



RESEARCH ARTICLE - WASPS

Two more social wasp species (Vespidae, Polistinae) collect mullerian bodies from *Cecropia* trees (Urticaceae)

SPS FELIZARDO, RC BORGES, JNA SANTOS, OT SILVEIRA

Museu Paraense Emilio Goeldi (MPEG), Belém-PA, Brazil

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Corresponding author

Orlando T Silveira

Museu Paraense Emilio Goeldi (MPEG-MCTI)

Coordenação de Zoologia

Av. Perimetral, 1901, Campus de Pesquisa

CEP 66077-830, Belém-PA, Brasil.

E-Mail: orlando@museu-goeldi.br

Abstract

The interactive behaviors of two species of social wasps, *Protopolybia chartergoides* (Gribodo) and *Charterginus fulvus* Fox with *Cecropia obtusifolia* (Cecropiaceae) are described. The use of *Cecropia* Müllerian bodies by both wasps is also described, as well as the distribution of this trait in *Protopolybia* and *Charterginus*.

Introduction

Associations between plants and insects have long been known to science and may be among the oldest co-evolved systems on our planet (Bronstein et al., 2006; Mishra et al., 2015). The evolution of both specific and generalist interactions has certainly facilitated great diversification among both plants and insects (Kasting & Catling, 2003). The Neotropical genus *Cecropia* Loefling (Cecropiaceae, Urticaceae) is commonly a participant in plant-insect mutualistic associations (Weiblen & Treiber 2015), with a large majority of its species (ca. 70) being myrmecophytic and developing specialized structures for sheltering ants (mostly the genus *Azteca* Roger; Dolichoderinae) (Treiber et al., 2016). *Cecropia* trees provide nesting domatia and food bodies (Mullerian bodies - MBds) produced by “trichilia” (sing. trichilium; pad-like organs located on the base of the leaf petiole) (Oliveira et al., 2015), and on the leaves (Gonçalves-Sousa & Paiva, 2016). In exchange, ants provide nutrients to the plants and help protect them against herbivory.

Three social wasp species have been reported to interact with *Cecropia*-ant associations. The first case was described by Richards (1978), who collected *Protopolybia chartergoides* (Gribodo) on the trichilia pads of a *Cecropia* sp. tree, and suggested that its trichomes were used in nest construction by the wasp. Lapierre et al. (2007) made intensive observations of *Charterginus xanthura* (de Saussure) and *C. nevermanni* Bequaert collecting Mullerian bodies (MBds) on different *Cecropia* species in French Guiana and Costa Rica. These wasps can collect MBds from trichilia pads while individually foraging (although they are susceptible to aggressive encounters by patrolling ants), or they may use a group strategy (*C. xanthura*) in which one of the wasps faces a domatia entrance and prevents any ants from leaving the nest, while other wasps collect MBds from the trichilium.

The present article originated from our observations of workers of *Charterginus fulvus* Fox bringing aggregates of minute whitish ovoid bodies to their nests. After consulting the paper by Lapierre et al. (2007) where use of MBds by *Charterginus* species is reported, the origin of this material was



confirmed by observations of *C. fulvus* and *P. chartergoides* visiting the trichilia of *Cecropia* trees at the Goeldi Museum campus, in Belém, Brazil. Both wasps are epiponini swarming species, with largely Amazonian distributions (Richards, 1978). We have observed those wasps collecting trichomes and Mullerian bodies; their use of trichomes in nest construction is treated in another paper (Borges et al., 2017). To better describe the interactions between these wasps and *Cecropia* trees and ants, we made additional observations of their activities (mainly on *Cecropia obtusifolia* Bertol. trees) in the city of Belém as well as other nearby localities in Pará State, Brazil. The following aspects were addressed: (1) frequency and associative aspects of the occurrence of wasps on MBd producing trees; (2) the wasps' foraging behaviors while on the trees; (3) interactions between wasps and ants.

