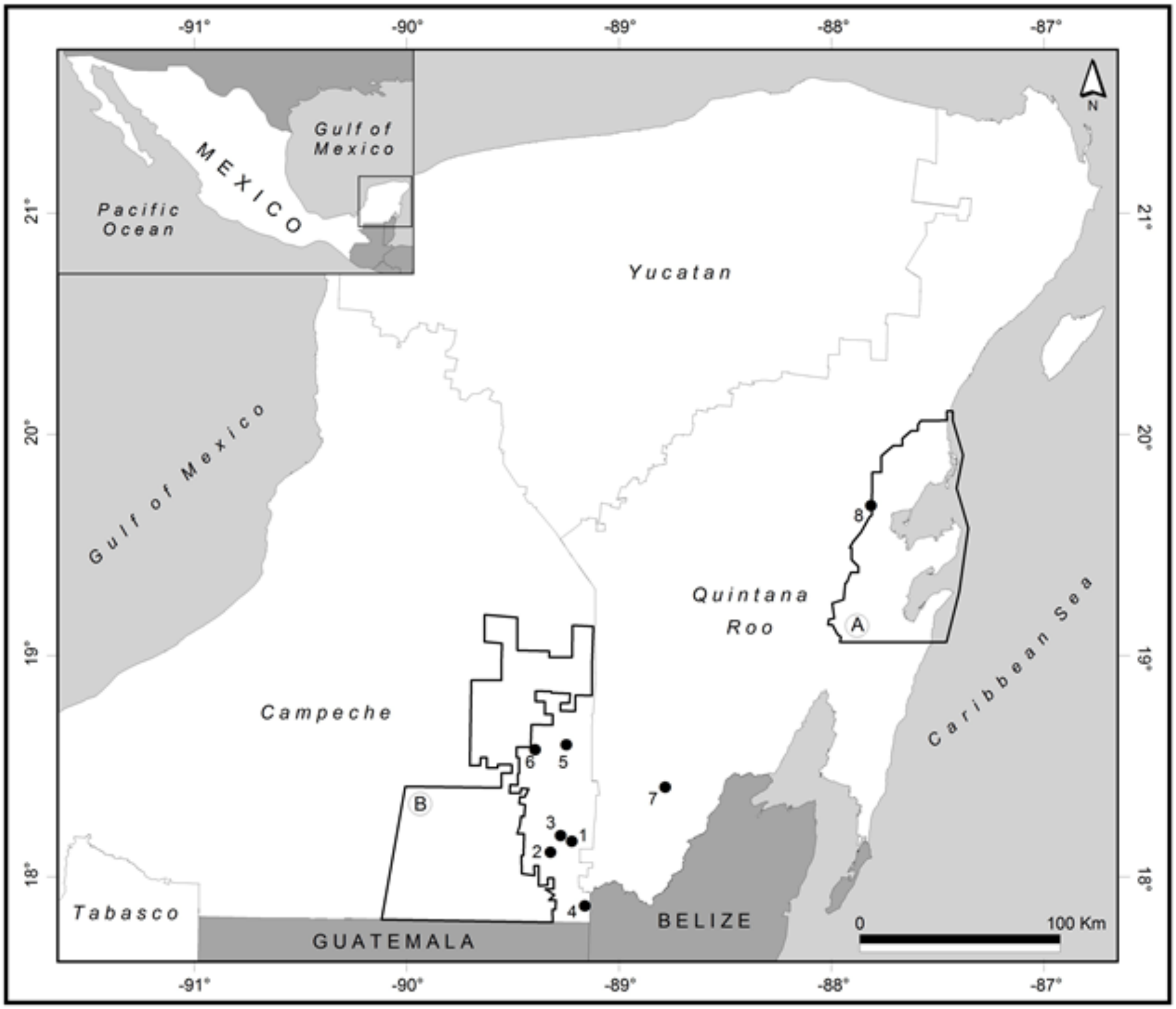

Fig. 1. Localization of *Neoponera villosa* collecting sites in the southern part of the Yucatan Peninsula, Mexico. Collecting sites: 1) Hermenegildo Galeana, 2) Ejido Blasillo, 3) Felipe Ángeles, 4) Pioneros del Río Xnohá, 5) Nuevo Becal, 6) Zoh-Laguna, 7) Kohunlich, 8) Sian Ka'an. Polygons: A) Sian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

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 (masl)** | **Number of colonies collected** |
|---|---|---|---|---|
| **Campeche** | | | | |
| 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 |
| **Quintana Roo** | | | | |
| 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 |

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 ± 2°C and 75 ± 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 (unpub. data; 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 eucharitid 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

### Ant colony composition and nesting behavior

A total of 72 queenright and 10 queenless colonies (or part 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 ± 0.5 queens (mean ± SEM, range: 0 – 20), 97.8 ± 7.9 workers (range: 3 – 322), 42.2 ± 4.9 pupae in cocoons (range: 2 – 261), and 41.1 ± 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 (Supplementary material Tab. S1). 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 leave 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.

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.

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.

| **Class** | **Order** | **Family** | **Taxon** | **Size (mm)** (mean ± SEM) | **Nb. of specimens** | **Nb. of occupied colonies** | **Observed relationship** | **Identification** | **References** |
|---|---|---|---|---|---|---|---|---|---|
| **Animalia, Arthropoda** | | | | | | | | | |
| Arachnida | Acari Mesostigmata | Laelapidae | *Cosmolaelaps* sp. | ≈ 0.4 mm | not estimated | 74 | Cleptoparasitic | H. Klömpen | This study |
| | | Oplitidae | *Oplitis* sp. | ≈ 0.4 mm | not estimated | 9 | Phoretic on both adults and larvae | H. Klömpen | This study |
| | Acari Trombidiformes Prostigmata | Scutacaridae | unidentified | < 0.3 mm | not estimated | 81 | Possibly fungivorous; found on the ventral side of the thorax of adult ants, between the coxae | H. Klömpen | This study |
| | Acari Sarcoptiformes Oribatida | Galumnidae | unidentified | ≈ 0.5 mm | 1 | 1 | Phoretic on larvae | H. Klömpen | This study |
| | Pseudoscorpiones | Chernetidae | *Chelodamus* sp. | 3.4 ± 0.15 (n = 22) | 223 | 52 | Brood predator | M. Harvey | This study |
| Insecta | Coleoptera | Staphylinidae | *Myrmigaster* sp. (Aleocarinae) | 2.05 ± 0.02 (n = 43) | 79 | 14 | Unknown | M. Maruyama | This study |
| | | Tenebrionidae | Unidentified (Alleculinae) | 8.0 | 1 | 1 | Possible brood predator | Triplehorn & Johnson 2005 | This study |
| | Diptera | Syrphidae | *Hypselosyrphus trigonus* 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 | *Trichopria* sp. (Diapriinae) | 2.3 ± 0.06 (n = 7) | 14 | 4 | Unknown; found exploring ant cocoons | L. Masner | This study |
| | | Eucharitidae | *Kapala* sp. (Eucharitinae) | 4.3 ± 0.12 (n = 6) | 8 | 5 | Solitary ectoparasitoid, larval-pupal | J. Heraty | This study |
| | Lepidoptera | Riodinidae | Unidentified | 9.0 | 1 | 1 | Brood predator | L.A. Kaminski | This study |
| **Fungi, Ascomycota** | | | | | | | | | |
| Sordariomycetes | Hypocreales | Ophiocordycipitaceae | *Ophiocordyceps* sp. | | 1 | 1 | Parasitic on adults | J. Arau | This study |

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. Representative publications for each group are shown; where possible, biological studies or works that provide summaries of the known biology are listed. The column 'Identification' refers to the taxonomist who identified the taxon or to the resources used by the authors. 1) Jałoszyński & Olszanowski 2015; 2) Wojcik & Naves 1992; 3) Ishii & Yamaoka 1982; 4) Castaño-Meneses & al. 2015b; 5) Bell & al. 2007; 6) Kistner 1982; 7) Hölldobler & Kwapich 2017; 8) Ashe & Kistner 2005; 9) Irmler 2010; 10) Betz & al. 2018; 11); Beutelspacher Baigts 1999; 12) Park 1942; 13) Park 1947; 14) Molleman & Walter 2001; 15) Rotheray & al. 2007; 16) Ross 2000; 17) Mariño P. 1994; 18) Mackay & Mackay 2002; 19) Roeder & al 2018; 20) Murakami & Higashi 1997; 21) Blüthgen & al. 2000; 22) Sarnat & al. 2015; 23) DaRocha & al. 2015; 24) Longino & Fernández 2007; 25) Molero-Baltanás & al. 2017; 26) Araujo & al. 1996.

| Class | Order | Family | Subfamily | Taxon | Size (mm) (mean ± SEM) | Nb. of specimens | Nb. 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 Scydmaeninae[1] | 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[2], including arboreal ant nests[3] | Bueno-Villegas & al. 2004 |
| Entognatha | Collembola | unidentified | unidentified | unidentified (at least 2 spp.) | 1.0 – 2.0 | not estimated | 57 | Detritivorous[4] | Triplehorn & Johnson 2005 |
| Insecta | Archaeognatha | unidentified | unidentified | unidentified | | 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[5] | Triplehorn & Johnson 2005 |
| | Coleoptera | Histeridae | unidentified | unidentified | 1.0 ± 0.02 ( n = 35) | 98 | 23 | Predaceous; some species feed on ant prey or beg for food (trophallaxis)[6] | Triplehorn & Johnson 2005 |
| | | Nitidulidae | unidentified | unidentified | 1.4 ± 0.02 (n = 8) | 8 | 6 | Scavengers or fungivorous. *Amphotis marginata* is a cleptoparasite of *Lasius* fuliginosus[7] | Triplehorn & Johnson 2005 |
| | | | unidentified | | < 1.0 | 3 | 2 | Fungivorous; found in | Triplehorn & |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Ptiliidae | | unidentified | | | | refuse piles in ant nests[6] | JOHNSON 2005 |
| | | Staphylinidae | Aleocharinae | *Myrmigaster* sp. 2 | 3.7 ± 0.04 (n = 38) | 85 | 23 | Generalist predator[8] | M. MARUYAMA |
| | | | Osoriinae | *Thoracophorus* sp. | 2.4 (n = 1) | 2 | 2 | Fungivorous; at least two neotropical species associated with termites. Association with canopy ants is suspected[9] | M. MARUYAMA |
| | | | Paederinae | unidentified sp. 1 | 2.9 ± 0.05 (n = 23) | 30 | 13 | Generalist predators in decaying plant material; common in damp habitats[10] | M. MARUYAMA |
| | | | | unidentified sp. 2 | | 1 | 1 | | |
| | | | | unidentified sp. 3 | 5.2 (n = 1) | 1 | 1 | | |
| | | | Pselaphinae | *Oxarthrius* sp. (Batrisini) | 2.0 ± 0.04 (n = 15) | 15 | 12 | Litter dwelling, predaceous; occurs in *A. bracteata* bromeliads[11]; *O. attaphilus* Bruch 1933 associated with *Atta sexdens* L.[12] | ASENJO & al. 2018 |
| | | | | *Rhytus* sp. (Arhytodini) | 1.7 | 1 | 1 | Generalist predators[13]; *R. myrmecophilous* is associated with *W. auropunctata*[12] | M. MARUYAMA |
| | | | | *Tyropsis* sp. (Tyrini) | 2.5 ± 0.08 (n = 21) | 32 | 20 | Generalist predators; litter dwelling[12] | M. MARUYAMA |
| | | | | unidentified | 1.2 – 1.3 | 2 | 2 | | |
| | | | Scydmaeninae | *Euconnus* sp. | 1.2 ± 0.05 (n = 10) | 15 | 7 | Mite and springtail predators; scavenger on dead ants [14] | FERRO & al. 2015 |
| | Diptera | Syrphidae | Eristalinae | *Copestylum* sp. | 9.9 ± 0.21 (n = 5) | 5 (larvae) | 2 | Larvae develop upon decomposing plant material[15] | G. ROTHERAY |
| | | Unidentified | | unidentified | | 2 | 2 | ? (poorly preserved) | |
| | Embioptera | unidentified | unidentified | unidentified | 4 – 4.9 | 2 | 1 | Nymphs and adult | TRIPLEHORN & |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | females are herbivorous, feeding on leaf litter, moss, bark and lichen[16]. Some species have been found in bromeliads (*Tillandsia*)[17] | JOHNSON 2005 |
| | Hymenoptera | Formicidae | Dolichoderinae | *Forelius pruinosus* (ROGER, 1863 | 1.8 ± 0.06 (n = 9) | 17 adults + brood | 1 | Omnivorous[18]; a termophilic species[19] | J.-P. LACHAUD |
| | | | Myrmicinae | *Cyphomyrmex rimosus* (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[20]; found nesting in bromeliads[21] | J.-P. LACHAUD |
| | | | | *Pheidole flavens* ROGER, 1863 | minors: 1.2 ± 0.02 (n = 12)<br>majors: 2.0 ± 0.09 (n = 3) | 133 adults + brood | 3 | Predators or scavengers on other arthropods; nests in the soil[22] and also in bromeliads in the canopy[23] | J.-P. LACHAUD |
| | | | | *Wasmannia auropunctata* (ROGER, 1863) | 1.4 ± 0.02 (n = 15) | 277 adults + brood | 3 | Omnivorous scavengers and predators; nests are almost anywhere, including epiphytes[24] | J.-P. LACHAUD |
| | | | Ponerinae | *Hypoponera opacior* (FOREL, 1893) | 3.8 | 1 queen | 1 | Predator; nests under stones but also in bromeliads[23] | J.-P. LACHAUD |
| | Zygentoma | Nicoletiidae | unidentified | unidentified | 4.5 ± 0.26 (n = 8) | 15 | 11 | Some species are ant cleptoparasites/ commensals[25] | TRIPLEHORN & JOHNSON 2005 |
| Malacostraca | Isopoda (Oniscidea) | unidentified | unidentified | unidentified | 4 – 4.5 mm | 4 | 3 | Detritivorous; leaf litter decomposers; some species found in bromeliads[26] | TRIPLEHORN & JOHNSON 2005 |

**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).

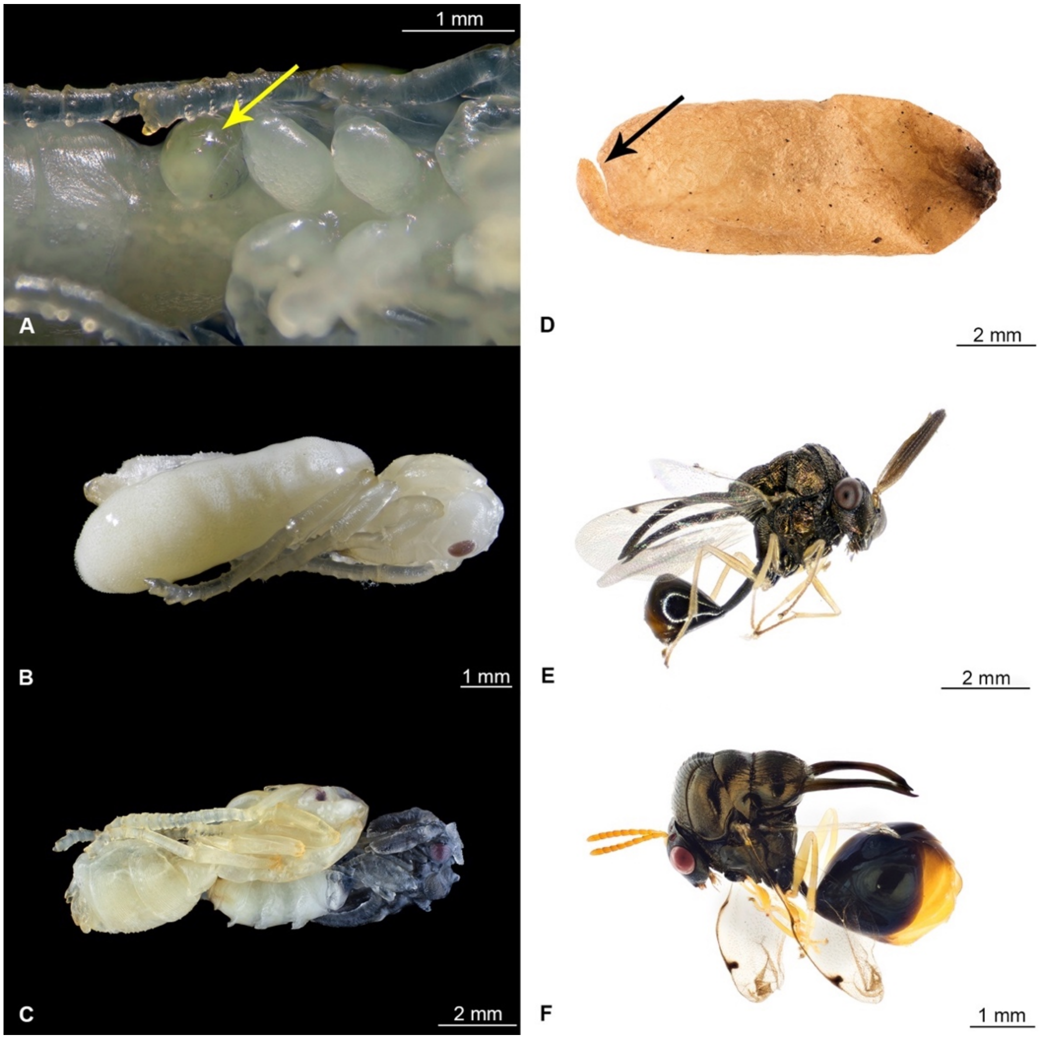

Fig. 3. The ectoparasitoid eucharitid wasp *Kapala* sp. A) Second larval instar (arrow) under the third coxa of a host pupa. B) Third larval instar upon a host pupa. C) A *Kapala* sp. male pupa and the host remains. D) Operculum made by the wasp (arrow) on emergence from the host cocoon. E) Male, lateral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

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. 3 A-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, 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 5.6% of the pupae at the population level, with a mean parasitism rate of 26.3 ± 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. 4 A-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.

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 has been removed to show the developing parasitoid.

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, $P < 0.05$, Supplementary material Fig. S1 A).

**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* (Pseudoscorpiones: Chernetidae). This genus has been previously reported as inhabiting *A. bracteata* (BEUTELSPACHER BAIGTS 1999). In the nests of *Neoponera villosa* that species was observed unequivocally feeding on ant larvae (Fig. 5 A-B). This furtive predator was far more common than the riodinid caterpillar as a total of 223 specimens (adults and immatures, 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 ± 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 ($r = 0.44$, t = 3.46, d.f. = 50, $P < 0.05$; and $r = 0.52$, t = 3.78, d.f. = 50, $P < 0.05$, respectively). (Supplementary material Fig. S1 B-C) in occupied colonies, but no pattern of occupancy was observed according to abiotic parameters or season.

Finally, a single beetle pupa was found among the cocoons (Fig. 5 D), and could be reared up to the adult stage and determined as belonging to the Tenebrionidae (Alleculine subfamily) (Fig. 5 C). 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.

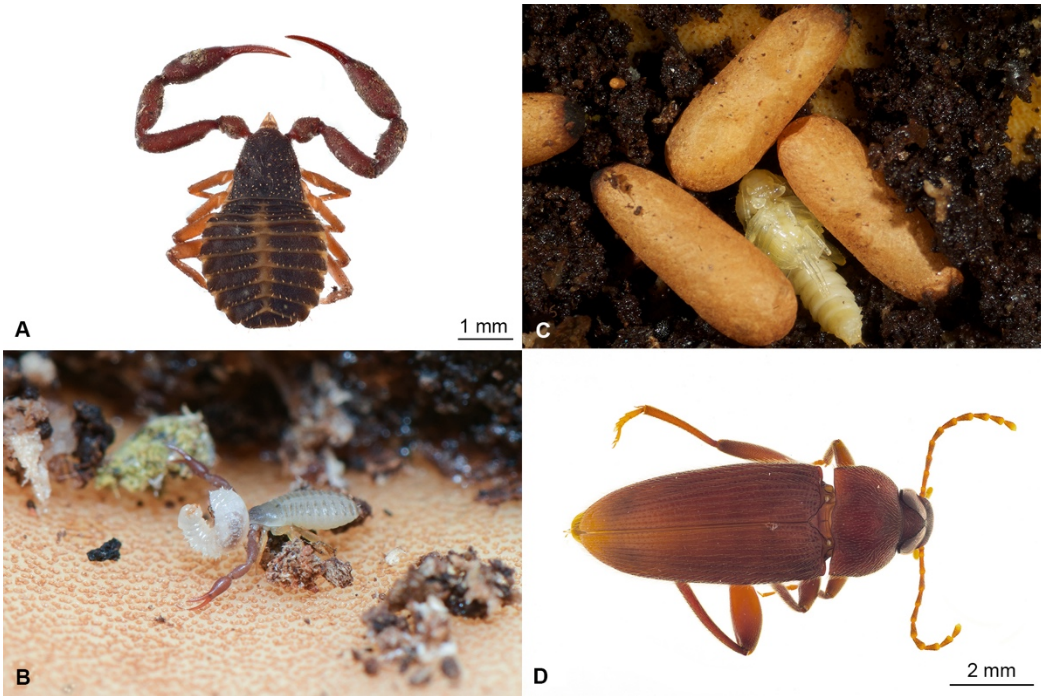

Fig. 5. Predatory myrmecophiles. A) *Chelodamus* sp. (Pseudoscorpiones: Chernetidae). B) Same species feeding on a *Neoponera villosa* larva. C) An unidentified species of Tenebrionidae (Alleculinae). D) Same species; pupa among ant cocoons.

**Cleptoparasites:** Mites of a species of the genus *Cosmolaelaps* Berlese, 1903 (Mesostigmata: Laelapidae) (Fig. 6 A) 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 colonies and sporadically on eggs, in spite of the hygienic and defensive behaviors of the host workers; however, their prevalence among 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. 6 B), 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. 6 C). 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. 6 D). 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 ± 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. 6 E) 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. 6 F, Supplementary material 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.

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.

Similarly, a new species (Maruyama, pers. comm.) of myrmecophilous beetle, belonging to the genus *Myrmigaster* SHARP, 1876 (Coleoptera: Staphylinidae) (Fig. 6 G-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 ± 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 (Supplementary material Video S1). Most Diapriinae 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 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, Fig. 7) and Hymenoptera (other ant species) 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, 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. However, 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. 8), 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 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*, *Myrmegaster* 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.

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-E) Unidentified Paederinae. F) *Rhytus* sp. (formerly *Arhytodes*) (Pselaphinae). G) *Tyropsis* sp. (Pselaphinae). H) *Oxarthrius* sp. (Pselaphinae). I) Unidentified Pselaphinae. J) *Euconnus* sp. (Scydmaeninae).

Fig. 8. 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.

## 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. 9 A). Likewise, the global species accumulation curve including all the species found in the central part of the nest (myrmecophiles and not integrated associates; Fig. 9 B), did not reveal a tendency to reach an asymptote. The Chao 2 estimator gave a 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 ± 2.1, range: 1 - 43, n = 23) might have been decisive in failing to recover this parasitoid species.

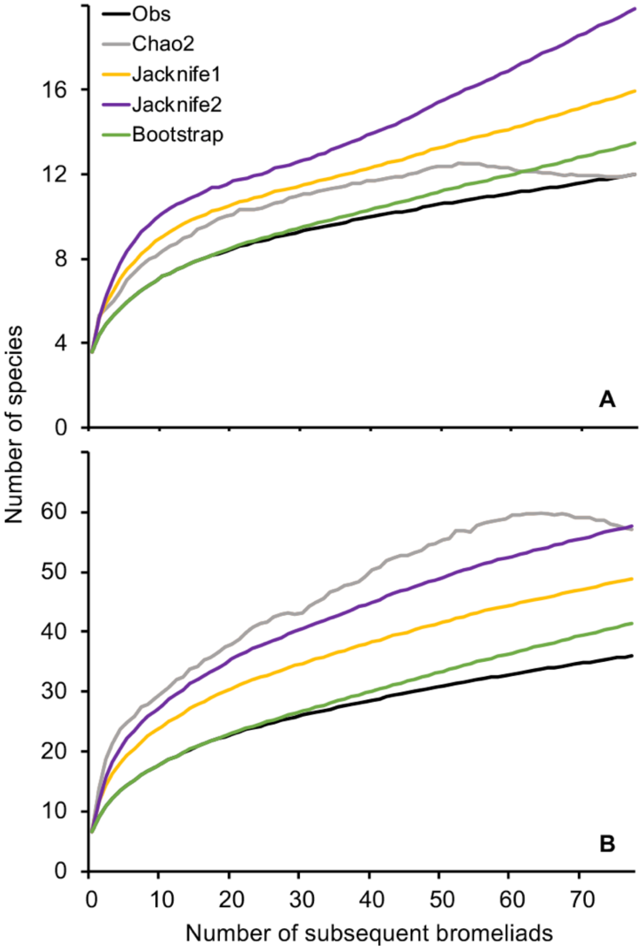

Fig. 9. Species accumulation curves showing number of taxa associated with *Neoponera villosa* ants. A) True myrmecophiles, B) Curves for all taxa (both myrmecophiles and not integrated associates) found inside the nest chambers. Graphs show observed species richness and estimated species richness calculated with several estimators.

## 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 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 (HÖLLDOBLER & 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 (VAZQUEZ-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 (Supplementary material 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, ROCHA & al. 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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**Appendix, as digital supplementary material to this article, at the journal's web pages**

Table S1. *Neoponera villosa* colonies composition.

| Colony number | Queens | Gynes | Males | Workers | Larvae | Cocoons |
|---|---|---|---|---|---|---|
| 1 | 16 | 1 | 0 | 322 | 5 | 65 |
| 2 | 13 | 4 | 0 | 226 | 8 | 13 |
| 3 | 4 | 0 | 0 | 75 | 28 | 20 |
| 4 | 8 | 10 | 22 | 185 | 161 | 261 |
| 5 | 5 | 3 | 0 | 107 | 63 | 51 |
| 6 | 10 | 0 | 0 | 122 | 32 | 22 |
| 7 | 0 | 0 | 6 | 5 | 1 | 5 |
| 8 | 17 | 4 | 0 | 206 | 45 | 29 |
| 9 | 1 | 0 | 0 | 186 | 51 | 97 |
| 10 | 20 | 21 | 0 | 73 | 49 | 44 |
| 11 | 6 | 0 | 0 | 84 | 3 | 3 |
| 12 | 1 | 0 | 0 | 115 | 101 | 40 |
| 13 | 1 | 0 | 0 | 143 | 44 | 74 |
| 14 | 1 | 0 | 0 | 180 | 69 | 65 |
| 15 | 7 | 1 | 0 | 145 | 72 | 9 |
| 16 | 10 | 0 | 0 | 283 | 22 | 8 |
| 17 | 8 | 0 | 0 | 285 | 72 | 19 |
| 18 | 2 | 0 | 0 | 74 | 1 | 4 |
| 19 | 1 | 0 | 0 | 27 | 15 | 4 |
| 20 | 7 | 0 | 0 | 117 | 4 | 3 |
| 21 | 1 | 0 | 0 | 47 | 39 | 27 |
| 22 | 11 | 3 | 0 | 165 | 265 | 173 |
| 23 | 4 | 0 | 0 | 33 | 19 | 25 |
| 24 | 0 | 0 | 0 | 10 | 2 | 4 |
| 25 | 1 | 0 | 0 | 113 | 43 | 94 |
| 26 | 0 | 0 | 0 | 40 | 58 | 41 |
| 27 | 5 | 1 | 0 | 131 | 70 | 150 |
| 28 | 2 | 0 | 0 | 65 | 39 | 79 |
| 29 | 1 | 0 | 0 | 104 | 42 | 86 |
| 30 | 7 | 4 | 0 | 150 | 38 | 62 |
| 31 | 4 | 0 | 0 | 196 | 132 | 173 |
| 32 | 0 | 0 | 0 | 3 | 0 | 2 |
| 33 | 0 | 0 | 26 | 60 | 0 | 2 |
| 34 | 2 | 0 | 0 | 160 | 78 | 103 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 35 | 0 | 0 | 0 | 57 | 9 | 19 |
| 36 | 6 | 0 | 0 | 40 | 17 | 13 |
| 37 | 3 | 0 | 0 | 62 | 17 | 25 |
| 38 | 2 | 0 | 0 | 142 | 43 | 91 |
| 39 | 1 | 0 | 0 | 21 | 9 | 14 |
| 40 | 0 | 0 | 0 | 7 | 6 | 3 |
| 41 | 1 | 0 | 0 | 82 | 14 | 20 |
| 42 | 1 | 0 | 0 | 44 | 16 | 21 |
| 43 | 1 | 0 | 0 | 84 | 46 | 40 |
| 44 | 11 | 0 | 0 | 46 | 58 | 35 |
| 45 | 0 | 0 | 0 | 43 | 15 | 19 |
| 46 | 3 | 0 | 0 | 82 | 55 | 32 |
| 47 | 5 | 1 | 0 | 107 | 74 | 30 |
| 48 | 2 | 1 | 0 | 43 | 8 | 7 |
| 49 | 1 | 0 | 0 | 21 | 15 | 6 |
| 50 | 1 | 0 | 0 | 12 | 9 | 2 |
| 51 | 10 | 0 | 0 | 280 | 21 | 78 |
| 52 | 3 | 0 | 0 | 82 | 34 | 32 |
| 53 | 1 | 0 | 0 | 29 | 43 | 15 |
| 54 | 0 | 0 | 0 | 43 | 44 | 16 |
| 55 | 3 | 0 | 0 | 17 | 8 | 4 |
| 56 | 1 | 0 | 0 | 40 | 26 | 39 |
| 57 | 1 | 0 | 0 | 133 | 37 | 52 |
| 58 | 1 | 0 | 0 | 83 | 14 | 25 |
| 59 | 2 | 0 | 0 | 18 | 16 | 8 |
| 60 | 1 | 0 | 0 | 32 | 10 | 3 |
| 61 | 0 | 0 | 0 | 51 | 12 | 3 |
| 62 | 1 | 3 | 0 | 62 | 7 | 25 |
| 63 | 2 | 2 | 0 | 70 | 16 | 64 |
| 64 | 1 | 0 | 0 | 116 | 40 | 77 |
| 65 | 6 | 2 | 0 | 223 | 163 | 69 |
| 66 | 1 | 6 | 29 | 53 | 38 | 28 |
| 67 | 1 | 0 | 0 | 207 | 34 | 80 |
| 68 | 1 | 0 | 0 | 91 | 42 | 83 |
| 69 | 1 | 8 | 12 | 48 | 22 | 49 |
| 70 | 1 | 6 | 4 | 126 | 40 | 35 |
| 71 | 1 | 1 | 39 | 74 | 21 | 23 |
| 72 | 1 | 2 | 0 | 87 | 37 | 47 |
| 73 | 1 | 0 | 0 | 43 | 29 | 24 |
| 74 | 2 | 5 | 2 | 77 | 88 | 24 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 75 | 1 | 1 | 0 | 45 | 4 | 15 |
| 76 | 1 | 0 | 0 | 50 | 37 | 28 |
| 77 | 4 | 2 | 1 | 119 | 58 | 55 |
| 78 | 1 | 2 | 3 | 99 | 56 | 38 |
| 79 | 1 | 0 | 0 | 171 | 93 | 61 |
| 80 | 2 | 0 | 0 | 197 | 149 | 31 |
| 81 | 6 | 4 | 0 | 52 | 15 | 20 |
| 82 | 1 | 0 | 0 | 74 | 30 | 78 |

Table S2. Organisms reported in the literature as associated with ants of the genus *Neoponera* EMERY, 1901, and type of interaction.

| Class | Order | Family | Taxon | Host | Interaction with ants | Country | Reference |
|---|---|---|---|---|---|---|---|
| Nematoda | Mermithida | Mermithidae | Unidentified | *N. inversa N. villosa* | Parasite of workers | Colombia, Venezuela | EMERY 1904; WHEELER 1928; |
| Arachnida | Sarcoptiformes | Histiostomatidae | *Histiostoma* spp. | *N. apicalis*<br>*N. inversa*<br>*N. verenae*<br>*N. villosa* | Hypopus1 phoretic on workers (mandibles) | Brazil | LOPES & al. 2015a |
| | Mesostigmata | Laelapidae | *Cosmolaelaps* spp. | *N. apicalis*<br>*N. verenae*<br>*N. villosa* | Phoretic? Adults and immatures found upon cocoons | Brazil | LOPES & al. 2015a |
| | | | *Cosmolaelaps pronex* SILVA, MOREIRA & OLIVEIRA, 2018 | *N. inversa* | Females and males phoretic on ant brood (mostly cocoons) | Brazil | SILVA & al. 2018 |
| | | Oplitidae | *Oplitis* spp. | *N. apicalis*<br>*N. inversa*<br>*N. verenae*<br>*N. villosa* | Females phoretic on workers (legs) | Brazil | LOPES & al. 2015a |
| | | | *Oplitis apicalis* LOPES, OLIVEIRA DELABIE & KLOMPEN, 2015 | *N. apicalis*<br>*N. inversa*<br>*N. verenae* | Females phoretic on workers (legs) | Brazil | LOPES & al. 2015b |
| | Trombidiformes | Neopygmephoridae | *Petalomium* spp. | *N. apicalis*<br>*N. inversa*<br>*N. verenae*<br>*N. villosa* | Females phoretic on workers (between the coxae) | Brazil | LOPES & al. 2015a |
| | | | *Petalomium verenae* | *N. verenae* | Females phoretic on | Brazil | SILVA & al. 2017 |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
|  |  |  | SILVA, KHAUSTOV & OLIVEIRA, 2017 |  | workers (thorax, ventrad) |  |  |
| Entognatha | Collembola | Cyphoderidae<br>Isotomidae<br>Brachystomellidae<br>Neanuridae | *Cyphoderus similis* FOLSOM, 1927<br>*Folsomides parvulus* STACH, 1922<br>*Proisotoma minima* (ABSOLON, 1901)<br>*Brachystomella* sp.<br>*Arlesia albipes* (FOLSOM, 1927) | *N. inversa*<br>*N villos* | Ant associates | Brazil | CASTAÑO-MENESES & al. 2015 |
|  |  | Entomobryidae<br>Sminthurididae<br>Neelidae | *Pseudosinella* sp.<br>*Sphaeridia serrata* FOLSOM & MILLS, 1938<br>*Megalothorax* sp. | *N. inversa* | Ant associates | Brazil | CASTAÑO-MENESES & AL. 2015 |
| Insecta | Hymenoptera | Encyrtidae | *Blanchardiscus* sp. *ca pollux* NOYES, 2004 | *N. goeldii* | Gregarious endoparasitoid of pupae | French Guiana | PÉREZ - LACHAUD & al. 2012 |
|  |  |  | *Blanchardiscus* sp. | *N. villosa* | Gregarious endoparasitoid of male pupae | Mexico | PÉREZ - LACHAUD & LACHAUD 2017 |
|  |  | Perilampidae | Unidentified | *N. luteola* | Pupal parasitoid | Peru | DAVIDSON & FISHER 1991 |
|  |  | Eucharitidae | *Kapala* sp.1 | *N. apicalis* | Solitary, pupal ectoparasitoid | Mexico<br>French Guiana | DE LA MORA & PHILPOTT 2010; LACHAUD & al. 2012 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Kapala* sp.2 | *N. verenae* | Solitary, pupal ectoparasitoid | French Guiana | LACHAUD & al. 2012 |
| | Diptera | Syrphidae | *Hypselosyrphus trigonus* HULL, 1937 | *N. villosa* | Solitary, pupal ectoparasitoid | Mexico | PÉREZ-LACHAUD & al. 2014 |
| | | Phoridae | *Apocephalus paraponerae* BORGMEIER, 1958<br>*A. crassilatus* BROWN, 2000<br>*A. globosus* BROWN, 2000 | *N. villosa* | Females are attracted to injured ants (oviposition not confirmed)2 | Brazil<br>Colombia<br>Costa Rica | BROWN 2000 |
| | | | *Cataclinusa bucki* SCHMITZ, 1927 | *N. crenata* | Males and females attracted to ant nest | | BORGMEIER 1962 in DISNEY 1994 |
| | | | *Ecitomyia juxtaposita* BORGMEIER, 1960 | *N. laevigata* | Adult female resident in the nest | | BORGMEIER 1960 in DISNEY 1994 |
| | | unidentified | unidentified | *N. commutata* | Gregarious dipteran parasitoid of ant pupae | French Guiana | LACHAUD & al. 2012 |

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[1] Hypopu: deutonymph with a sucker, a morphological adaptation for attaching to host.

[2] Several other phorid species are attracted to injured Neoponera spp. See BROWn & al. 2015 (Suppl. material 1) for an exhaustive list.

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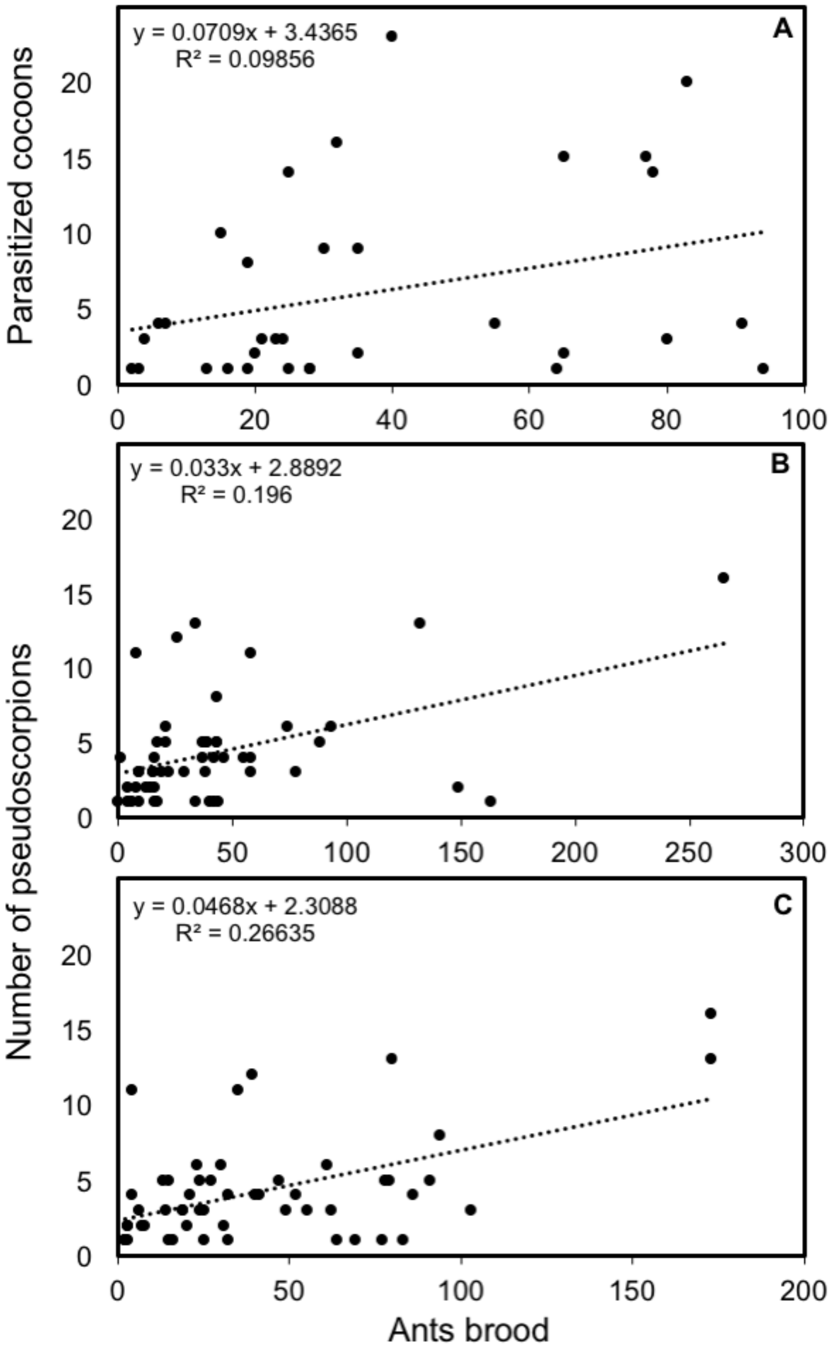


Figure S1. Relationship between available host brood and selected myrmecophiles. A) Relationship between the number of parasitized cocoons and the number of available cocoons in colonies parasitized by the ectoparasitoids *Kapala* n. sp. and *Hypselosyrphus trigonus*. B-C) Relationship between the number of pseudoscorpions and the target brood stages: B) ant larvae, and C) ant cocoons.

Figure S2. *Neoponera villosa* myrmecophiles exploring the host cocoons. A) *Trichopria* sp., B) *Myrmigaster* n. sp.