Methods

Observations were carried out mainly at the Museu Paraense Emílio Goeldi research campus (MPEG) (1°27'52"S x 48°26'36"W), Belém, Pará State, Brazil. At the MPEG campus, data were collected on working days, during fifteen months, from September/2015 to December/2016, focusing on a set of seven *Cecropia* trees, generally 2 m tall, spatially arranged in four groups, up to 350 m from each other, in an approximately 3.5 ha triangular area in the campus. However, not all of the trees were inspected during the entire 15-month observation period, for reasons varying from pruning by the campus administration (two cases), temporary inaccessibility because of a building reform (one case), or flooding (three cases). In general, all of the trees were observed for at least three months; three of them were observed for six months; and one tree was accompanied during the full observation period. The trees were inspected three times along each working day in a week (early in the morning, around midday, and late in the afternoon). Each observation event lasted for approximately one hour, during which time all accessible plants were inspected. The times of MBd formation, as well as wasp foraging activities, and ant and wasp interactions were registered.

The pruning of some of the trees eventually turned out to be a convenient circumstance because it "reset" the plant-ant interaction systems, and because observations became easier on lower trees. Being a pioneer species, *Cecropia* trees rapidly initiated regrowth (with some exceptions), with subsequent development of myrmecophytic structures for recolonization by ants. Weekly inspections were performed during the initial phases of regrowth to accompany tree and trichilia development and reveal the presence of colonizing ants. Regular observations reintiated once the trees began to produce Mullerian bodies.

Wasps and ants were collected directly on the plants (using entomological forceps) and subsequently identified in the laboratory using the keys published by Richards (1978) for wasps, and by Longino (2010) for ants. Plant identifications

were made by Dr. Mario Jardim, of the Botany Department of the Goeldi Museum. Photographs documenting wasp and ant activities on trees were taken using a Canon 600D digital camera.

Additional observations were made on trees in other localities in Belém and other cities in Pará state (Abaetetuba, Acará, and Barcarena), and in São José de Ribamar, Maranhão State. Observations were carried out for only one day in those areas.

Results

Wasps' visits to the trees

Visits by the two vespidae wasps to *C. obtusifolia* were largely limited to trees still unoccupied by *Azteca* ants, with both wasp species being reasonably common in those cases; the visits occurred even during moderate rains. Visitation frequencies could be very high (ca 80% of inspected trees) before tree colonization by the ants, and at the times of day when MBd production was most intense (mainly between 11:30 and 13:30). Mature MBds were not commonly found in the early morning and late afternoon, and wasps were present in lower numbers at those times (mostly *C. fulvus* collecting trichomes).

P. chartergoides was by far the most frequent vespidae species, and the exclusive wasp in ca. 87% of the observed visits. In 10% of the cases, both *P. chartergoides* and *C. fulvus* were observed foraging together on trees (rarely on the same trichilium), and *C. fulvus* was the sole visiting wasp species in only 3% of cases.

Ants

The ant species *Camponotus* sp. and *Nylanderia* sp. (Formicinae), *Solenopsis* cf *invicta* and *Crematogaster erecta* Mayr (Myrmicinae), and *Azteca alfari* Emery (Dolichoderinae) were commonly observed on focal trees. Ants other than *Azteca* species did not form resident colonies.

Protopolybia chartergoides behavior

Before occupation by *Azteca* ants, *P. chartergoides* wasp foragers (from only a few, up to seven individuals) were commonly seen on *Cecropia* trees (on nearby leaves) just before the appearance of MBds. The wasps remained largely inactive before the MBds matured, only sporadically inspecting the trichilia pads for MBd availability (Fig 1). After the emergence of MBds on any given trichilium, individual foraging wasps would collect the corpuscles and then fly away. After a successful MBd harvesting event, the number of visiting wasps on a tree commonly increased, and two wasps could collect MBds from a single trichilium (Fig 2).

P. chartergoides wasps visiting a productive trichilium would quickly collect and swallow a small number of mature MBds (estimated between five and ten). The wasp would then regurgitate the material and masticate it for some time, without leaving the plant; they would then swallow it again and fly away.

Charterginus fulvus behavior

Most *C. fulvus* foragers were observed alone on the trees. They could occasionally be seen in small groups of up to three foragers, but they would forage independently on different trichilia.

Unlike *P. chartergoides*, *C. fulvus* foragers were not observed to remain inactive and wait on a plant for the emergence of mature MBds. They would arrive and then immediately search the trichilia, collecting either trichomes or MBds, if the latter were available.

To harvest the MBds, *C. fulvus* foragers would remove them with their mandibles and mash them into a ball using their mouthparts and forelegs. They were never observed swallowing MBd aggregates. Observations on material brought by foragers to a *C. fulvus* nest on the MPEG campus (see Borges et al., 2017), indicated that MBd masses were shared with workers in the nest, in the same way as other collected materials.

Wasp – ant encounters (non-*Azteca*)

Before *A. alfari* colonies were established, other ant species were commonly observed on *Cecropia* trees, *Camponotus* sp. being the most common. Encounters between

vespid wasps and *Camponotus* ants were clearly antagonistic, with ants consistently being chased away by the wasps. The wasps would typically flap their wings vigorously while facing the ants, but without leaving the trichilium (Figs 3 and 4).

Resident ants

After the trees had fully developed myrmecophytic structures, *A. alfari* ants became more frequent and *Camponotus* ants became scarcer, eventually disappearing. When the domatia were open, *Azteca* ants patrolled the entire plant and collected MBds on trichillia.

Once *A. alfari* had established their nests on the trees, wasp visitation frequencies diminished, until they completely stopped. No significant interactions were observed between wasps and *A. alfari* individuals.

Observations of plants in different stages of development, and the “resetting” produced by pruning the trees on the MPEG campus, showed that the interrelated phenomena of tree development and colonization by *A. alfari* limit the use of trichilia resources by the wasp species studied. While MBds are known to be mutualistic rewards for *Azteca* ants, during the early stages of plant development those ants were absent from the trees and *P. chartergoides* and *C. fulvus* could freely exploit the trichilia MBds and trichomes.



Figs 1–4. *Protopolybia chartergoides* and *Camponotus* sp. on *Cecropia obtusifolia*. 1-4, *P. chartergoides* individuals collecting Mullerian bodies; 3-4, interaction between wasp and ant near a trichilium, with evasive behavior of ant. (scales in photographs are similar but not identical)

Observations in other areas

The same social wasp species were observed using *Cecropia* tree resources in all of the other study locations, except Maranhão State, as *C. fulvus* was not observed in São José de Ribamar. Actually, this species has not been recorded from that state (see Andena et al., 2009; Richards, 1978). Observations in these places also focused on relatively young trees (less than three meters tall, with diameters ranging from 1 to 10 cm). As observed in the MPEG campus, the wasps in other areas collected both MBds and trichomes, and trees with resident and patrolling ants showed reduced numbers of visiting wasps.

Discussion

Our observations showed that at least two more social wasp species harvest Mullerian bodies on *Cecropia* trees in the Neotropics, one of them being a member of the genus *Protopolybia* (with no previously published reports on that behavior). *P. chartergoides* was a far more frequent visitor to *Cecropia* than *C. fulvus*, and its MBd collecting behavior was more elaborate, involving sequential acts of swallowing-regurgitating-masticating and then swallowing the material again, to achieve a full load to transport to the nest. *C. fulvus* transported MBds externally (as aggregates) held between their mouthparts and legs. Different from *C. xanthura* (Lapierre, 2007), neither of these species was a frequent visitor, nor were they successful at harvesting MBds during later plant developmental stages when colonies of *Azteca* ants became well-established; likewise, they did not demonstrate group strategies for coping with ant defenses.

At least in that studied part of northeastern Amazonia, visiting by the two focal social wasps to *Cecropia* trees is certainly far from being occasional. To the contrary, based on the regularity of their visits, it appears that MBds constitute a significant proportion of the foraging items of those wasps, and undoubtedly represent a very important food resource for their colonies. In that respect, it is important to note that during parallel observations of a colony of *C. fulvus* at the Goeldi Museum campus, a large proportion of the observed foragers returned to the nest carrying trichomes and MBds. Further investigation showed that the observed nest was entirely constructed with trichomes collected on trichilia of *Cecropia* trees (Borges et al., 2017). Quantitative measurement of the relative importance of MBds as forage items should be undertaken in future studies of the foraging activities of those wasps.