# CAPÍTULO III

# ¿Cómo escapar del nido del hospedero? El caso de la mosca *Hypselosyrphus trigonus,* parasitoide de *Neoponera villosa*

## Borrador

## Resumen

Las larvas/prepupas de la hormiga Neotropical *Neoponera villosa* que nidifican en la bromelia *Aechmea bracteata* son parasitadas por la mosca *Hypselosyrphus trigonus*. Los estados inmaduros del parasitoide se desarrollan bien protegidos dentro del cocón del hospedero; sin embargo, la coexistencia con estas hormigas agresivas implica dos problemas para los adultos: i) encontrar un hospedero adecuado para ovipositar (en el caso de las hembras), y ii) escapar del nido natal para reproducirse. El objetivo de este trabajo fue determinar cómo las moscas adultas logran escapar ilesas del cocón y nido de un hospedero tan agresivo, ya que los adultos de esta especie tienen un aparato bucal esponjoso que les impide cortar un agujero de escape en el cocón del hospedero y salir por sí mismas. Se realizaron observaciones y bioensayos en el laboratorio utilizando cocones parasitados y sin parasitar. Para cada bioensayo se confinaron en frascos de vidrio cinco obreras de *N. villosa* junto con un cocón parasitado y uno no parasitado procedentes de la misma colonia. El comportamiento de las obreras se registró y el estado de los cocones se evaluó 24 h después (intacto vs. abierto). Los cocones que no fueron abiertos por las hormigas fueron posteriormente disectados para confirmar la presencia de la cría de hormiga o del parasitoide. En todos los bioensayos (n = 30), las obreras de *N. villosa* abrieron el cocón parasitado y depositaron el pupario del parasitoide en el basurero; por el contrario, todos los cocones no parasitados permanecieron intactos después de 24 h de confinamiento con las obreras. De los puparios descartados en la basura, en el 90% de los casos, las moscas adultas emergieron sin daño. En un solo caso, una hembra emergió estando aún expuesta a las hormigas, y fue atacada hasta su muerte. Adicionalmente, durante el muestreo y colecta de *N. villosa*, se hallaron siete puparios intactos y cuatro vacíos en la pila de desechos de la colonia, entre las hojas de la planta hospedera, lo que confirma el comportamiento de rechazo de los puparios en condiciones naturales. Por otro lado, para determinar qué estímulos desencadenan el comportamiento del hospedero, se extrajeron y analizaron en cromatografía de gases los hidrocarburos cuticulares (HC) y otros compuestos presentes en la cutícula de inmaduros del parasitoide y de inmaduros y adultos de la hormiga hospedera. Los cromatogramas señalaron diferencias entre los HC de cocones parasitados y no parasitados, con la presencia de compuestos producidos por el parasitoide. Los resultados de las observaciones y experimentos sugieren que las obreras de *N. villosa* abren sólo los cocones parasitados y sacan del nido los puparios del parasitoide. La cutícula rígida de los puparios representa una defensa mecánica exitosa al ser manipulados por las hormigas. El rechazo de los puparios permite a la mosca parasitoide completar su desarrollo y emerger en un lugar seguro, evadiendo la interacción directa con las hormigas. En muchos casos, los individuos parasitados suelen modificar su comportamiento como resultado de la manipulación por sus parásitos/parasitoides, aumentando así la supervivencia de estos últimos. En este trabajo se demostró la manipulación de la colonia completa del hospedero por parte del parasitoide al manipular el comportamiento de hormigas no parasitadas.

**Palabras clave:** hormiga, interacción, manipulación del comportamiento, mirmecófilo, señales químicas, Syrphidae.

## Introducción

Las hormigas se distinguen particularmente de otros insectos en sus complejas sociedades cerradas (Emerson 1939; Hölldobler y Wilson 1990). Sus colonias y nidos ofrecen un microhábitat con condiciones ambientales estables y con abundantes recursos (Hölldobler y Wilson 1990) que son atractivos para una amplia gama de otros organismos generalmente denominados mirmecófilos (Donisthorpe 1927; Kistner 1979; Hölldobler y Wilson 1990; Kronauer y Pierce 2011). Como todos los insectos eusociales, las hormigas presentan una variedad de canales de comunicación que les permiten regular su organización social y actuar como un superorganismo (Hölldobler y Wilson 1990). El principal canal de comunicación se basa en señales químicas a través de perfiles de hidrocarburos cuticulares compartidos por todos los miembros de la colonia (Liu et al. 2000; Howard y Blomquist 2005; d'Ettorre y Lenoir 2010), por el cual las obreras pueden discriminar entre individuos que pertenecen a la misma colonia de aquellos ajenos (Hölldobler y Wilson 1990; Lenoir et al. 2001; Breed 2003; Bagnères y Lorenzi 2010; d'Ettorre y Lenoir 2010), aunque también pueden intervenir otros estímulos (p. ej. estímulos táctiles, visuales y acústicos: ver Le Masne 1953; Hickling y Brown 2000; Nelson y Jackson 2006; Cammaerts 2007; Barbero et al. 2009, 2012; Di Giulio et al. 2015).

En las hormigas, los mecanismos de reconocimiento del congénere son esenciales para evitar que los intrusos exploten los recursos de la colonia. En general, se conoce como inmunidad social a cualquier defensa a nivel de la colonia contra patógenos e intrusos (Cremer et al. 2007), incluyendo la necroforesis (expulsión de cadáveres fuera del nido) (Diez et al. 2011, 2014), el comportamiento profiláctico (limpieza de desechos potencialmente patógenos) (Diez et al. 2012), y el rechazo de intrusos que rompen los sistemas de defensa de las hormigas (Rocha et al. 2014; Pérez-Lachaud et al. 2015, 2019). Sin embargo, muchos intrusos mirmecófilos han desarrollado diversas adaptaciones (incluyendo adaptaciones comportamentales, anatómicas, acústicas o químicas) que les permiten romper los complejos sistemas de comunicación y las sofisticadas defensas de las hormigas y ser tolerados mientras explotan los recursos dentro de los nidos hospederos (Hölldobler 1971; Vander Meer y Wojcik 1982; Steidle y Dettner 1993; Fiedler et al. 1996; Akino 2002; Pierce et al. 2002; Barbero et al. 2009; Hojo et al. 2009; Bagnères y Lorenzi 2010; Riva et al. 2016). Las estrategias de infiltración han sido muy estudiadas en varios mirmecófilos (p. ej. Allies et al. 1986; Akino et al. 1996; Martin et al. 2007; Barbero et al. 2009; Hojo et al. 2009; Witte et al. 2009; Bagnères y Lorenzi 2010; Parker 2016; Riva et al. 2016; de Bekker et al. 2018; Neupert et al. 2018); sin embargo, los estudios sobre las estrategias utilizadas por los intrusos (o sus crías) para abandonar los nidos de la hormiga hospedera siguen siendo poco comunes (Lhomme et al. 2012).

Hasta la fecha, las especies conocidas de mirmecófilos se incluyen en 39 órdenes de artrópodos (Kistner 1982; Hölldobler y Wilson 1990; Schmidt-Hempel 1998; Rettenmeyer et al. 2011; Lachaud y Pérez-Lachaud 2012; Maruyama et al. 2013; Lachaud et al. 2016; Quevillon y Hughes 2018; Castaño-Meneses et al. 2019). Las interacciones con las hormigas van desde el mutualismo y el comensalismo hasta la depredación y el parasitismo o parasitoidismo (Kistner 1982; Hölldobler y Wilson 1990; Feener 2000; Lachaud y Pérez-Lachaud 2012; Poinar 2012; Lachaud et al. 2013; Pérez-Lachaud et al. 2014). Los parasitoides de hormigas incluyen representantes de varias familias de avispas, ácaros, estrepsípteros, moscas y nemátodos (Gösswald 1950; Feener y Brown 1997; Brown 2000; Kathirithamby 2009; Lachaud y Pérez-Lachaud 2012; Pérez-Lachaud et al. 2012; Poinar 2012; Pérez-Lachaud et al. 2014; González et al. 2016; Lachaud et al. 2016), que atacan a la cría de las hormigas o a los adultos y causan daño directo a la colonia. De éstos, la mosca *Hypselosyrphus trigonus* Hull, 1937 (Diptera: Syrphidae), un microdontino neotropical que actúa como parasitoide primario, es el único sírfido parasitoide reportado que ataca las etapas inmaduras de las hormigas (Pérez-Lachaud et al. 2014; Pérez-Lachaud y Lachaud 2017). Los estados inmaduros de la mosca sírfida se desarrollan bien protegidos dentro del cocón del hospedero; sin embargo, la coexistencia con estas hormigas agresivas implica dos desafíos: encontrar un hospedero adecuado para ovipositar y escapar del nido natal.

En este estudio, realizamos bioensayos para esclarecer cómo las moscas adultas de *H. trigonus* logran escapar ilesas del cocón y del nido de un hospedero tan agresivo, ya que además tienen un aparato bucal esponjoso que les impide cortar un agujero de escape en el cocón del hospedero (Labandeira 1997). Observaciones iniciales sugieren que las hormigas mismas pueden intervenir en este proceso. Este estudio pretende responder a las siguientes preguntas: 1) ¿Pueden los parasitoides de *H. trigonus* romper el cocón del hospedero para escapar del nido? 2) ¿Utiliza el parasitoide señales químicas para manipular el comportamiento del hospedero?

## Materiales y métodos

### *Hormiga hospedera*

La hormiga ponerina *Neoponera villosa* presenta una amplia distribución desde Texas hasta Argentina (Mackay y Mackay 2010). En el sur de la Península de Yucatán en México, *N. villosa* nidifica principalmente en la bromelia-tanque *Aechmea bracteata* (Sw.) Griseb (Dejean 1990; Dejean et al. 1995; Dejean y Olmsted 1997). Estas hormigas son depredadoras generalistas. Presentan una estrategia de caza individual, sin reclutamiento (Lachaud et al. 1984; Pérez-Bautista et al. 1985; García-Pérez 1989; Dejean y Corbara 1990) y pueden responder de manera diferente a distintos tipos de presas (Dejean y Corbara 1990).

Para la obtención del material biológico, se colectaron cinco colonias de *N. villosa*, las cuales estaban parasitadas por *H. trigonus*. Se registró la composición de cada colonia de hormiga hospedera y para evaluar cuales cocones estaban parasitados por *H. trigonus*, se revisaron a contraluz bajo un estéreo-microscopio (Cuadro 1).

Cuadro 1. Composición de las colonias de *N. villosa* y número de cocones parasitados.

| **Sitio de colecta** | **Fecha de colecta** | **Reinas** | **Hembras aladas** | **Machos** | **Obreras** | **Larvas** | **Cocones** | **Cocones parasitados** |
|---|---|---|---|---|---|---|---|---|
| Nuevo Becal | 17/01/17 | 1 | 0 | 0 | 180 | 69 | 65 | 13 |
| Sian Ka´an | 21/11/17 | 1 | 0 | 0 | 84 | 46 | 40 | 23 |
| Nuevo Becal | 04/12/17 | 3 | 0 | 0 | 82 | 55 | 32 | 16 |
| Nuevo Becal | 04/12/17 | 5 | 1 | 0 | 107 | 74 | 30 | 9 |
| Nuevo Becal | 04/12/17 | 2 | 1 | 0 | 43 | 8 | 7 | 4 |

### *Bioensayos*

Los bioensayos se llevaron a cabo después de un período de 7 días de aclimatación de las hormigas a las condiciones de laboratorio. Las hormigas de una misma colonia y las larvas y huevos, se mantuvieron en nidos artificiales (recipientes de plástico de 14 cm de diámetro x 25 cm de altura) provistos de bebederos (tubos de vidrio con agua tapados con algodón) bajo iluminación natural y temperatura ambiente (28 ºC ± 2 °C y 75% ± 5% humedad relativa). Las hormigas se alimentaron con pulpa de manzana como fuente azucarada y con larvas de *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae) como fuente de proteínas. Las obreras fueron seleccionadas al azar del nido original y solo fueron evaluadas una vez. Las observaciones se llevaron a cabo del 27 de enero al 18 de diciembre de 2018, y se realizó un total de 30 repeticiones.

Para evaluar si las moscas adultas pueden romper y escapar del cocón del hospedero, se confinaron seis cocones parasitados en frascos de vidrio, por un período de 30 días.

Después de ese período de tiempo, se evaluó si los cocones habían sido abiertos o permanecían intactos; posteriormente, se disectaron los cocones intactos y se evaluó el estado de desarrollo del parasitoide. Adicionalmente, se realizó la disección de otros tres puparios que fueron expulsados por las obreras para evaluar el estado de desarrollo del parasitoide.

Los bioensayos se realizaron en cámaras de observación (frascos de vidrio transparente, 9 cm de diámetro x 15 cm de longitud). Para cada bioensayo, un grupo de cinco obreras de *N. villosa* junto con un cocón parasitado y uno no parasitado (control) procedentes de una misma colonia, fueron confinados en una cámara de observación que incluía un vial de 10-ml con agua tapado con algodón en un extremo. Las hormigas fueron alimentadas con pulpa de manzana como fuente azucarada, la cual fue colocada en el contenedor durante el tiempo del bioensayo. La cámara de observación se cerró y se apartó durante 24 horas. El comportamiento de las obreras se registró y el estado de los cocones se evaluó 24 horas después (intacto vs. abierto). Se recuperaron los puparios que fueron expulsados por las hormigas, se mantuvieron en confinamiento en espera de la emergencia de los adultos, y se registró su sexo. Los cocones que no fueron abiertos por las hormigas, fueron disectados para confirmar la presencia de la cría de hormigas o del parasitoide.

### *Extracción y análisis de hidrocarburos cuticulares*

Para evaluar si las señales químicas desencadenan el comportamiento de rechazo y con el objetivo de determinar si los cocones parasitados difieren de los cocones no parasitados, se analizaron extractos en hexano de cocones parasitados (n = 5), puparios del parasitoide *H. trigonus* (n = 5) y, cocones no parasitados (n = 5), así como obreras de la hormiga hospedera (n = 5).

La extracción de los hidrocarburos cuticulares (HC) y otros compuestos presentes también en la cutícula, se realizó sumergiendo individualmente los puparios, cocones y adultos en 200 microlitros de hexano durante 24 horas. Antes de la extracción, las hormigas vivas fueron sacrificadas por congelación durante 1 minuto. Después de 24 horas, los organismos fueron retirados del hexano, y los viales se mantuvieron en un congelador (-20 ºC) para evitar la evaporación. Los extractos y la pureza del solvente, fueron examinados a través del método analítico de cromatografía de gases acoplado a espectrometría de masas (GC/MS, por sus siglas en inglés), en el laboratorio del Departamento de Zoología de la Universidad de Tel Aviv, en Tel Aviv, Israel.

## Resultados

### *Respuesta conductual de la hormiga hospedera*

El repertorio conductual observado en las obreras de *N. villosa* al entrar en contacto con cocones parasitados, involucró un repertorio de siete comportamientos distintos (información obtenida a partir de tres videos): deambulación, detección por contacto, antenación, apertura del cocón, sujeta y levanta, transporte y rechazo (Cuadro 2). La detección de los cocones parasitados generalmente es realizado por más de una obrera, las cuales inmediatamente realizan la exploración prolongada de los cocones a través de la antenación, hasta que finalmente una de ellas comienza a romper el cocón parasitado (Figura 1). Una vez que el cocón ha sido abierto, la obrera sujeta y levanta el pupario con las mandíbulas para después realizar el transporte. El rechazo del intruso se lleva a cabo cuando el pupario es arrojado a la pila de basura donde se desechan los cadáveres de miembros de la colonia. Por el contrario, los cocones sanos no generaron la atención de las obreras en el tiempo de confinamiento. Estos permanecieron intactos y generalmente no fueron manipulados por las obreras.

Cuadro 2. Repertorio conductual observado en las obreras de *N. villosa* al encontrarse con cocones parasitados.

| Evento comportamental | Descripción |
|---|---|
| Deambulación | Las obreras deambulan sin objetivo fijo dentro de la cámara de observación. |
| Detección por contacto | Durante la deambulación dentro de la cámara de observación, las obreras hacen contacto con el cocón parasitado y/o el cocón control. |
| Antenación | La obrera realiza golpes repetidos con sus antenas (exploración). |
| Apertura del cocón | La obrera rompe con las mandíbulas el cocón parasitado y saca el pupario. |
| Sujeta y levanta | La obrera agarra y levanta entre sus mandíbulas el pupario del parasitoide. |
| Transporte | La obrera transporta el pupario del parasitoide, sujetándolo con las mandíbulas. |
| Rechazo del pupario | La obrera trasporta el pupario, y lo deposita en el basurero establecido en la cámara de observación. |

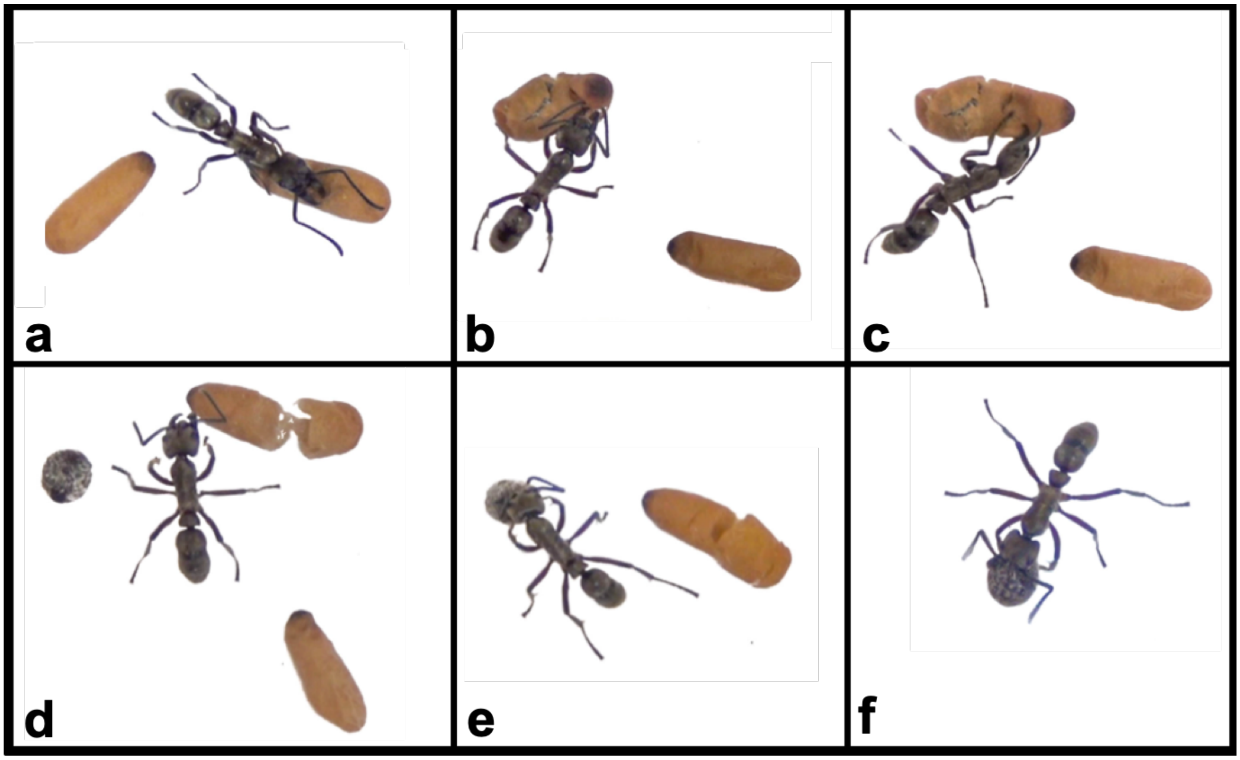


Figura 1. Ilustración del comportamiento de obreras de *N. villosa* en el reconocimiento y la apertura de cocones parasitados por *Hypselosyrphus trigonus*. a) exploración del cocón; b) y c) apertura del cocón parasitado; d) cocón abierto; el pupario ha sido ya extraído del cocón; e) la obrera retiene el cocón con las mandíbulas; f) la obrera transporta el pupario entre sus mandíbulas. Instantáneas tomadas a partir de uno de los videos.

Después de 30 días de confinamiento, los seis cocones parasitados que fueron aislados para comprobar si las moscas adultas podían cortar el cocón para escapar, permanecieron intactos. Las disecciones de los cocones revelaron que 4 adultos emergieron exitosamente de los puparios pero fueron incapaces de abrir los cocones del hospedero y murieron dentro (2 machos, 2 hembras) y una hembra fue encontrada muerta dentro del pupario; mientras que el pupario restante ya no prosiguió su desarrollo. Por otro lado, tres puparios que habían sido expulsados por las hormigas fueron disectados para verificar el estado de desarrollo del parasitoide al momento de ser descartados de la colonia hospedera. Las disecciones mostraron que los parasitoides seguian siendo larvas/prepupas.

*Bioensayos y supervivencia del parasitoide*

En todos los bioensayos (n = 30), las obreras de *N. villosa* abrieron el cocón parasitado y arrojaron los puparios de las moscas detrás del depósito de agua; por el contrario, todos los cocones sanos permanecieron intactos después de 24 horas de confinamiento con las obreras. En el 90 % de los puparios descartados, las moscas adultas emergieron sin daños (12 machos, 15 hembras) mientras que en los tres casos restantes, el parasitoide no prosiguió su desarrollo. El tiempo promedio de emergencia de pupas a adultos fue de 20 ± 0.46 días (N = 24). En un caso, una hembra emergió mientras estaba expuesta a las hormigas y éstas la mataron.

Adicionalmente, durante el muestreo de las bromelias en marzo de 2018 en la localidad de Nuevo Becal, encontramos siete puparios de estos sírfidos en tres colonias (nido 1: 4 puparios, nido 2: 2, nido 3: 1) entre las hojas externas de la planta hospedera que contenían los desechos de la colonia (cutícula de insectos, tierra, etc.). Todos estos puparios continuaron su desarrollo y los adultos emergieron de 15 a 19 días después de haberlos colectado (machos: 4, hembras: 3). Además, se encontraron cuatro puparios vacíos en la pila de basura entre las hojas externas de la planta hospedera de una colonia, lo que corrobora el rechazo por las obreras en condiciones naturales.

### *Cromatogramas y comparación de los compuestos químicos*

Se identificaron un total de 90 compuestos procedentes de obreras, cocones sanos y parasitados, así como de puparios de *H. trigonus* (Cuadro 3). Estos compuestos estuvieron agrupados en nueve grupos funcionales. Los hidrocarburos cuticulares representaron el 60% (n = 54) de los compuestos de la cutícula (alcanos: 18.9%, n = 17; alquenos: 8.9%, n = 8 y alcanos metilados: 32.2%, n = 29). Otros compuestos presentes en la cutícula fueron los aldehídos con el 11.1% (n = 10), seguido de los alcoholes con el 8.9% (n = 8). Cada uno de los grupos restantes presentaron menos del 7.8% (n = 1 a 7) de los compuestos químicos.

Cuadro 3. Hidrocarburos y otros compuestos cuticulares reportados en los análisis en cromatografía de gases para inmaduros del parasitoide e inmaduros y adultos de la hormiga hospedera. + = baja, ++ = media, +++ = alta (abundancia).

| Tiempo de retención | Compuesto | Cocón parasitado | Cocón no parasitado | Pupario | Obrera | Grupo funcional |
|---|---|---|---|---|---|---|
| 5 | 4-methyl-3-heptanone | | | | + | Cetona |
| 5.4 | 4-methyl-3-heptanol | | | | + | Alcohol |
| 6.4 | 2-ethyl-1-hexanol | | | | + | Alcohol |
| 7.6 | Nonanal | + | + | | | Aldehído |
| 7.8 | Phenethyl alcohol | | | | + | Alcohol |
| 9 | Dodecane | + | + | | | Alcano |
| 10.4 | Tridecane | | | | + | Alcano |
| 11.2 | 2-methyl tridecane | | | | + | Alcano |
| 11.7 | Tetradecane | | | | + | Alcano |
| 12.4 | 2-methyl tetradecane | | | | + | Alcano |
| 12.8 | Pentadecadiene | | | | + | Alqueno |
| 12.8 | Pentadecene | | | | + | Alqueno |
| 13 | Pentadecane | + | + | | ++ | Alcano |
| 13.2 | Dodecanoic acid | + | + | | | Ácido carboxílico |
| 13.8 | 3-methyl pentadecane | | | | + | Alcano |
| 13.9 | Tetradecanol | | | | + | Alcohol |
| 14 | Hexadecene | | | | + | Alqueno |
| 14.1 | Heptadecadiene | + | + | | | Alqueno |
| 14.2 | Hexadecane | | | | + | Alcano |
| 15 | Oleyl alcohol | | | | + | Alcohol |
| 15 | Heptadecene | + | + | | +++ | Alqueno |
| 15.2 | Heptadecane | + | + | | + | Alcano |
| 15.6 | Unidentified sesquiterpene | | | | + | Terpeno |
| 16 | Palmitic acid | | + | | | Ácido carboxílico |
| 16.2 | Farnesol | | | | + | Alcohol |
| 17.1 | Nonadecene | + | | | | Alqueno |
| 17.4 | Nonadecane | + | + | | | Alcano |
| 17.8 | Palmitoleic acid | | + | | | Ácido carboxílico |
| 18 | Palmitic acid | + | + | | + | Ácido carboxílico |
| 18.4 | Octadecanal | | | | + | Aldehído |
| 18.4 | Ethyl palmitate | + | + | | | Éster |
| 18.7 | 5,9-dimethyl pentatriacontane + octadecanal | | | ++ | | Aldehído |
| 19.2 | Geranyl geraniol | | | | + | Terpeno |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| 19.2 | Heneicosane | + |  | + |  | Alcano |
| 20 | Linoleic acid |  | ++ |  |  | Ácido carboxílico |
| 20 | Oleic acid | + | ++ |  | +++ | Ácido carboxílico |
| 20 | Stearic acid |  |  |  | + | Ácido carboxílico |
| 20 | Ethyl linoleate | + | + |  |  | Éster |
| 20 | Ethyl oleate |  | + |  |  | Éster |
| 20 | Ethyl stearate | + |  |  |  | Éster |
| 20.5 | Eicosanal |  |  | + |  | Aldehído |
| 21 | Tricosane | + |  | ++ |  | Alcano |
| 21.2 | Arachidonic acid acid |  |  |  | + | Ácido carboxílico |
| 21.6 | Ethyl eicosanoate | + |  |  |  | Éster |
| 21.8 | Tetracosane | + |  | + |  | Alcano |
| 22 | Eicosyl acetate |  |  | + |  | Éster |
| 22.2 | Docosanal |  |  | + |  | Aldehído |
| 22.6 | Pentacosane | + | + | +++ |  | Alcano |
| 23.2 | Ethyl docosanoate | + |  |  |  | Éster |
| 23.5 | Hexacosane |  | + | + |  | Alcano |
| 23.8 | Tetracosane | + |  |  |  | Alcano |
| 23.8 | Tetracosanal |  |  | + |  | Aldehído |
| 24 | 1-Heptacosene |  |  | + |  | Alqueno |
| 24 | 2-Heptacosene |  |  | + |  | Alqueno |
| 24.2 | Heptacosane | + | + | ++ |  | Alcano |
| 24.4 | 11 + 13-methyl heptacosane | + |  |  |  | Alcano |
| 24.9 | Octacosane | + | + | + |  | Alcano |
| 25 | Squalene |  |  | + |  | Terpeno |
| 25.2 | Hexacosanal |  |  | + |  | Aldehído |
| 25.6 | Nonacosane | + | ++ | + | + | Alcano |
| 25.8 | 13-Methyl nonacosane |  |  | + |  | Alcano |
| 25.8 | 11 + 13-methyl nonacosane | + |  |  |  | Alcano |
| 26 | 4-methyl nonacosane |  | + |  |  | Alcano |
| 26.2 | 3-methyl nonacosane | + | + |  |  | Alcano |
| 26.4 | Triacontane |  | + |  | + | Alcano |
| 26.8 | Dotriacontanal | + |  |  |  | Aldehído |
| 26.8 | Octacosanal |  | + |  |  | Aldehído |
| 27 | 2-methyl triacontane | + | + |  | ++ | Alcano |
| 27.2 | Hentriacontane | + | + |  | + | Alcano |
| 27.8 | 11-methyl hentriacontane |  |  |  | + | Alcano |
| 27.8 | Cholesterol |  |  |  | + | Alcohol |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| 28 | 3-methyl hentriacontane |  | + |  | + | Alcano |
| 28.4 | 5,9-dimethyl hentriacontane |  |  |  | + | Alcano |
| 28.8 | 3,9-dimethyl hentriacontane |  |  |  | + | Alcano |
| 28.8 | Dotriacontanal | + | + |  |  | Aldehído |
| 29.2 | 10-methyl dotriacontane |  |  |  | + | Alcano |
| 29.6 | 4,10-dimethyl dotriacontane |  |  |  | + | Alcano |
| 30 | 2,10,14-trimethyl dotriacontane |  |  |  | + | Alcano |
| 30.2 | 11-methyl tritriacontane |  |  |  | + | Alcano |
| 30.6 | 11,15-dimethyl tritriacontane |  |  |  | +++ | Alcano |
| 30.8 | 5,11-dimethyl tritriacontane |  |  |  | + | Alcano |
| 31 | 7,11,15-trimethyl tritriacontane |  |  |  | + | Alcano |
| 31.2 | 5,11,15-trimethyl tritriacontane |  |  |  | ++ | Alcano |
| 31.8 | 3,11,15-trimethyl tritriacontane |  |  |  | + | Alcano |
| 32 | 1,30-triacontanediol |  | + |  |  | Alcohol |
| 32.4 | 10 + 12 + 14-methyl tetratriacontane |  |  |  | + | Alcano |
| 33.8 | 11-methyl pentatriacontane |  |  |  | + | Alcano |
| 34.4 | 11,15-dimethyl pentatriacontane |  |  |  | ++ | Alcano |
| 35 | 9,13-dimethyl pentatriacontane |  |  |  | + | Alcano |
| 35.2 | 7,11-dimethyl pentatriacontane |  |  |  | + | Alcano |
| 35.6 | 5,9-dimethyl pentatriacontane |  |  |  | + | Alcano |
| 36.8 | 7,9,13-trimethyl pentatriacontane |  |  |  | + | Alcano |

Los cocones no parasitados y parasitados presentaron 29 diferentes compuestos químicos cada uno, los puparios del parasitoide 18 y las obreras 51. Cabe señalar que, los cocones parasitados presentaron siete compuestos exclusivos que no estaban presentes en los cocones no parasitados, en puparios ni en obreras (Fig. 2, 3 y 5). Los cocones parasitados y no parasitados compartieron 20 compuestos químicos y difirieron en 18 (9 específicos de los cocones parasitados y 9 específicos de los cocones no parasitados). Por otra parte, los puparios del parasitoide presentaron un perfil específico, con 10 compuestos exclusivos y difieren en 11, 13 y 17 compuestos que no fueron hallados en los cocones parasitados, cocones no parasitados y obreras, respectivamente.

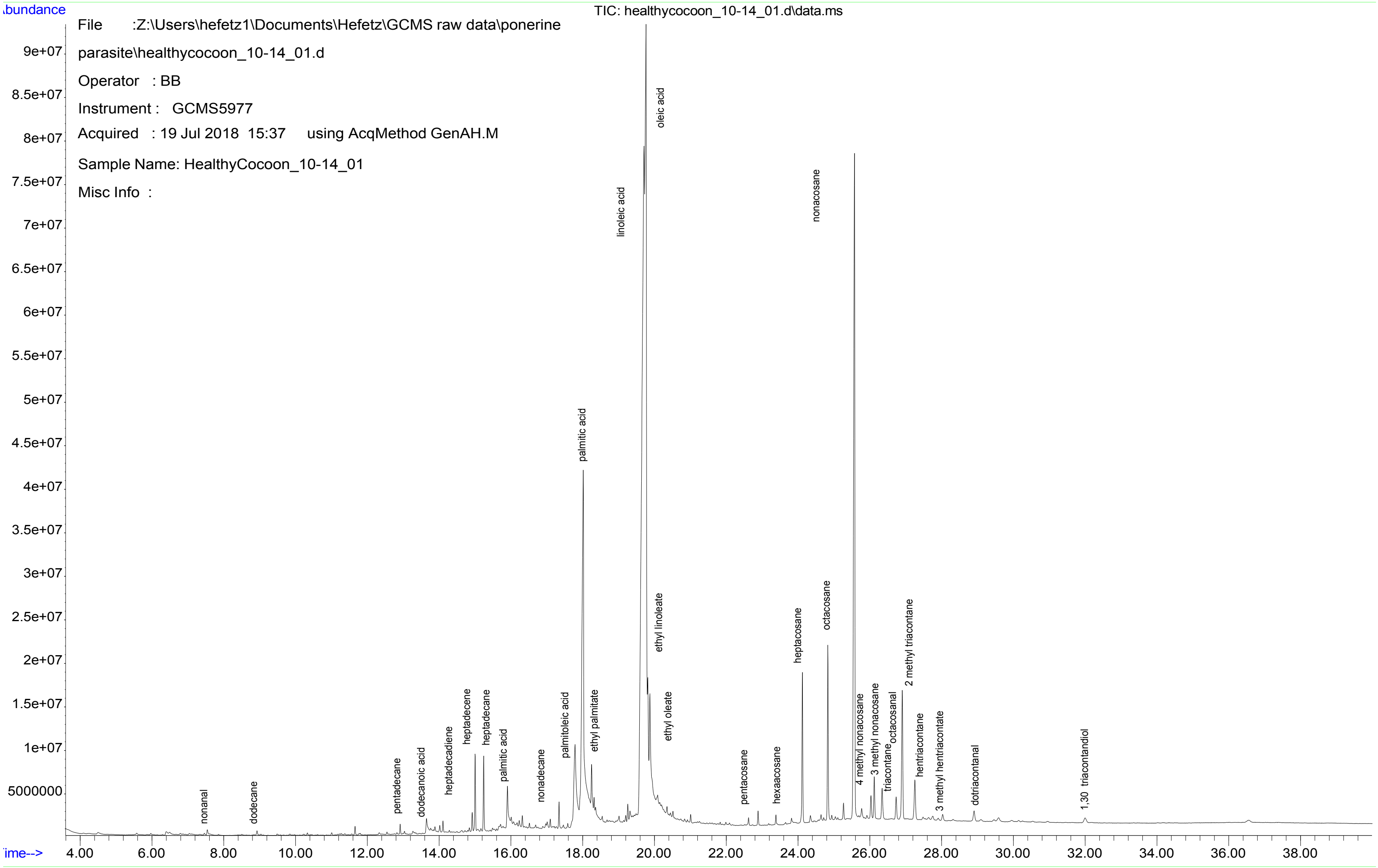


Figura 2. Cromatograma del perfil de hidrocarburos cuticulares y otros compuestos presentes en los cocones de *N. villosa* (control).

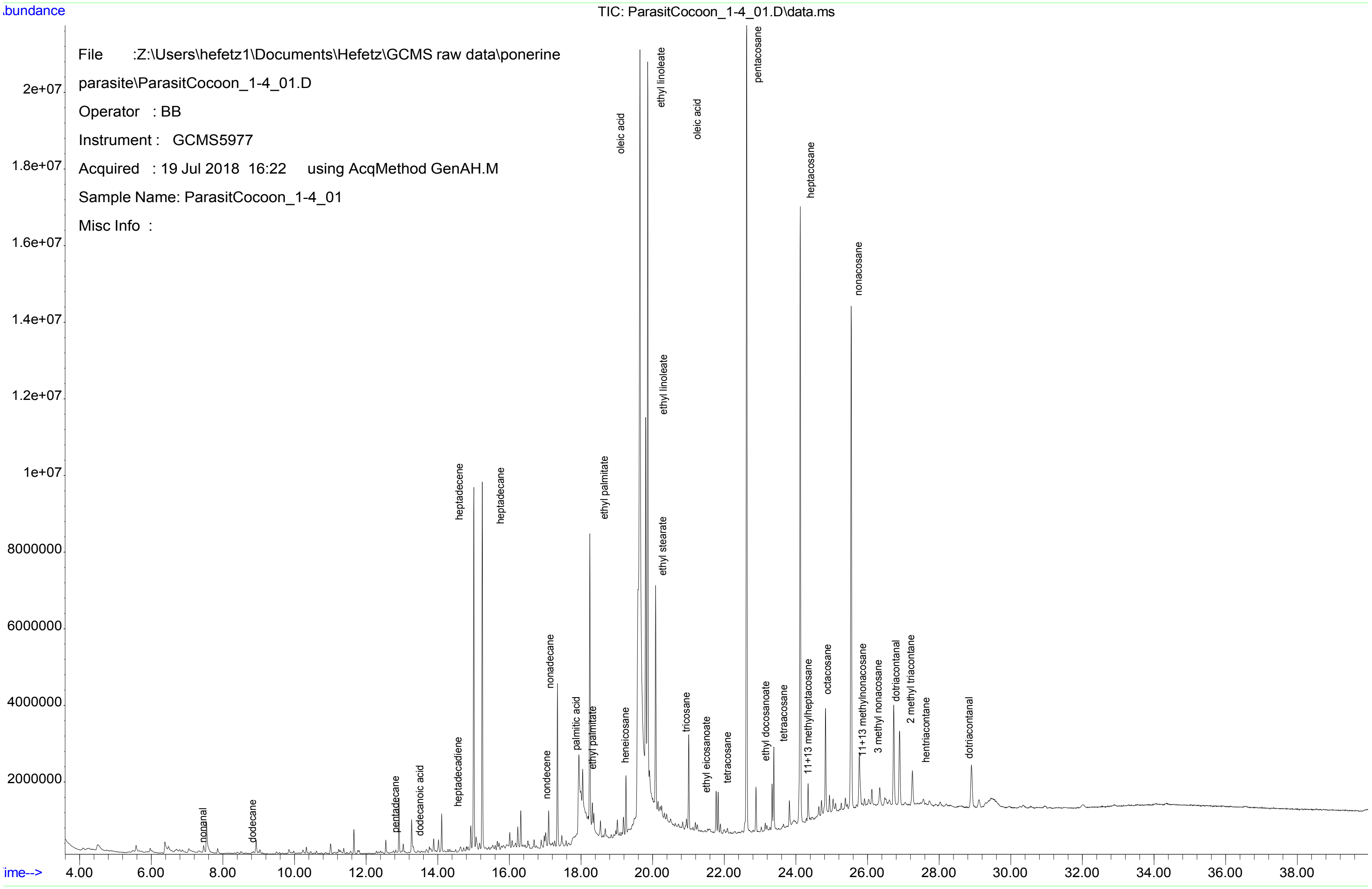
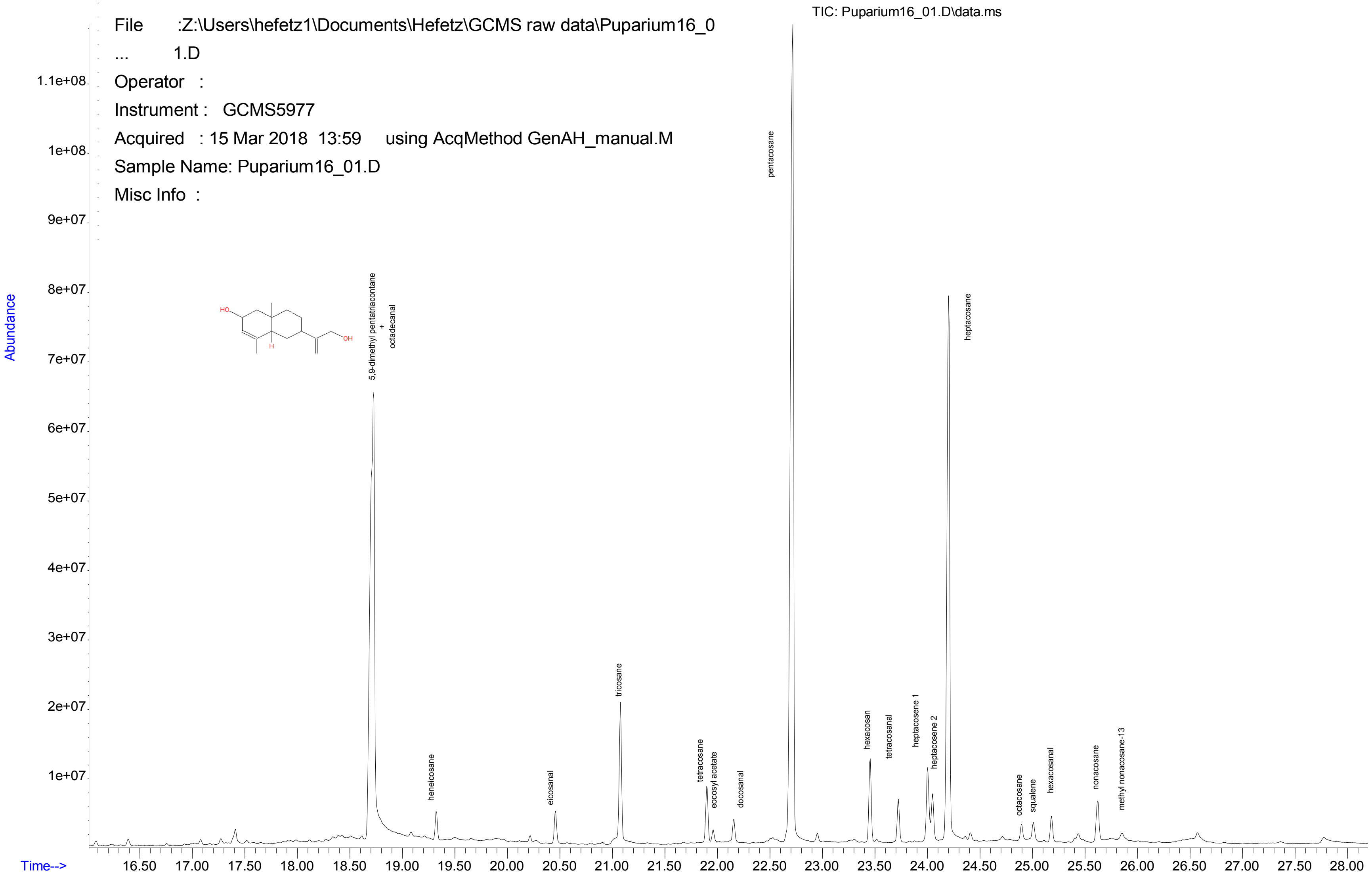