Field observations by one of the present authors (OTS) in the eastern Amazonia region suggest that both wasp species studied here preferentially occupy habitats at rain forest edges (or in clearings), even in sites experiencing moderate anthropogenic pressure (but in those cases, always in areas with access to reasonably preserved forest patches; e.g., the Goeldi Museum campus). Ecological information published

earlier (Lapierre et al., 2007), and now here, present a possible specific functional reason for the association of those wasp species with the early successional vegetation typical of edge habitats – given the strong character of *Cecropia* species as pioneer plants in the Neotropics.

One might ask if foraging for *Cecropia* MBds is a trait inherited from some common ancestor (to both *P. chartergoides* and the *Charterginus* species) at some level in the Polistinae phylogeny, or a convergent feature in those taxa. Relationships between *Charterginus* and *Protopolybia* are probably “close”, in the sense that their respective branches in published trees have often appeared in relatively nearby positions. Carpenter (1991) placed *Charterginus* as a sister group to a component (arranged as a polytomy) that included *Protopolybia* and several other epiponine genera. More recently, in a study based on both morphological and molecular characters (Pickett & Carpenter, 2010), the species *C. xanthura* was found to be sister to a group formed by three *Protopolybia* species, and *Brachygastra lecheguana* Latreille and *Protonectarina sylveirae* (de Saussure), the whole clade embedded within a large epiponine component (composed of 34 species from 12 of the total 19 genera).

Phylogenetic relationships in the genus *Charterginus*, as studied by Andena et al. (2009), showed two main components – one formed by *C. fulvus* and *C. xanthura* as sister taxa, and another formed by four remaining species, with the pattern *C. weyrauchi* (*C. nevermanni* (*C. carinatus* + *C. zavattari*)). The currently known distribution for the trait “MBd collecting” in *fulvus*, *xanthura*, and *nevermanni* indicates that it is possibly a primitive feature in the genus (expected to occur also in the other three species of the larger *weyrauchi-zavattari* clade).

The situation in the larger genus *Protopolybia* is quite different, with “MBd collecting” behavior by *P. chartergoides* being the only known occurrence of that trait in a genus with a slightly more than 30 species. *P. chartergoides* together with four other species form a small reasonably differentiated group formerly classified as the genus *Pseudochartergus* Ducke, 1905 (synonymized to *Protopolybia* by Carpenter & Wenzel, 1989). A preliminary phylogenetic study of *Protopolybia* by Santos et al. (2015), suggested that the “*Pseudochartergus*” group is probably monophyletic, although its relationships with other species-groups in *Protopolybia* are still uncertain. However, in none of the alternative most parsimonious phylogenetic hypotheses evaluated by Santos et al. (2015) the “*Pseudochartergus*” group appeared as a basal (or primitive) lineage, which, taken with current evidence on the distribution of “MBd collecting” behavior, indicates that this trait would not be part of the ground plan of *Protopolybia*. So, at present, this behavior would best be considered a convergent trait in *P. chartergoides*, independent of any close relationship between *Protopolybia* and *Charterginus*.

It is interesting to note, in respect of possibility of independent origins of the “MBd collecting” trait, that

Charterginus and some *Protopolybia* species (especially those of the “Pseudochartergus” group) share a peculiar (and certainly derived in Epiponini) feature related to head morphology, with an extremely narrow gena (the lateral part of the head behind the composite eyes) (see Andena et al., 2009; Richards, 1978). The inner side of genal wall, jointly with the occipital and post occipital areas, form the attachment surfaces for the mandible musculature, principally the large adductor muscles (Duncan, 1939; Silveira & Santos, 2011). Additionally, in respect of the female mandible morphology, the species *C. xanthura*, *C. fulvus*, *P. chartergoides*, *P. fuscatus*, *P. duckeianus*, *P. sedula*, and several other of the *P. exigua* group studied by Silveira and Santos (2011) have very similar morphologies, with well-developed apical teeth, but considerably reduced mesial (inner) denticles. It therefore seems reasonable to speculate whether these particular morphologies (i.e. regarding to reduced head wall surfaces, and attenuated mandible mesial relief) may be adaptively related to a biological role of collecting relatively soft materials such as plant trichomes for nest construction as well as Mullerian bodies as food items (see Sarmiento, 2004).

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