Figura 3. Cromatograma del perfil de hidrocarburos cuticulares y otros compuestos presentes en los cocones parasitados por *H. trigonus.*



File :Z:\Users\hefetz1\Documents\Hefetz\GCMS raw data\Puparium16_0
... 1.D
Operator :
Instrument : GCMS5977
Acquired : 15 Mar 2018 13:59 using AcqMethod GenAH_manual.M
Sample Name: Puparium16_01.D
Misc Info :

Figura 4. Cromatograma del perfil de hidrocarburos cuticulares y otros compuestos presentes en los puparios del parasitoide *H. trigonus.*

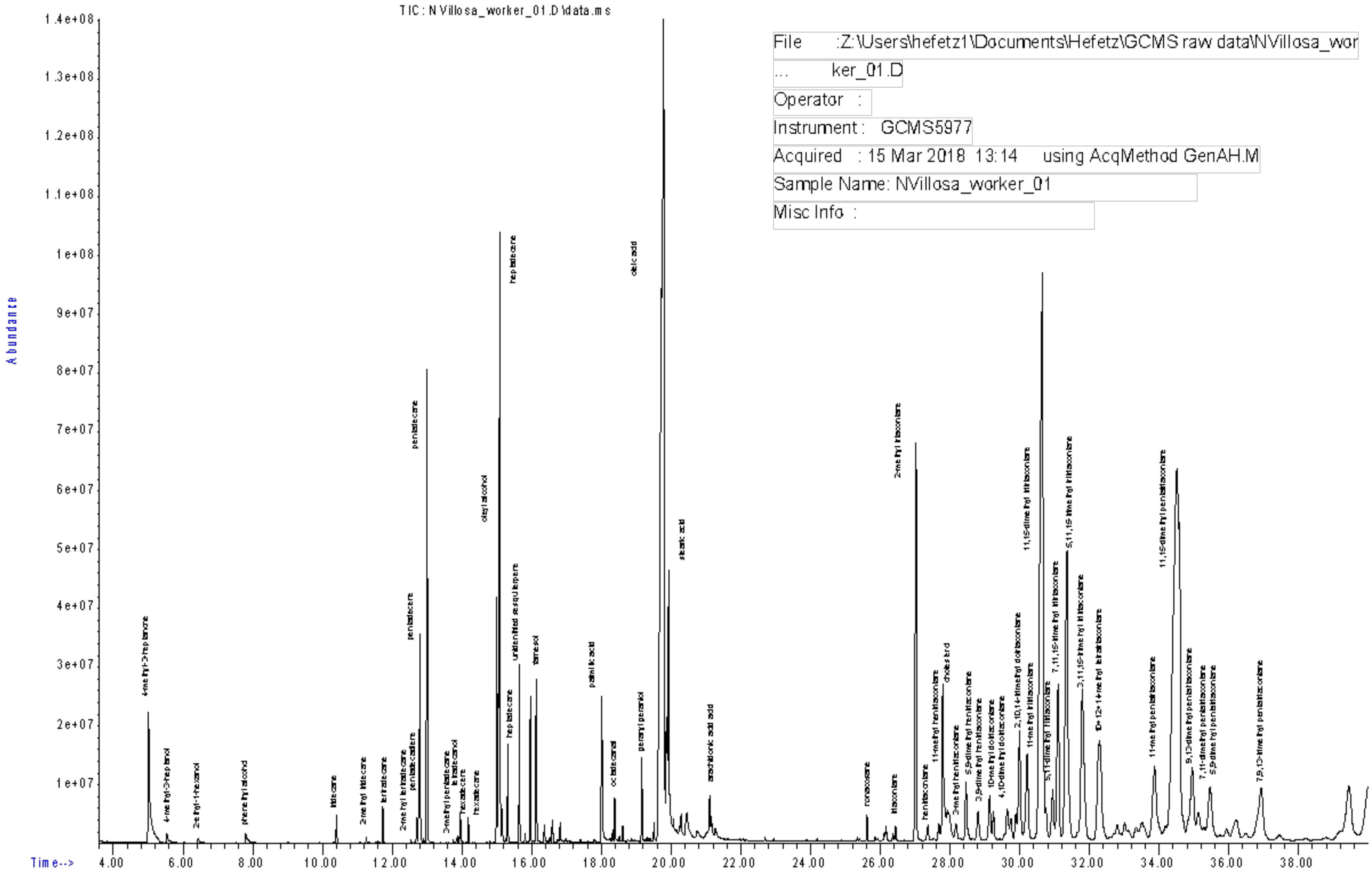


Figura 5. Cromatograma del perfil de hidrocarburos cuticulares y otros compuestos presentes en las obreras de *N. villosa.*

## Discusión

Los resultados de las observaciones y experimentos sugieren que las obreras de *N. villosa* abren sólo los cocones parasitados y transportan fuera del nido los puparios del parasitoide, lo cual permite que los adultos emerjan en un lugar alejado, permitiéndoles completar su ciclo de vida, y trae como consecuencia un aumento del fitness del parasitoide. El comportamiento de rechazo como respuesta de la colonia ante un intruso es, aparentemente, una respuesta de defensa generalizada (Rocha et al. 2019). Por ejemplo, las avispas parasitoides *Dilocantha lachaudii* Heraty, 1998 e *Isomerala coronata* (Westwood, 1874) (Hymenoptera: Eucharitidae) que emergen dentro del nido de su hormiga hospedera *Ectatomma tuberculatum* (Olivier, 1792), son capturados y transportados fuera del nido (Pérez-Lachaud et al. 2015). De la misma manera, observamos que las obreras de *N. villosa* expulsan los puparios de *H. trigonus* al exterior del nido. Este comportamiento de rechazo también se observó en condiciones naturales, ya que en varias ocasiones se registraron obreras de *N. villosa* transportando entre las hojas externas de la planta hospedera el pupario del parasitoide. Nuestros resultados señalan que el comportamiento de rechazo de los puparios por las obreras de *N. villosa,* es similar al reportado en el transporte de obreras conespecíficas muertas (Wilson et al. 1958; Haskins y Haskins 1974; Pérez-Lachaud et al. 2019) y en el rechazo de objetos que no pertenecen a la colonia (Howard y Tschinkel 1976; Pérez-Lachaud et al. 2019). Sin embargo, en nuestro caso, este proceso difiere en que las obreras, además, abren los cocones. Tanto las obreras conespecíficas muertas (ver Pérez-Lachaud et al. 2019) como los cocones parasitados reportados en este estudio generaron fuerte interés en las obreras, las cuales realizaron recurrente exploración mediante el anteneo, en algunos casos por más de una obrera; además, en ambos casos no se detectó ningún comportamiento agonístico.

A diferencia de las avispas parasíticas que tienen aparato bucal con mandíbulas falcadas con las que cortan el cocón del hospedero para escapar (Buys et al. 2010; Peeters et al. 2015), las moscas sírfidas presentan aparato bucal esponjoso (Labandeira 1997), por lo que previamente se formuló la hipótesis que los parasitoides adultos de *H. trigonus* no podrían cortar el cocón de la hormiga hospedera para poder escapar (Pérez-Lachaud et al. 2014). En nuestros bioensayos, todas las moscas adultas que emergieron de los puparios fueron incapaces de abrir los cocones del hospedero y murieron dentro. Esto contrasta con la alta supervivencia de los puparios descartados por las hormigas (90%), y sugiere que para poder escapar de los cocones y del nido, *H. trigonus* manipula el comportamiento de las hormigas. La comparación de los perfiles cuticulares de cocones parasitados y no parasitados sugiere además que esta manipulación podría ser a través de señales químicas.

La comunicación a través de señales químicas juega un papel importante en la organización social de las hormigas (Liu et al. 2000; Howard y Blomquist 2005; d'Ettorre y Lenoir 2010). Sin embargo, este canal puede ser explotado tanto por adultos como por estados inmaduros de varias especies mirmecófilas (Hölldobler 1971; Vander Meer y Wojcik 1982; Steidle y Dettner 1993; Akino 2002; Pierce et al. 2002; Hojo et al. 2009; Bagnères y Lorenzi 2010). En los resultados de GC/MS los cocones parasitados y no parasitados presentaron 19 compuestos químicos que diferían entre ellos. De la misma manera, los puparios (sin el cocón del hospedero) presentaron 11 y 13 compuestos que no estuvieron presentes en los cocones parasitados y no parasitados. Los diferentes compuestos propios del pupario del parasitoide, probablemente representan el estímulo para desencadenar el comportamiento de apertura de los cocones en las obreras. En los insectos sociales, para proteger a la colonia de la transmisión de enfermedades y parásitos, las obreras identifican a través de señales químicas a los congéneres enfermos (Masterman et al. 2001; Spivak et al. 2003; McAfee et al. 2018). Por ejemplo, *Apis mellifera* L. (Hymenoptera: Apidae), puede discriminar entre los olores emitidos por la cría sana y la cría que ha sido infectada por una enfermedad micótica causada por *Ascosphaera apis* (Onygenales: Ascosphaeraceae) (Masterman et al. 2001; Spivak et al. 2003). De la misma manera, se ha reportado el comportamiento necroforético de la hormiga granívora, *Pogonomyrmex badius* (Latreille, 1802) (Myrmicinae), desencadenado por el ácido oleico (Gordon 1983). El ácido oleico y el ácido linoleico son los dos ácidos grasos insaturados más importantes que actúan como una señal de muerte durante la descomposición de cadáveres de artrópodos. Estos ácidos grasos son utilizados por múltiples especies de hormigas (Wilson et al. 1958; Gordon 1983;

Akino y Yamaoka 1996; Qiu et al. 2015) y otros taxones de artrópodos (Rollo et al. 1994; Abbott 2006; Yao et al. 2009; Chouvenc et al. 2012; Sun y Zhou 2013; Aksenov y Rollo 2017) en el reconocimiento de cadáveres. En nuestros resultados de GC/MS, de manera inesperada, el ácido oleico se encontró en abundancias similares en obreras, cocones sanos y parasitados, pero no se encontró en los puparios del parasitoide; de la misma manera, el ácido linoleico se encontró en cocones sanos, pero no en los otros grupos analizados (ver Cuadro 3). Es probable que el ácido oleico que se registró en los cocones parasitados se explique a través de contaminación con los olores presentes en el cocón del hospedero. Los puparios, por su parte, no presentaron compuestos con ácido carboxílico como grupo funcional (ver Cuadro 3), por lo que se descarta que la remoción y transporte del pupario esté influenciada por estos ácidos insaturados.

Sin embargo, como se mencionó anteriormente, el comportamiento de rechazo del pupario de *H. trigonus* pudiera ser similar al rechazo de objetos ajenos reportado en *E. tubercultum* (Pérez-Lachaud et al. 2019) con la diferencia que el repertorio de eventos conductuales en la interacción hormiga-microdontino incluye la apertura del cocón previa al rechazo. Es probable que los diferentes compuestos encontrados de manera exclusiva en los cocones parasitados, así como los ausentes y reportados en los cocones no parasitados hayan desencadenado una respuesta más compleja que en lo reportado por Pérez-Lachaud et al. (2019) en el rechazo de objetos por la hormiga *E. tuberculatum*. En el Cuadro 3 se puede observar que los puparios al igual que las obreras contenían el compuesto Octadecanal, pero no los cocones parasitados y no parasitados. Este aldehído ha sido reportado como feromona sexual en algunos insectos (Tatsuki et al. 1983; Ho et al. 1996). Los puparios rechazados en los experimentos, generalmente contenían pupas maduras que posiblemente empiezan a liberar el compuesto Octadecanal como feromona sexual (o su precursor), la cual podría ser detectada por las obreras del hospedero y reconocida como diferente al olor de los cocones. El uso de señales químicas para desencadenar el comportamiento de apertura del cocón de la hormiga hospedera no se ha señalado anteriormente. Sin embargo, es necesario precisar que los comportamientos de apertura y de rechazo observados pueden ser el resultado de una verdadera manipulación por parte del parasitoide, o bien pueden ser la consecuencia del desarrollo normal de *H. trigonus*, que en algún punto en su desarrollo comienza a producir compuestos específicos.

En nuestros experimentos, en un solo caso, después de que una obrera abrió un cocón parasitado y transportó el pupario en la pila de basura, una hembra adulta del parasitoide emergió mientras aún estaba expuesta a las hormigas y fue atacada. Por el contrario, en la mayoría de los experimentos, después de que las obreras manipularon con las mandíbulas los puparios, las moscas adultas emergieron sin daños. Estos resultados sugieren que la cutícula rígida del pupario puede representar una defensa exitosa durante la manipulación por las hormigas. El papel de este tipo de defensas estructurales (cutícula gruesa o rígida, cuerpo ovoide, etc.) durante las interacciones con hormigas agresivas también ha sido reportado en otros mirmecófilos (Vander Meer y Wojcik 1982; Heraty et al. 2015; Pérez-Lachaud et al. 2019). Por ejemplo, las orugas de *Liphyra brassolis* Westwood 1864 (Lycaenidae), pasan la mayor parte de su vida dentro de las colonias de la hormiga *Oecophylla smaragdina* Fabricius, 1775 (Formicinae), alimentándose de las larvas de esta agresiva hormiga tejedora (Dupont et al. 2016). *Liphyra brassolis*, no parece ser tolerada en el nido de la hormiga hospedera, sin embargo, está protegida contra los ataques de las obreras por una cutícula gruesa en forma de caparazón (Johnson y Valentine 1986; Ballmer y Pratt 1988). Aparentemente, las obreras no abren cocones parasitados por *H. trigonus* en estado larvario. De hecho, en un caso, se expuso un cocón parasitado por una larva de *H. trigonus* y las obreras no abrieron el cocón hasta que la larva se transformó en pupario (seis días más tarde).

Los cocones de *N. villosa* parasitados por la mosca *H. trigonus* presentaron diferencias en el perfil de compuestos químicos a los encontrados en cocones sanos. Es probable que, la presencia (o ausencia) de determinados compuestos haya desencadenado el comportamiento de rechazo en las obreras de *N. villosa*, estimulándolas a realizar la apertura del cocón y a reconocer a los puparios del parasitoide como ajenos a la colonia, para después rápidamente expulsarlos del nido. Es notable que el momento en que las obreras abrieron los cocones parasitados coincide con una etapa avanzada en el desarrollo del parasitoide, en donde el adulto aún en desarrollo se encuentra protegido por el pupario. Esta sincronización, probablemente es una ventaja para que las moscas de *H. trigonus* completen su desarrollo y emerjan en un lugar seguro, evitando interacciones agonísticas con las hormigas, lo que contribuye al éxito reproductivo de esta especie.

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# CAPÍTULO IV

# Nest site selection during colony relocation in Yucatan Peninsula populations of the ponerine ant *Neoponera villosa* (Hymenoptera: Formicidae)

**SOMETIDO A:** Insects

# CAPÍTULO IV

# Nest-site selection during colony relocation in Yucatan Peninsula populations of the ponerine ant *Neoponera villosa* (Hymenoptera: Formicidae)

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**Abstract:** In the Yucatan Peninsula, the ponerine ant *Neoponera villosa* nests almost exclusively in tank bromeliads, *Aechmea bracteata.* We aimed at determining factors influencing nest-site selection during nest relocation regularly promoted by hurricanes in this area. Using ants with and without previous experience of *Ae. bracteata*, we tested their preference for refuges consisting of *Ae. bracteata* leaves over two other bromeliads, *Ae. bromeliifolia* and *Ananas comosus*. We further evaluated bromeliad-associated traits that might influence nest-site selection (form, size). Workers with and without previous contact with *Ae. bracteata* significantly preferred this species over others, suggesting the existence of an innate attraction to this bromeliad. However, preference was higher in ants that had experienced previous contact with *Ae. bracteata*. Workers easily discriminated between shelters of *Ae. bracteata* and *A. comosus*, but not those of the closely related *Ae. bromeliifolia*. In marked contrast, ants discriminated between similar sized *Ae. bracteata* and *Ae. bromeliifolia* plants, suggesting that chemical cues and plant structure play an important role. Size was also significant as they selected the largest plant when provided two dissimilar *Ae. bracteata* plants. Nest-site selection by *N. villosa* workers depends on both innate preferences and familiarization with plant stimuli during larval development and early adulthood.

**Keywords:** *Aechmea bracteata*; bromeliad; Ponerinae; tandem running; colony relocation, adaptation.

## 1. Introduction

Many species of social hymenoptera frequently move to new nests sites although emigration presents significant challenges and risks [1,2], and often implies a fitness cost [3,4]. Colony relocation is a common phenomenon in ants [1,5]. Some ant species move their nests as part of their life history (e.g. army ants), but the majority do so in response to numerous biotic and abiotic factors, including microclimate fluctuation [6–8], physical disturbance [9,10], intra and interspecific competition [1,4,11–13], resource availability [14,15], and predator or parasite pressure [5,12,14,16]. Arboreal ants are particularly prone to move their colonies from one site to another [1] as occurs commonly in the Neotropical ponerine ant, *Neoponera villosa* (Fabricius) (Hymenoptera: Formicidae).

*Neoponera villosa* is a generalist arboreal predatory ant [17–19] with a wide geographical distribution, from Texas to Argentina [20]. This species occurs both in wet and dry forests [21], and is an opportunistic cavity-breeder that nests in dead and live trees, and in bromeliads [22–24]. In the southern part of the Yucatan Peninsula, Mexico, *N. villosa* nests mainly in the epiphytic bromeliad *Aechmea bracteata* (Sw.) Griseb [23,25,26] although other species of *Aechmea* with the same type of growth are available in this area [27]. *Aechmea bracteata* is a "phytotelm tank" type bromeliad; mature plants present a waterproof central cavity suitable for housing ants [23,26]; large groups of shoots at different stages of maturity develop from a rhizome [28]. This bromeliad is characteristic of the inundated forest of the Sian Ka'an Biosphere Reserve where clusters are found established at a mean height of 1.3 m [26]. Like other large tank bromeliads, *Ae. bracteata* individuals offer permanent shelter to a wide diversity of organisms, both specialists and opportunists [23,26,29,30], and during extreme flooding and other climatic events they constitute ecological refuges for many other ground-dwelling arthropods [31]. As with most myrmecophytes, *Ae. bracteata* can be associated with several ant species, including *N. villosa* [26]; however, it does not depend on ants for its germination [32].

*Neoponera villosa* is not an obligate inhabitant of myrmecophytes; however, in the southern region of the Yucatan Peninsula, this ant uses the tank-bromeliad as a nest throughout the year, displaying a very marked local specialization [25]. There is little knowledge regarding the evolution of host–plant specialization between plants and ants in facultative associations. In the case of ants that nest in specific plants, it has been shown that host plant recognition is primarily based on two factors: an innate (genetically determined) attraction towards certain plants rather than others, and the influence of the environment during development and early adult life (pre-imaginal learning and conditioning through contact with the host plant during larval life and the first days of adult life), that may even supplant a genetically determined attraction or deterrence [25,33–35]. For instance, the African arboreal ants *Tetramorium aculeatum* (Mayr) (Myrmicinae) and *Oecophylla longinoda* (Latreille) (Formicinae) present a familiarization process (early learning) that can replace the innate attraction of both species [34,35]. This learning only takes place during the neonatal stage, a sensitive period after which the influence of the environment ceases [see 33]. Attraction to *Ae. bracteata* by alate queens (gynes) and young *N. villosa* workers (nurses) has been studied in the context of new colony foundation by foundress females [25]. Gynes from colonies nesting in *Ae. bracteata* are attracted to this bromeliad, a preference that appears to be learned during the larval stage. This pre-imaginal learning may be further strengthened at the beginning of the imago life, causing local fidelity toward *Ae. bracteata* over other available species [25]. However, nest-site selection in *N. villosa* has not been studied in the context of nest-relocation, a distributed, nonhierarchical decision-making process which is performed by several scouts who find potential nest sites. Informed scouts lead nestmates to the chosen new nest sites through tandem running, with only one nest mate being recruited at a time. The new nest site is defined by a quorum sensing mechanism, i.e. when more ants are present at one of the alternatives [5].

The Yucatan Peninsula has been identified as a region that is affected by hurricanes and droughts [36], which can result in bromeliads dislodging from their host tree and falling to the ground, thus requiring complete ant colonies to relocate. For cavity-nesting species such as *N. villosa,* there is only a limited number of potential nest sites that can meet the requirements of a mature colony. Furthermore, nesting sites are competitively searched for by other species, specifically *Dolichoderus bispinosus* (Olivier) and *Nasutitermes* sp. [23,26]. In most cases, scouts encounter various candidate shelters and have to decide which is the most suitable. Some characteristics of the potential nest site, in particular the size of the nesting cavity, can constrain colony growth [37–41] and this factor is expected to influence nest-site choice in *N. villosa* [23]. Furthermore, some ant species can assess nest-site suitability through various physical characteristics such as darkness, cavity height, entrance width and configuration [42,43]. However, with regard to *N. villosa*, the stimuli which intervene during nest-site selection have not been identified. In various species of ants that establish obligate interactions with plants, it has been demonstrated that host plant recognition is primarily based on chemical cues that attract foundresses [44–49]. However, plant height, nest-site geometry or clear areas around trees that provide information on the size of the potential nest candidate or on its protective potential, are used by various animal species as cues during nest-site selection [40,50–53] and may also play an important role during nest relocation in *N. villosa*.

In the present study, we performed different experiments (two-choice bioassays) to determine how *N. villosa* workers select a nest-site in the eventuality of nest relocation. Because rearing workers from egg to adult was not feasible, we took advantage of the fact that *N. villosa* nests almost exclusively in cavities of live trees in northern Yucatan where *Ae. bracteata* is rare, to investigate nest-site selection of *N. villosa* workers without previous contact with this bromeliad. Our research addressed the following questions: 1) Do *N. villos*a workers have an innate preference for *Ae. bracteata*? 2) Is the preference modulated by the pre-imaginal or neonatal ant experience linked to the origin of the colony (workers with or without previous contact with *Ae. bracteata*)? 3) Are the recognition and localization of *Ae. bracteata* regulated by chemical stimuli? 4) Does *Ae. bracteata* size influence nest-site selection?

## 2. Materials and Methods

### *2.1. Ant collection and identification*

Ants in bromeliads were collected in five sites in the southern part of the Yucatan Peninsula: Ejido Blasillo (18°7'37.98"N, 89°20'20.93"W, 261 m.a.s.l.), Nuevo Becal (18°36'39.36"N, 89°16'15.54"W, 239 m.a.s.l.) and Zoh-Laguna (18°35'11.61"N, 89°25'4.67"W, 257 m a.s.l.) in Campeche; Kohunlich (18°25'31.08"N, 88°48'9.89"W, 143 m.a.s.l.) and Sian Ka'an Biosphere Reserve (19°41'56.17"N, 87°50'18.31"W, 18 m.a.s.l.)

in Quintana Roo. Ants nesting in tree cavities (mainly *Lysiloma latisiliquum* (L.) Benth., *Caesalpinia gaumeri* (Britton and Rose) Greenm., and *Leucaena leucocephala* (Lam.) de Wit (Fabaceae), and *Bursera simaruba* (L.) Sarg. (Burseraceae)) were collected essentially in Cuxtal Ecological Reserve (20°51'46.58"N, 89°36'40.68"W, 17 m.a.s.l.) in Yucatan, in the northern part of the Peninsula, but a few were collected in the south, in Chetumal (18°32'37.90"N, 88°15'46.38"W, 10 m.a.s.l.) in Quintana Roo. In the latter two sites, *Ae. bracteata* is rare or absent. Each epiphyte was cut off from the supporting branch, dismantled leaf-by-leaf in plastic bins coated with Fluon (Whitford GmbH, Diez, Germany), and all *N. villosa* ants and their brood were collected. Both ants from epiphytes and those from tree cavities were housed in plastic jars under laboratory conditions until bioassays (see below).

*Neoponera villosa* belongs to the neotropical species complex of *N. foetida* (L.), which includes 12 other species [54,55]. Due to their morphological similarity, *N. villosa* has been confused in the past with two other species with a wide distribution, *N. inversa* (Smith) and *N. curvinodis* (Forel). Until now, however, only *N. villosa* has been reported in the Yucatan Peninsula [56]. Nevertheless, in order to confirm ant identity and further support our comparisons, five workers nesting in bromeliads and five workers nesting in live trees were DNA extracted and barcoded as part of an independent study (Lachaud and Pérez-Lachaud, unpubl.). DNA extraction and amplification followed the protocol in [57], with a freezing step after initial incubation according to the recommendation of [58] for Hymenoptera. Sequences were edited using CodonCode v. 3.0.1 (CodonCode Corporation, Dedham, MA, USA) and uploaded to Barcode of Life Database (BOLD, boldsystems.org).

### *2.2. Nest site selection*

As our study is focused on nest relocation, only workers were used for the two-choice tests implemented to evaluate nest-site selection. This parallels nest relocation following disturbance or destruction of the old nest whereby emigrations are organized by workers (scouts) that set out from the damaged nest to find a new home, thoroughly inspecting any candidate nest that they find [2]. As in various other ponerine species such as *N. verenae* (Forel) (referred to as *Pachycondyla obscuricornis* Emery; [59]), *N.*

*apicalis* (Latreille) [60], or *Diacamma indicum* (Santschi) [61,62], *N. villosa* uses a specific behavior called tandem running in which a single worker attracts a single recruit (or two in some occasions) and leads her towards the new nest site. As in other *Neoponera* [59,60] such recruitment by *N. villosa* is exclusively used during nest relocation and never during foraging.

### 2.2.1 Experimental set up

Ants from 35 colonies living in *Ae. bracteata* and 11 colonies (or parts of colonies) nesting in tree cavities were used in bioassays (Table S1). Observations were conducted from 26 January 2017 to 12 February 2018. Bioassays were carried out after a two-week period of acclimatization of the ants to laboratory conditions under natural illumination and at room temperature (26 ± 1 ºC). Workers were randomly selected from the original nest and only evaluated once. Two-choice tests were conducted using transparent plastic jars (14 cm in diameter x 25 cm in height, 3 l vol.) into which the ants were deposited. Each jar was connected via a transparent plastic tube (1.5 cm in diameter x 20 cm in length) to an election chamber (bioassays with live plants: 45 x 30 x 60 cm plastic box; bioassays with parts of plants (leaves): 40 x 21 x 14 cm). Each election chamber included a glass tube (2 cm in diameter x 8 cm in length) filled with water and stuffed with cotton at one end. The ants were fed sliced apple pulp, which was placed in the election chamber for the duration of the bioassay.

The protocol for the observations followed that of [25]. For each bioassay, two different refuges or “nest-sites”, consisting in tubular shelters to eliminate the influence of plant architecture, were placed in the election chamber. Subsequently, a group of 20 workers randomly obtained from those foraging and some brood were gently placed into the adjacent transparent jar. As colony size varies greatly from one colony to another, and because it was not feasible to collect complete colonies to perform the required number of replicates per bioassay, a fixed sample-size of 20 ants per bioassay was used. This is a common procedure in experiments with large ants (see for example: [34,35]). We carried out 21 to 30 replicates for each comparison and each replicate consisted of individuals from the same colony. The experimental device was then closed and set aside for 24 h, allowing the ants to install themselves in one shelter along with the brood (see [34]). The stimulus for the initiation of movement towards a potential nest (no shelter in the jar and artificial illumination) was constant across experiments and across replicates within experiments. This is a standard procedure used to trigger colony relocation in ants [63]. After 24 hours we evaluated the number of workers in any of the two refuge options ("nest-site") and those that remained in the jar or that were wandering or foraging.

### 2.2.2 Experiment 1.

To evaluate whether *N. villosa* nests in any available cavity or whether it prefers the refuge provided by *Ae. bracteata*, workers were presented with two tubular shelters (4 cm in diameter x 10 cm in length, with only one opening) made from: a) the rolled leaves of *Ae. bracteata* (treatment) and b) from a cardboard (control). Thirty replicates were performed with workers originating from colonies living in bromeliads and thirty with workers from colonies collected in tree cavities.

### 2.2.3. Experiment 2.

To evaluate whether *N. villosa* workers are able to discriminate *Ae. bracteata* through chemical stimuli emitted by the plant, shelters made of leaves of two other species belonging also to the Bromeliaceae family (*Ae. bromeliifolia* (Rudge) Baker, and *Ananas comosus* (L.) Merr.) where offered in combination with *Ae. bracteata* in two-choice bioassays as in the previous experiment. These bromeliads share similar traits (e.g. long and narrow leaves), and the texture of their leaves and general architecture are similar, but the nature and composition of their chemical signals are different, particularly between species from different genera [64]. Thirty replicates were performed for each possible comparison with workers originating from colonies living in bromeliads, and 21 to 26 replicates with ants from colonies collected in tree cavities. For each replicate, the ants had the choice between two shelters: one shelter made from the leaves of *Ae. bracteata* (control), and another made from the leaves of one of the two other bromeliad species.

2.2.4. Experiment 3.

To evaluate the influence of other bromeliad-related traits (structure of the plant), ants were offered the choice between whole plants of similar size of the two *Aechmea* species. Thirty replicates were performed with both types of workers.

2.2.5. Experiment 4.

As the results of the previous experiments showed that both shelters made of *Ae. bracteata* leaves and whole plants of this species were preferred (see Results), we evaluated whether the choice by *N. villosa* ants could be influenced by the size of the available *Ae. bracteata*. Twenty-nine replicates with workers from colonies nesting in *Ae. bracteata* were set up. In each replicate, *Ae. bracteata* bromeliads of two different sizes were offered: small (25 cm) vs. large (80 cm).

### *2.3 Statistical analyses*

Not all ants were found inside refuges; some workers were foraging and others were inactive. Inactivity is very common in social insects and is an intrinsic feature of the ants' behavior [65], making up to 40% of the members in a colony (e.g. [66]); furthermore, specific workers are consistently inactive [67]. Inactive workers are quantitatively important in *N. villosa* colonies [18]. To avoid inconsistencies due to a number of ants not choosing or performing other activities, we calculated the total number of ants making a choice in each bioassay (found inside the proposed refuges or plants) and used proportions of ants as the variable response. Data were analyzed with one sample t tests in order to determine whether the proportion of ants that preferred the treatment vs. the control differed from the expected mean value of 0.5 (random distribution). Analyses were performed in R [68].

### *2.4. Ethics statement*

Sampling comply with the current laws of Mexico and was carried out under permit number FAUT-0277 from Secretaría de Medio Ambiente y Recursos Naturales, Dirección General de Vida Silvestre, granted to G.P.-L. Only the biological material required for this study was collected.

## 3. Results

### *3.1. Species identification*

DNA sequences generated in the present work (Genbank accession numbers MK779595 to MK779604) confirmed that both populations (ants nesting in *Ae. bracteata* and ants nesting in live tree cavities) did not diverge genetically: all ants used in this work belonged to *N. villosa*. DNA sequences of both populations represent a single molecular operational taxonomic unit, and cluster with all other *N. villosa* molecular public data (Figure S1).

### *3.2. Tandem running behavior*

In the bioassays, *N. villosa* workers began exploring the new area (election chamber) and the proposed refuges, then selected one shelter. Afterwards, workers returned to the nest box and recruited nestmates through tandem running behavior. These recruited workers moved to the selected refuge, inspected it, and returned to the "old nest" to recruit new nestmates. Qualitatively we found that several tandem running ants followed the same path, suggesting trail laying behavior, although marking of the trail was not observed (Video S1). Similar trail laying through hind gut fluids or pygidial gland secretions has been reported in other ponerine species using tandem running [69–72]. The recruitment process was initiated through a "jerking" movement of a recruiting ant stimulating a nestmate to follow her to the new nest site, whereby the ant performs a rapid and vertical shaking of the body. Such rapid, vertical shaking of the body displayed by the recruiting ant to enhance the chemical signal has been reported in other ant species from various subfamilies [71,73,74]. Nestmates reacted by replicating the jerking movements and then initiated tandem running along the trail of the recruiting ant toward the new nest site. A single worker, or occasionally a maximum of two, were recruited and travelled in a single column. In some cases, when contact between the scout and the follower was broken, the recruiting ant pulled the legs of the nestmate with their jaws to reinforce the recruitment signal.

3.3 *Nest site selection*

3.3.1 Experiment 1.

There was no effect of the original nesting site (whether in bromeliads or in tree cavities) upon the choice of ants. Ants from both origins significantly preferred refuges made up of the leaves of *Ae. bracteata* over cardboard shelters (one sample t tests, t = 37.3, df = 29, p < 0. 0001; and t = 68.69, df= 29, p < 0. 0001 for ants originally nesting in *Ae. bracteata* and those nesting in tree cavities, respectively; Figure 1).

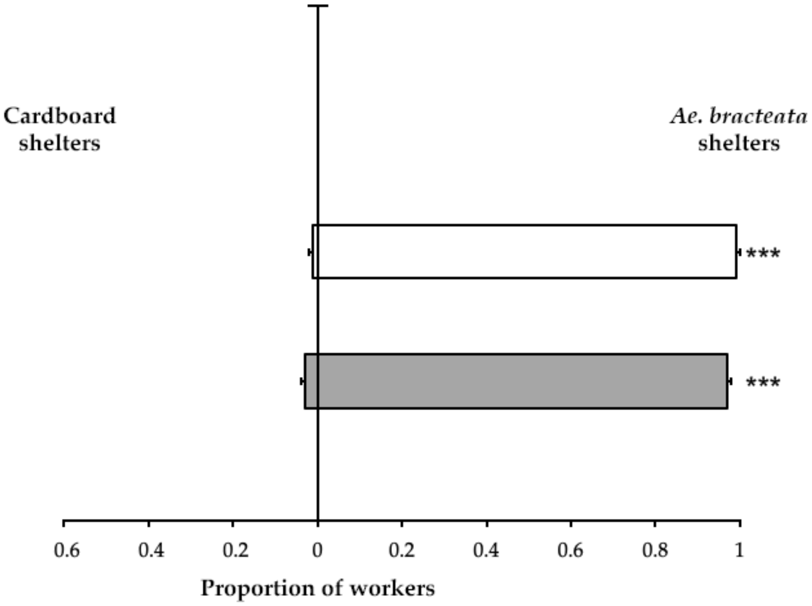

**Figure 1.** Proportion of *N. villosa* workers choosing refuges consisting of *Ae. bracteata* leaves vs. cardboard refuges. Workers from colonies of two distinct origins were tested: workers originally nesting in *Ae. bracteata* (n = 30 trials, grey), and workers from colonies previously nesting in tree cavities (n = 30 trials, white). *** = p < 0. 0001, one sample t test, mean proportion differs from the expected mean value of 0.5.

3.3.2 Experiment 2.

When ants had to select between refuges made up of leaves of two A*echmea* species, their choice was significantly influenced by their previous nesting site: ants from tree cavities, which did not have any previous contact with *Ae. bracteata*, preferred *Ae. bracteata* over *Ae. bromeliifolia* (t = 8.66, df = 20, p < 0.0001) while ants from colonies nesting in *Ae. bracteata* did not show any marked preference (t = 1.83, df = 29, p > 0.05; Figure 2). However, when the choice concerned refuges made up of leaves of species from two different Bromeliaceae genera, ants from both origins preferred *Ae. bracteata* over *A. comosus* (t = 25.26; df = 29, p < 0. 0001; and t = 7.41, df = 25, p < 0. 0001 for ants originally nesting in *Ae. bracteata* and those nesting in tree cavities, respectively; Figure 2); and this pattern was stronger in ants originally nesting in the bromeliad.

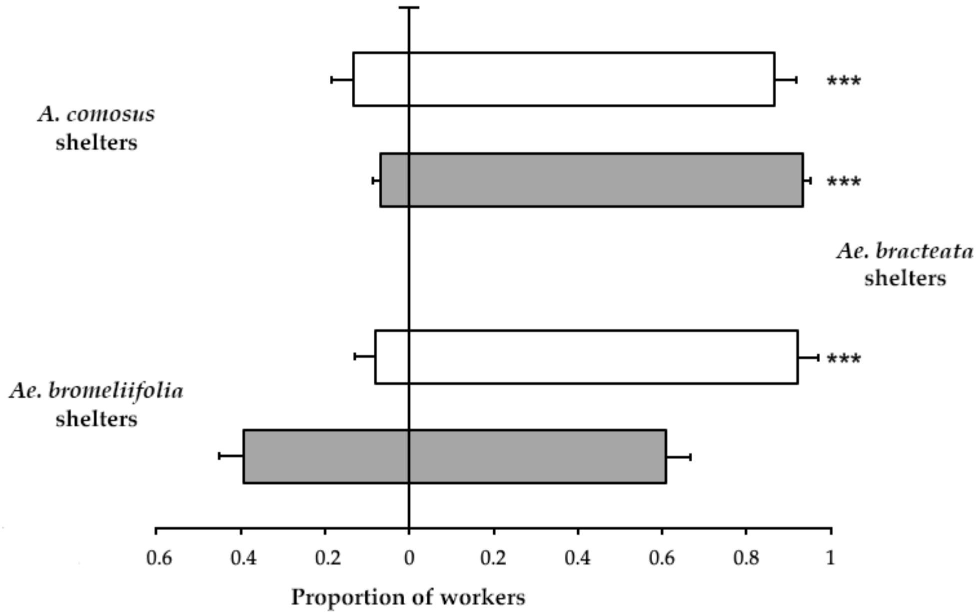

**Figure 2.** Proportion of *N. villosa* workers choosing between refuges consisting of leaves of *Aechmea bracteata* vs. leaves of two other bromeliads: *Ae. bromeliifolia* or *Ananas comosus*. Workers from colonies of two distinct origins were tested: workers originally nesting in *Ae. bracteata* (n = 30 trials, grey), and workers from colonies previously nesting in tree cavities (n = 21 or n = 26 trials, white). *** = *p* < 0. 0001, one sample t test, mean proportion differs from the expected mean value of 0.5.

### 3.3.3 Experiment 3.

When offered a choice between whole plants of *Ae. bracteata* and *Ae. bromeliifolia* of the same size, ants of both origins preferred also *Ae. bracteata* over *Ae. bromeliifolia* (t = 3.36; df = 29, p < 0. 01; and t = 5.79, df = 29, p < 0. 0001 for ants originally nesting in *Ae. bracteata* and those nesting in tree cavities, respectively; Figure 3).

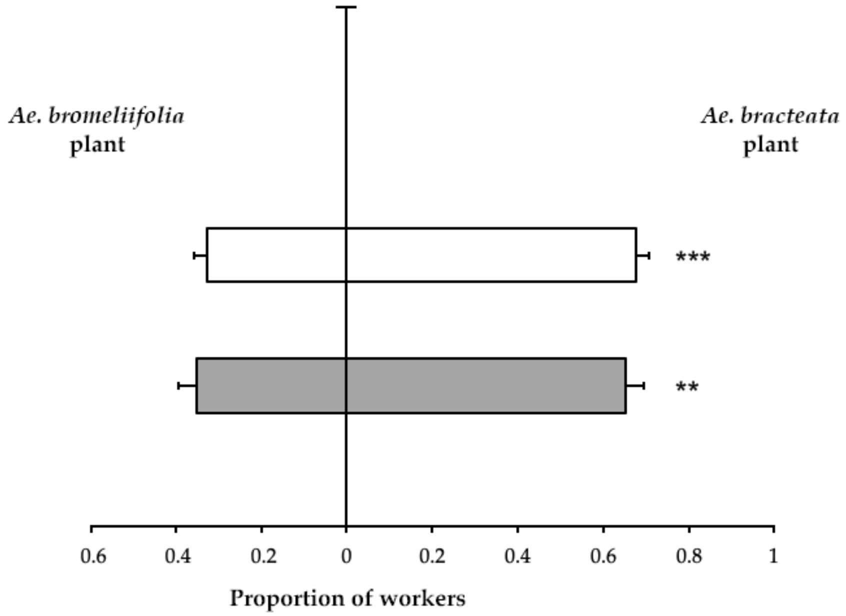

**Figure 3.** Proportion of *N. villosa* workers choosing between whole plants of *Ae. bracteata vs. Ae. bromeliifolia* of the same size. Bioassays were performed with two different experimental groups: workers originating from colonies originally nesting in *Ae. bracteata* (n = 30 trials, grey) and workers from colonies collected in tree cavities (n = 30 trials, white). ** $p$ = < 0.01 and *** = $p$ < 0. 0001; one sample t test, mean proportion differs from the expected mean value of 0.5.

### 3.3.4 Experiment 4.

*Neoponera villosa* workers originating from colonies established in *Ae. bracteata* significantly chose large *Ae. bracteata* bromeliads over small ones (One sample t test, t = 16.04, df =28, p < 0.0001; Figure 4). The mean proportion of ants choosing the large over the small bromeliad was 0.89.

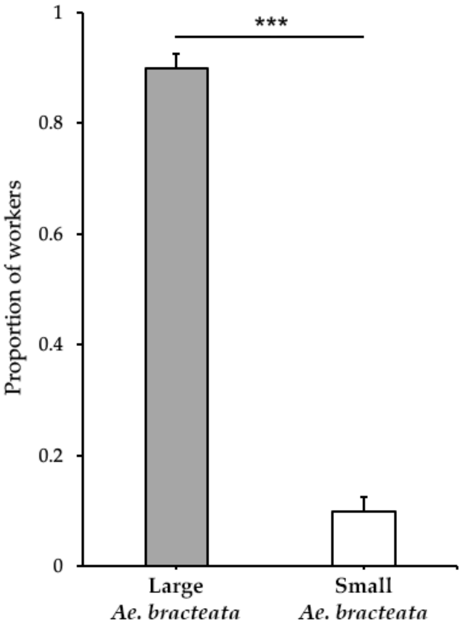

**Figure 4.** Proportion of *N. villosa* workers choosing between two *Ae. bracteata* of different sizes: large (80 cm) vs. small (25 cm). All workers tested were originally nesting in *Ae. bracteata* (n = 29 trials). one sample t test, mean proportion differs from the expected mean value of 0.5, *** = $p < 0.001$.

## 4. Discussion

Most animals, if not all, exhibit innate behaviors in response to specific sensory stimuli [75,76]. Bumble bees and honeybees, for example, exhibit innate color preferences, notably yellow and purple, which reflects the peak sensitivity of their color receptors [77–80], while the pseudomyrmecine ant *Tetraponera aethiops* Smith shows an innate attraction to its natural host plant *Barteria fistulosa* Mast (Passifloraceae) even when young callows were reared in laboratory conditions without any further contact with this plant post emergence [35]. Our experiments provide a similar example of innate attraction of *N. villosa* towards the bromeliad *Ae. bracteata*. Whatever the origin of the ants (with or without previous experience with *Ae. bracteata*), our data show that *N. villosa* workers exhibit a significant preference for refuges made up of leaves of *Ae. bracteata* over other available potential refuges consisting of leaves of two other bromeliad species, *Ae. bromeliifolia* and *A. comosus*, or of cardboard. Contrary to the results obtained by [25], which did not demonstrate any spontaneous preference of *N. villosa* for *Ae. bracteata* (workers reared in the laboratory without any contact with plants were attracted indifferently towards *Ae. bracteata* or towards the orchid *Myrmecophila* *tibicinis* (Batem.) Rolfe), our results point to the existence of an innate preference for *Ae. bracteata*. Although, in most experiments, the preference for *Ae. bracteata* was higher in ants with previous experience with this plant, even individuals without previous experience with this bromeliad exhibited such preference.

The influence of the environment, through pre-imaginal and neonatal learning (early experience), can interfere and replace any innate attraction or repulsion [81,82]. In ants, environmental induction of adult choices by passive familiarization during early adulthood has been demonstrated for various species. For example, although under natural conditions thyme (*Thymus vulgaris* L.) repels adult workers of the formicines *Formica polyctena* Foerster and *Camponotus vagus* Scopoli, workers of these species chose to settle in tubes that contained this plant if they have been reared in its presence when they were callow neonates [33,83]. Similarly, in the African arboreal ants *T. aculeatum* and *O. longinoda*, early learning during the first part of the life of adult workers and gynes can supersede innate attraction to guava (*Psidium guajava* L.) and cocoa (*Theobroma cacao* L.) or to mango leaves (*Mangifera indica* L.), respectively [34]. Early learning during the larval life and just after adult emergence appears to occur in *N. villosa*, as our data show that the preference for *Ae. bracteata* was modulated by the original nesting substrate: previous experience with *Ae. bracteata* enhancing the preference towards this plant over cardboard or *A. comosus*. These results confirm previous studies on foundresses of *N. villosa* which have shown that the influence of the original nest site environment on subsequent individual choice during nest site selection for colony foundation is due to an imprinting during larval life, strengthened at the beginning of the imago life through early learning [25]. When given the choice between a refuge containing *Ae. bracteata* or nothing, gynes of *N. villosa* that have previously experienced contact with the bromeliad during their larval and the first part of their nymphal life significantly preferred *Ae. bracteata*, whereas gynes which completed their nymphal life on another epiphyte (*M. tibicinis*) did not discriminate between both options. However, gynes which completed their nymphal life on M*. tibicinis* displayed a significant preference to this plant when presented with a choice between *Ae. bracteata* and *M. tibicinis* [25]. The local fidelity towards *Ae. bracteata* over other available nest-sites observed in our study area and the marked preference for this epiphyte during our experiments might be explained through early learning by winged queens as suggested by [25], and additionally through a combined effect: an innate attraction to *Ae. bracteata* by workers, reinforced by pre-imaginal learning and early learning during adulthood, influencing their choice during nest relocation events. The evolution of such an innate attraction, leading to local specialization in *Ae. bracteata* as a nesting-site, may be due to the predominance of this particular bromeliad throughout the biogeographic area of *N. villosa* (*Ae. bracteata* occurs from E Mexico to N Colombia and NW Venezuela [84]), and also because this bromeliad as microhabitat provides specific benefits to ants, both as shelter and as foraging site, and further constitutes refuge during extreme stochastic climatic events [31,85]. Preference in *N. villosa* workers for *Ae. bracteata* may be an adaptive response driven by climatic events in our study area, promoting the selection of such a stable microhabitat.

Various other bromeliad associated traits (size, chemical profile) may also be involved in nest-site selection as demonstrated by the preference of *N. villosa* workers for large *Ae. bracteata* when presented with small and large plants, or by the differences in preference demonstrated when choosing between closely related bromeliad species (both when offered as refuges and complete plants), most likely related to the chemical substances they produce. The architectural form and complexity of the plant may facilitate the emergence of different temperature and humidity microhabitats suitable for ants to settle, and it is known that other specialist arthropods (e.g. bromeliad-dwelling salticids) choose bromeliads based on rosette and leaf architectures [86]; furthermore, larger individual plants with a complex structure may facilitate the development of large ant populations and promote the maintenance of a diverse community of potential prey [87]. The use of chemical cues for host localization has been reported in a wide range of insects, including both herbivores [88–91] and predators or parasites [91–94]. In ants, chemical volatiles are also used to identify potential host plants. For example, *Crematogaster* spp. foundress queens can recognize their *Macaranga* host plant species, identifying chemical compounds of the stem surfaces of seedlings [44]; and queens of *Azteca* spp. and *Allomerus octoarticulatus* Mayr use chemical cues to select their myrmecophyte *Cordia nodosa* Lam. [45]. In our experiments, it was evident that *N. villosa* workers originally nesting in *Ae. bracteata*, had difficulty in arriving at a consensus when in the presence of shelters of two *Aechmea* species, without further information on the suitability of the potential nest-sites (plant structure or size of the cavity, for example) and therefore, the probabilities of choosing either bromeliad were similar. Contrastingly, choosing between complete plants of these two bromeliads was straight forward and significantly in favor of *Ae. bracteata* over the other species. These results suggest that workers of *N. villosa* select *Ae. bracteata* through some plant stimuli, probably of chemical nature, supplemented with information provided by the whole plant. The ability to discriminate between distinct plant species and genera is an obvious advantage, as the time and energy to find a suitable nest-site is minimized.

Evidently, nearly all ant species have the capability of shifting their nests if they become unsuitable [1], and selecting the best nest-site among numerous alternatives may be critical to the success and survival of the colony. As our results show, *N. villosa* workers preferred to settle in *Ae. bracteata* shelters and plants over other possibilities, and preferred large *Aechmea* plants over small ones. Behavioral flexibility constitutes an essential component of the adaptive repertoires of animals. In this context, the modulation of the innate attraction of *N. villosa* workers towards *Ae. bracteata* through early experience probably facilitates the replacement of this plant as the most suitable nest-site in habitats where *Ae. bracteata* is rare, as occurs in the northern part of the Yucatan Peninsula where this species nests in cavities of several live trees.

## 4. Conclusions

In our experiments, nest-site choice by *N. villosa* workers was influenced by an innate attraction to the bromeliad *Aechmea bracteata*. The local fidelity towards *Ae. bracteata* over other available nest-sites in the southern region of the Yucatan Peninsula and the marked preference for this epiphyte during our experiments might be explained through a combined effect: an innate attraction to *Ae. bracteata* by workers, reinforced by pre-imaginal and early learning during adulthood, influencing their choice during nest relocation events. Preference of *N. villosa* workers for *Ae. bracteata* may be an adaptive response driven by extreme climatic events in our study area, promoting the selection of such a stable microhabitat as nesting-site.

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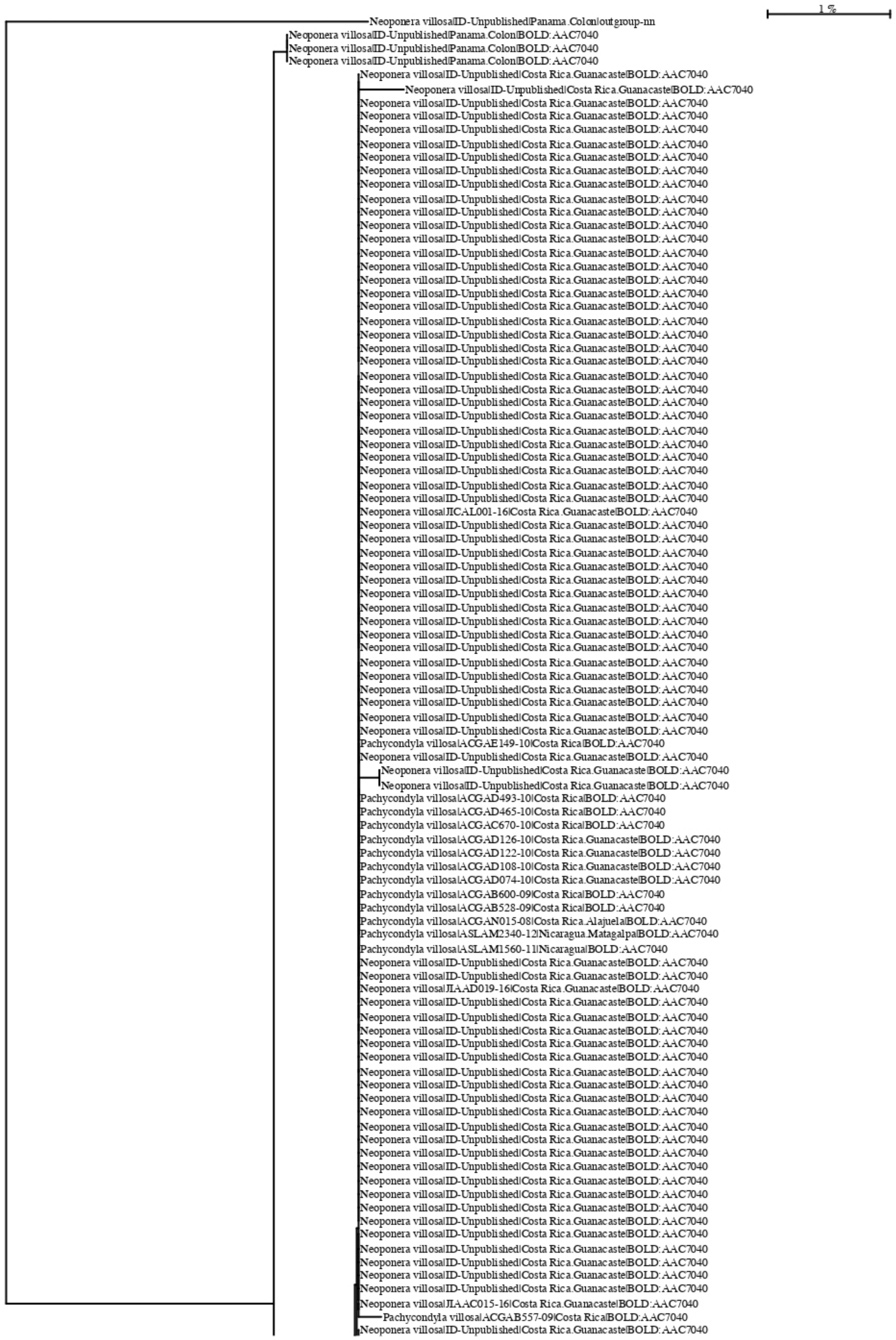
## Supplementary Materials:



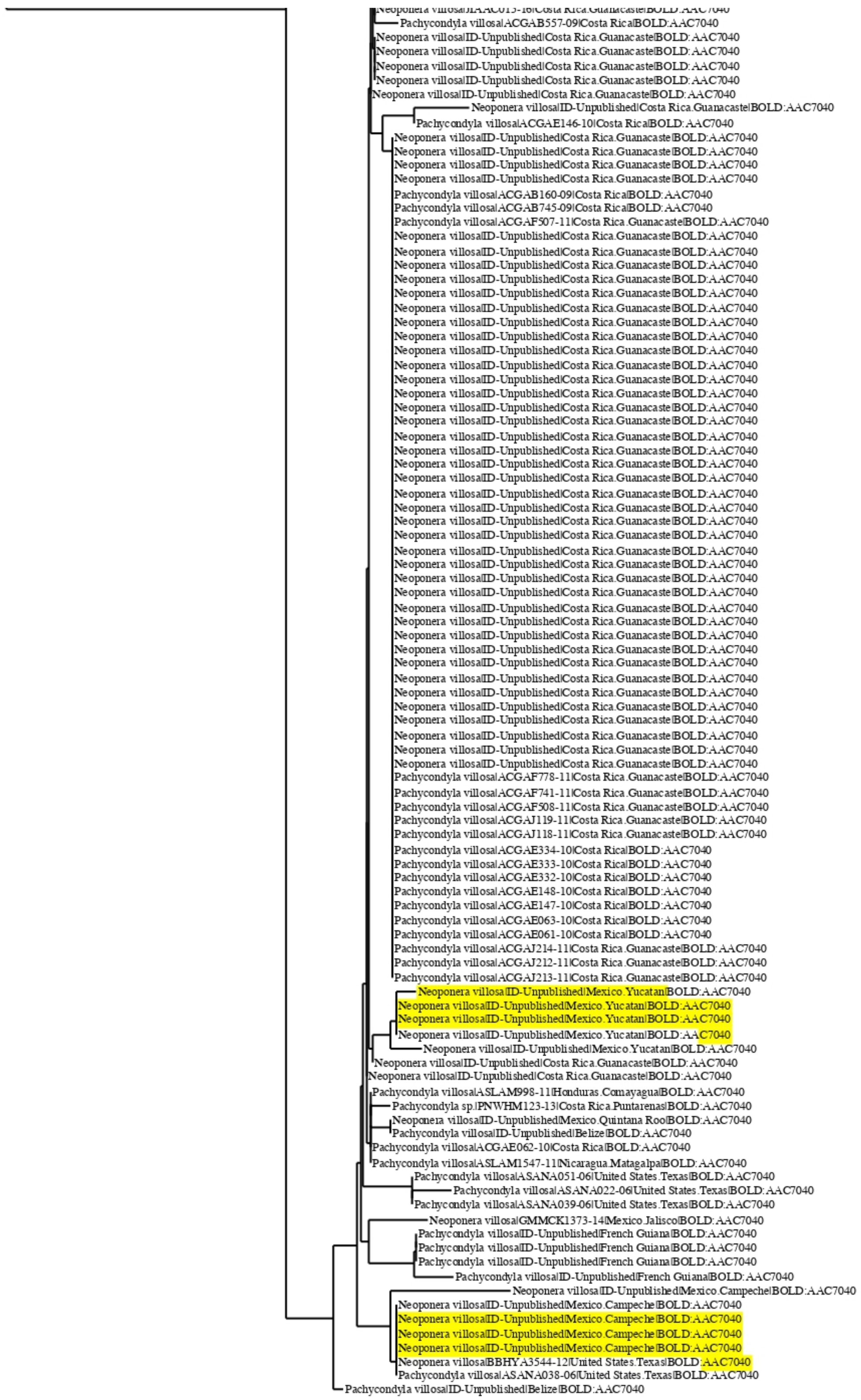


Figure S1. Taxon ID tree of *N. villosa* molecular public data, including the 10 sequences from this study (highlighted in yellow).

Table S1. Original composition of the *Neoponera villosa* colonies used in the two-choice bioassays.

| Type of colony | n | Mean number of queens | Mean number of workers | Mean number of larvae | Mean number of cocoons |
|---|---|---|---|---|---|
| Monogynic | 10 | 1 | 84.5 ± 16.4 | 33.7 ± 6.1 | 44.5 ± 10.3 |
| Polygynic | 21 | 5.4 ± 0.7 | 128 ±17.4 | 54.2 ± 12.6 | 54.8 ± 12 |
| Queenless | 4 | - | 50 ± 5.0 | 20.5 ± 12.9 | 20.3 ± 8. |

# CAPÍTULO V

## 5.1. Discusión general

Entre los objetivos de esta investigación se propuso catalogar a los organismos mirmecófilos que habitan dentro de los nidos y colonias de la hormiga *N. villosa*, así como aportar al conocimiento sobre las interacciones comportamentales que ocurren entre estos mirmecófilos y su hormiga hospedera, con énfasis en los organismos con hábito parasitoide. De un total de 84 nidos de *N. villosa* colectados en la bromelia-tanque *A. bracteata*, se registraron 43 taxones diferentes de organismos asociados a *N. villosa* pertenecientes a cinco clases de artrópodos y una clase de hongo. Al menos 12 especies son mirmecófilos obligados de esta hormiga, y fueron encontradas en asociación física con la cría y/o los adultos de *N. villosa* (Capítulo II: Tabla 2). Estos organismos presentaron diversas interacciones tróficas con la hormiga hospedera, principalmente asociaciones de tipo antagónico: depredación, parasitismo, cleptoparasitismo y parasitoidismo. La comunidad de mirmecófilos de *N. villosa* estuvo compuesta por especies que se alimentaban de la cría del hospedero, dos de estos (*Hypselosyrphus trigonus* y *Kapala* n. sp) parasitando el estado de desarrollo de larva/prepupa de la hormiga y coexistiendo en la misma población y, ocasionalmente, en el mismo nido. Asimismo, se registró la presencia de pseudoscorpiones depredando sobre la cría de la hormiga, y tres especies de ácaros con comportamiento forético y uno de ellos con hábito cleptoparásito. Además, se reporta la primera asociación de una larva de mariposa riodínida (posiblemente un depredador de cría) con una hormiga ponerina. Aunque la prevalencia de la mayoría de estas especies en las colonias de *N. villosa* fue muy baja, su efecto combinado a nivel de la población hospedera podría ser importante. Por otra parte, también se registraron otros mirmecófilos en asociación con las colonias de *N. villosa* aunque no estaban intimamente integrados a la colonia y pertenecen a la comunidad de mirmecófilos facultativos (Capítulo II: Tabla 3).

Se han propuesto diversas hipótesis para explicar el por qué algunas especies de hormigas parecen establecer más relaciones simbióticas que otras. Se ha considerado que las especies de hormigas que forman colonias muy grandes o aquellas con amplia distribución y abundancia albergan más organismos mirmecófilos que aquellas con colonias muy pequeñas (Hölldobler y Wilson 1990; Rettenmeyer et al. 2011; Kronauer y Pierce 2011). Por otro lado, debido a una diversidad genética mayor y menor agresividad, se ha sugerido también que especies de hormigas poligínicas tienen menos mirmecófilos que especies monogínicas (Päivinen et al. 2003). Sin embargo, los datos obtenidos hasta el momento sugieren que también las hormigas ponerinas con colonias de tamaño moderado como *N. villosa*, albergan una comunidad de mirmecófilos importante. Con la excepción de la mosca parasitoide *H. trigonus*, la cual fue previamente reportada atacando a la cría de *N. villosa* en Campeche y Quintana Roo (Pérez-Lachaud et al. 2014; Pérez-Lachaud y Lachaud 2017), todas las demás asociaciones reportadas son nuevas para la ciencia, incluyendo nuevas especies por describir (*Kapala* n. sp, *Myrmigaster* n. sp.).

Algunos de los mirmecófilos reportados en este estudio presentaron sorprendentes adaptaciones a la vida de la colonia del hospedero. Por ejemplo, en el Capítulo III se describe el caso de la mosca *H. trigonus*, parasitoide cuyos estados inmaduros atacan larvas/prepupas de *N. villosa* y se desarrollan bien protegidos dentro del cocón del hospedero. Los adultos se reproducen fuera del nido natal, pero son incapaces de romper el cocón del hospedero por sí mismos. Los datos obtenidos hasta el momento sugieren que estas moscas podrían manipular el comportamiento de las obreras de *N. villosa* a través de señales químicas. La emisión de substancias volátiles en un momento preciso en el desarrollo del parasitoide, justo al finalizar la etapa larval, estimularía a las obreras a realizar la apertura del cocón y a reconocer a los puparios del parasitoide como ajenos a la colonia, para después rápidamente expulsarlos del nido. El uso de señales químicas para la manipulación de la hormiga hospedera ha sido reportado en varias especies de mirmecófilos (p. ej. Hölldobler 1971; Vander Meer y Wojcik 1982; Akino 2002; Hojo et al. 2009). Además, en los experimentos también se demostró que la cutícula rígida del pupario del parasitoide representa una defensa estructural exitosa durante la manipulación de las obreras al momento de expulsarlos del nido. El papel de esta defensa estructural durante las interacciones con hormigas agresivas también ha sido reportado en otros mirmecófilos incluyendo coleópteros y eucarítidos (Vander Meer y Wojcik 1982; Heraty et al. 2015; Pérez-Lachaud et al. 2019).

Cabe señalar que el momento en el que las obreras abrieron los cocones parasitados coincide con una etapa avanzada en el desarrollo del parasitoide, en donde el adulto aún en desarrollo se encuentra protegido por el pupario. Esta sincronización es una ventaja para que las moscas de *H. trigonus* completen su desarrollo y emerjan en un lugar seguro, evitando interacciones agonísticas con las hormigas, lo que contribuye al éxito reproductivo de esta especie.

Las hormigas del género *Neoponera* han sido poco estudiadas con respecto a la biota asociada a sus colonias. La mayoría de las especies de este género presentan hábitos arborícolas (Schmidt y Shattuck 2014), lo que posiblemente dificulta el acceso a sus nidos y, por consecuencia, su estudio. Una revisión de literatura arrojó un número muy bajo de mirmecófilos conocidos para solo 7 de las 54 especies del género *Neoponera* (ver Capítulo II: Material suplementario Tab. S2), con registros que consistieron en su mayoría de observaciones incidentales. Aparentemente, hasta la fecha, nuestro estudio es el primer intento de proporcionar una lista detallada de los mirmecófilos asociados a una hormiga ponerina, basado en una búsqueda dirigida de estos organismos y el registro de sus interacciones.

*Neoponera villosa* y *Aechmea bracteata* presentan casi la misma distribución biogeográfica (ver Ramírez-Morillo et al. 2004; Mackay y Mackay 2010), compartiendo una larga historia evolutiva. Esta historia evolutiva pudo haber servido de base para el surgimiento de una red de interacciones de esta hormiga exclusivas del microcosmos de la bromelia-tanque, en donde esta planta hospedera parece ser determinante.

Como se señaló anteriormente, en la región sur de la Península de Yucatán, *N. villosa* nidifica casi exclusivamente en la bromelia *A. bracteata*. Sin embargo, perturbaciones físicas ocasionadas por elementos climáticos o la senescencia natural de la planta de nidificación promueven regularmente la reubicación de los nidos de *N. villosa*. Por ello, en este estudio se planteó determinar los factores que influyen en la selección del sitio de nidificación durante la reubicación del nido y se centró en si las obreras de *N. villosa* presentan atracción innata hacia *A. bracteata*. En los bioensayos de elección pareados presentados en el Capítulo IV, se demostró que independientemente del origen de las obreras de *N. villosa* (con o sin experiencia previa con *A. bracteata*), estas presentaron preferencia significativa por los refugios hechos de hojas de *A. bracteata* sobre las otras opciones disponibles. Nuestros resultados indican la existencia de una clara preferencia innata por *A. bracteata*, ya que incluso, individuos sin experiencia previa con esta bromelia, exhibieron tal preferencia. El comportamiento innato a estímulos sensoriales ya ha sido demostrado en algunas especies de hormigas arborícolas hacia ciertas especies de plantas (Djieto-Lordon y Dejean 1999a, 1999b). No obstante, la influencia del ambiente, a través del aprendizaje temprano, puede interferir y reemplazar la atracción innata (Jaynes 1957; Caubet et al. 1992). En este estudio, se encontró que la preferencia de las obreras de *N. villosa* por *A. bracteata* fue modulada por el origen de la colonia, es decir, una experiencia previa de las obreras con esta bromelia potenció la preferencia hacia la planta. De igual manera, Dejean (1990) demostró que la selección del sitio de nidificación durante la fundación de la colonia estuvo influenciada por la impronta de las fundadoras durante el estado larvario, la cual es fortalecida a través del aprendizaje temprano durante los primeros días de la etapa adulta de la hormiga. Es probable que la fidelidad de *N. villosa* hacia *A. bracteata* sobre otros sitios potenciales de nidificación observados en nuestra área de estudio y durante nuestros experimentos, pueda explicarse a través de la atracción innata hacia esta planta y su modulación a través del aprendizaje temprano en las obreras

Asimismo, se comprobó que las obreras de *N. villosa* utilizan rasgos de la planta hospedera (p. ej. tamaño y perfil químico) para su reconocimiento y selección. En los experimentos de elección, las obreras eligieron significativamente bromelias de mayor tamaño. Las plantas de mayor tamaño pueden facilitar el desarrollo de colonias más grandes (Dejean et al. 2018). Por otra parte, las obreras presentaron dificultad para discriminar entre dos especies de *Aechmea* (tanto con refugios como con plantas vivas); por el contrario, la respuesta de las obreras al estar expuestas a refugios de dos géneros diferentes de Bromeliaceae fue más fuerte y clara. Estos resultados sugieren que las obreras de *N. villosa* reconocen a *A. bracteata* a través de estímulos de naturaleza química, complementados con información proporcionada por toda la planta

(p. ej. la arquitectura, tamaño). La capacidad de discriminar entre distintas especies y géneros de plantas es una ventaja adaptativa, que minimiza el tiempo y la energía para encontrar un lugar adecuado para nidificar.

La mayoría de las colonias de *N. villosa* colectadas en este estudio, estaban compuestas por más de una reina (colonias poligínicas) (ver Capítulo II), lo cual sugiere que la disponibilidad de *A. bracteata* como sitios de nidificación probablemente sea más limitada de lo esperado. La fragmentación y pérdida de hábitat amenazan a todas las hormigas arborícolas (Guénard et al. 2012; Pérez-Lachaud y Lachaud 2014) y a los organismos mirmecófilos que habitan en sus colonias y nidos. La baja abundancia y la alta especificidad de algunos de los mirmecófilos encontrados en este estudio los hacen aún más vulnerables a estas amenazas.

# CAPÍTULO VI

## 6.1. Conclusiones

*Neoponera villosa* se encontró nidificando en *A. bracteata* no solo durante la estación seca, sino durante todo el año. Dentro de los nidos establecidos en la bromelia encontramos un conjunto muy diverso de mirmecófilos asociados a *N. villosa,* algunos de los cuales son muy específicos y únicamente se encuentran en las colonias de estas hormigas (mirmecófilos obligados). Sin embargo, su baja prevalencia y naturaleza críptica los hace susceptibles a pasar desapercibidos y al igual que sus hormigas hospederas se encuentran amenazados por la fragmentación y pérdida de su hábitat. Cabe señalar, que con la excepción de la mosca parasitoide *H. trigonus*, todas las demás asociaciones registradas en este estudio, son nuevas para la ciencia, incluyendo nuevas especies que deberán ser descritas, por lo que en este trabajo se confirma a los nidos y colonias de *N. villosa* como hot-spots de biodiversidad. Estos mirmecófilos presentaron diversas interacciones tróficas con la hormiga hospedera, en su mayoría, interacciones antagónicas (parasitoidismo, depredación y cleptoparasitismo). Algunas de estas especies presentan notables adaptaciones a la vida de la colonia. Por ejemplo, es muy probable que la mosca parasitoide *H. trigonus* manipule el comportamiento de la hormiga *N. villosa* para escapar del cocón y del nido. Las adaptaciones de otros mirmecófilos para integrarse a la vida de las colonias de *N. villosa*, así como aquellas interacciones que no pudieron ser elucidadas aún, deberán ser estudiadas en el futuro.

La fidelidad de estas hormigas a la bromelia como sitio de nidificación parece explicarse, al menos en parte, por la existencia de una atracción innata hacia esta bromelia. Asimismo, se comprobó que las obreras utilizan rasgos de la planta hospedera para su reconocimiento y selección (tamaño, arquitectura, olores), los cuales pueden también ser usados por sus organismos mirmecófilos en la búsqueda de hospederos potenciales, por lo que *A. bracteata* puede tener un papel importante en el establecimiento de las interacciones mirmecófilos-*N. villosa*.

En este estudio, las curvas de acumulación de especies no alcanzaron una asíntota, esto sugiere que nuestro muestreo sistemático, aunque se realizó durante un período de tres años, fue insuficiente y se requiere de muestreos adicionales para ampliar la lista de mirmecófilos asociados a las colonias de *N. villosa* que nidifican en *A. bracteata*.

## 6.2. Recomendaciones y perspectivas futuras

En nuestro estudio, se reportó que la mayoría de las colonias de *N. villosa* que nidificaban en *A. bracteata* estaban compuestas por más de una reina (poligínicas). Se ha señalado que la pérdida de hábitat genera la pérdida de los sitios de nidificación, lo que ocasionaría la agrupación de las reinas en dichos sitios. Aunque algunas especies de hormigas están consideradas como amenazadas o con poblaciones vulnerables, ni una sola especie de hormiga en México está incluida en la lista roja de la Unión Internacional para la Conservación de la Naturaleza (UICN), principalmente porque para la mayoría de las especies no se cuenta con suficientes datos. Algunas especies de mirmecófilos reportados en este estudio, en específico, los parásitos sociales, presentaron asociaciones obligadas con la hormiga hospedera. El estilo de vida de estos mirmecófilos los hace fuertes candidatos para la Lista Roja.

Por lo anterior, se recomienda conservar los sitios de distribución de la bromelia-tanque *Aechmea bracteata*, ya que esta epífita genera directamente refugio o sitios de nidificación para varias especies de hormigas, incluyendo a *Neoponera villosa*. Además, la presencia de las colonias y nidos de *N. villosa* en estas bromelias componen un microcosmos que aumentan la complejidad de interacciones tróficas y relaciones ecológicas con otros organismos.

# CAPÍTULO VII

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**Anexo 1.** Características de los nidos de *Neoponera villosa* colectados para este estudio. Número de individuos por casta y por estado de desarrollo.

| Nido | Colecta | Sitio | Reinas | Hembras aladas | Obreras | Machos | Pupas | Larvas | Huevos |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20/01/2016 | Hermenegildo Galeana | 16 | 1 | 322 | 0 | 65 | 5 | si |
| 2 | 20/01/2016 | Hermenegildo Galeana | 13 | 4 | 226 | 0 | 13 | 8 | si |
| 3 | 18/02/2016 | Kohunlich | 4 | 0 | 75 | 0 | 20 | 28 | si |
| 4 | 17/05/2016 | Hermenegildo Galeana | 8 | 10 | 185 | 22 | 261 | 161 | si |
| 5 | 17/05/2016 | Hermenegildo Galeana | 5 | 3 | 107 | 0 | 51 | 63 | si |
| 6 | 05/07/2016 | Nuevo Becal | 10 | 0 | 122 | 0 | 22 | 32 | si |
| 7 | 05/07/2016 | Nuevo Becal | 0 | 0 | 5 | 6 | 5 | 1 | no |
| 8 | 05/07/2016 | Nuevo Becal | 17 | 4 | 206 | 0 | 29 | 45 | si |
| 9 | 05/07/2016 | Nuevo Becal | 1 | 0 | 186 | 0 | 97 | 51 | si |
| 10 | 05/07/2016 | Nuevo Becal | 20 | 21 | 73 | 0 | 44 | 49 | si |
| 11 | 29/11/2016 | Hermenegildo Galeana | 6 | 0 | 84 | 0 | 3 | 3 | si |
| 12 | 29/11/2016 | Hermenegildo Galeana | 0 | 0 | 59 | 0 | 0 | 0 | no |
| 13 | 29/11/2016 | Hermenegildo Galeana | 1 | 0 | 115 | 0 | 40 | 101 | si |
| *14 | 17/01/2017 | Nuevo Becal | 1 | 0 | 143 | 0 | 74 | 44 | si |
| *-**15 | 17/01/2017 | Nuevo Becal | 1 | 0 | 180 | 0 | 65 | 69 | si |
| *16 | 17/01/2017 | Nuevo Becal | 7 | 1 | 145 | 0 | 9 | 72 | si |
| *17 | 17/01/2017 | Zoh-Laguna | 10 | 0 | 283 | 0 | 8 | 22 | si |
| *18 | 17/01/2017 | Zoh-Laguna | 8 | 0 | 285 | 0 | 19 | 72 | si |
| *19 | 11/03/2017 | Ejido Blasillo | 2 | 0 | 74 | 0 | 4 | 1 | si |
| *20 | 11/03/2017 | Ejido Blasillo | 1 | 0 | 27 | 0 | 4 | 15 | si |
| *21 | 11/03/2017 | Ejido Blasillo | 7 | 0 | 117 | 0 | 3 | 4 | si |
| ***22 | 14/03/2017 | Cuxtal | 0 | 0 | 189 | 0 | 6 | 0 | no |
| ***23 | 14/03/2017 | Cuxtal | 0 | 0 | 506 | 0 | 61 | 2 | si |
| ***24 | 15/03/2017 | Cuxtal | 1 | 0 | 245 | 0 | 3 | 1 | si |
| *25 | 05/05/2017 | Kohunlich | 1 | 0 | 47 | 0 | 27 | 39 | si |
| *26 | 10/06/2017 | Ejido Blasillo | 11 | 3 | 165 | 0 | 173 | 265 | si |
| *27 | 10/06/2017 | Ejido Blasillo | 4 | 0 | 33 | 0 | 25 | 19 | si |
| 28 | 10/06/2017 | Ejido Blasillo | 0 | 0 | 2 | 0 | 0 | 0 | no |
| 29 | 10/06/2017 | Ejido Blasillo | 0 | 0 | 10 | 0 | 4 | 2 | no |
| *30 | 29/07/2017 | Felipe Ángeles | 1 | 0 | 113 | 0 | 94 | 43 | si |
| *31 | 30/07/2017 | Ejido Blasillo | 0 | 0 | 40 | 0 | 41 | 58 | si |
| *32 | 30/07/2017 | Ejido Blasillo | 5 | 1 | 131 | 0 | 150 | 70 | si |
| *33 | 30/07/2017 | Ejido Blasillo | 2 | 0 | 65 | 0 | 79 | 39 | si |
| *34 | 16/08/2017 | Sian Ka´an | 1 | 0 | 104 | 0 | 86 | 42 | si |
| *35 | 16/08/2017 | Sian Ka´an | 7 | 4 | 150 | 0 | 62 | 38 | si |
| *36 | 16/08/2017 | Sian Ka´an | 4 | 0 | 196 | 0 | 173 | 132 | si |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 37 | 16/08/2017 | Sian Ka´an | 0 | 0 | 3 | 0 | 2 | 0 | no |
| *38 | 16/08/2017 | Sian Ka´an | 0 | 0 | 60 | 26 | 2 | 0 | no |
| *39 | 16/08/2017 | Sian Ka´an | 2 | 0 | 160 | 0 | 103 | 78 | si |
| *40 | 16/08/2017 | Sian Ka´an | 0 | 0 | 57 | 0 | 19 | 9 | si |
| *41 | 16/08/2017 | Sian Ka´an | 6 | 0 | 40 | 0 | 13 | 17 | si |
| *42 | 16/08/2017 | Sian Ka´an | 3 | 0 | 62 | 0 | 25 | 17 | si |
| *43 | 16/08/2017 | Sian Ka´an | 2 | 0 | 142 | 0 | 91 | 43 | si |
| ***44 | 16/11/2017 | Chetumal | 2 | 0 | 43 | 0 | 26 | 7 | si |
| ***45 | 18/11/2017 | Chetumal | 1 | 0 | 64 | 0 | 30 | 34 | si |
| *46 | 21/11/2017 | Sian Ka´an | 1 | 0 | 21 | 0 | 14 | 9 | si |
| 47 | 21/11/2017 | Sian Ka´an | 0 | 0 | 7 | 0 | 3 | 6 | si |
| *48 | 21/11/2017 | Sian Ka´an | 1 | 0 | 82 | 0 | 20 | 14 | si |
| *49 | 21/11/2017 | Sian Ka´an | 1 | 0 | 44 | 0 | 21 | 16 | si |
| ***50 | 21/11/2017 | Sian Ka´an | 1 | 0 | 84 | 0 | 40 | 46 | si |
| *51 | 21/11/2017 | Sian Ka´an | 11 | 0 | 46 | 0 | 35 | 58 | si |
| *52 | 21/11/2017 | Sian Ka´an | 0 | 0 | 43 | 0 | 19 | 15 | si |
| ***53 | 04/12/2017 | Nuevo Becal | 3 | 0 | 82 | 0 | 32 | 55 | si |
| ***54 | 04/12/2017 | Nuevo Becal | 5 | 1 | 107 | 0 | 30 | 74 | si |
| ***55 | 04/12/2017 | Nuevo Becal | 2 | 1 | 43 | 0 | 7 | 8 | si |
| 56 | 04/12/2017 | Nuevo Becal | 1 | 0 | 21 | 0 | 6 | 15 | si |
| 57 | 04/12/2017 | Nuevo Becal | 1 | 0 | 12 | 0 | 2 | 9 | si |
| *58 | 24/01/2018 | Ejido Blasillo | 10 | 0 | 280 | 0 | 78 | 21 | si |
| *59 | 24/01/2018 | Ejido Blasillo | 3 | 0 | 82 | 0 | 32 | 34 | si |
| 60 | 09/03/2018 | Nuevo Becal | 1 | 0 | 29 | 0 | 15 | 43 | si |
| 61 | 09/03/2018 | Nuevo Becal | 0 | 0 | 43 | 0 | 16 | 44 | si |
| 62 | 09/03/2018 | Nuevo Becal | 3 | 0 | 17 | 0 | 4 | 8 | si |
| 63 | 09/03/2018 | Nuevo Becal | 1 | 0 | 40 | 0 | 39 | 26 | si |
| 64 | 09/03/2018 | Nuevo Becal | 1 | 0 | 133 | 0 | 52 | 37 | si |
| 65 | 09/03/2018 | Nuevo Becal | 1 | 0 | 83 | 0 | 25 | 14 | si |
| 66 | 09/03/2018 | Nuevo Becal | 2 | 0 | 18 | 0 | 8 | 16 | si |
| 67 | 09/03/2018 | Nuevo Becal | 1 | 0 | 32 | 0 | 3 | 10 | si |
| 68 | 09/03/2018 | Nuevo Becal | 0 | 0 | 51 | 0 | 3 | 12 | si |
| 69 | 09/03/2018 | Nuevo Becal | 1 | 3 | 62 | 0 | 25 | 7 | si |
| 70 | 19/03/2018 | Ejido Blasillo | 2 | 2 | 70 | 0 | 64 | 16 | si |
| 71 | 19/03/2018 | Ejido Blasillo | 1 | 0 | 116 | 0 | 77 | 40 | si |
| 72 | 19/03/2018 | Ejido Blasillo | 6 | 2 | 223 | 0 | 69 | 163 | si |
| 73 | 19/03/2018 | Ejido Blasillo | 1 | 6 | 53 | 29 | 28 | 38 | si |
| 74 | 19/03/2018 | Ejido Blasillo | 1 | 0 | 207 | 0 | 80 | 34 | si |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 75 | 19/03/2018 | Pioneros del río Xnoha | 1 | 0 | 91 | 0 | 83 | 42 | si |
| 76 | 12/04/2018 | Sian Ka´an | 1 | 8 | 48 | 12 | 49 | 22 | si |
| 77 | 12/04/2018 | Sian Ka´an | 1 | 6 | 126 | 4 | 35 | 40 | si |
| 78 | 12/04/2018 | Sian Ka´an | 1 | 1 | 74 | 39 | 23 | 21 | si |
| 79 | 12/04/2018 | Sian Ka´an | 1 | 2 | 87 | 0 | 47 | 37 | si |
| 80 | 12/04/2018 | Sian Ka´an | 1 | 0 | 43 | 0 | 24 | 29 | si |
| 81 | 12/04/2018 | Sian Ka´an | 2 | 5 | 77 | 2 | 24 | 88 | si |
| 82 | 12/04/2018 | Sian Ka´an | 1 | 1 | 45 | 0 | 15 | 4 | si |
| 83 | 12/04/2018 | Sian Ka´an | 1 | 0 | 50 | 0 | 28 | 37 | si |
| 84 | 12/04/2018 | Sian Ka´an | 4 | 2 | 119 | 1 | 55 | 58 | si |
| 85 | 12/04/2018 | Sian Ka´an | 1 | 2 | 99 | 3 | 38 | 56 | si |
| 86 | 16/01/2019 | Hermenegildo Galeana | 1 | 0 | 171 | 0 | 61 | 93 | si |
| 87 | 16/01/2019 | Ejido Blasillo | 2 | 0 | 197 | 0 | 31 | 149 | si |
| 88 | 16/01/2019 | Ejido Blasillo | 6 | 4 | 52 | 0 | 20 | 15 | si |
| 89 | 16/01/2019 | Ejido Blasillo | 1 | 0 | 74 | 0 | 78 | 30 | si |

*Usados en los bioensayos de elección

** Usados en los bioensayos de interacción

***Nidos colectados en troncos de árboles

**Anexo 2.** Artículo aceptado.

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Original Article

# 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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## 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

**Anexo 3.** Constancia de recepción del artículo por la revista Insects.

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