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Social wasps (Vespidae: Polistinae) on carcasses of *Rattus norvegicus* (Mammalia: Muridae) in the Central Amazonia, Brazil: possible forensic implications

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ABSTRACT

We evaluated the occurrence of social wasps in the decomposition process in tropical rainforest in central Amazonia (Ducke Reserve, Manaus, Brazil), using cadavers of rats as attractants, exposed in suspended cages. Ten species, in three genera, of social wasps were collected only in the initial stages of decay (fresh and bloated). Five species were collected, mainly in the fresh stage, feeding on flesh: *Agelaia angulata*, *Agelaia constructor*, *Agelaia fulvofasciata*, *Agelaia pallipes* and *Angiopolybia pallens*. Five species were collected, mainly in the bloated stage, feeding on flesh and eggs and first instar larvae of dipteran: *Agelaia testacea*, *Angiopolybia obidensis*, *Apoica arborea*, *Apoica pallens* and *Apoica thoracica*. Due to the aspect of the injuries caused by the wasps to the carcass, they may be mistaken as skin ulcers, burns or abrasions, which may mislead a forensic investigation.

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Introduction

Studies of entomofauna associated to decaying vertebrate carcasses are of great importance to forensic science, since insects can aid in criminal and non-criminal investigations by estimating the time passed since death, also known as minimum *post-mortem* interval (PMI_{min}) (Oliveira-Costa, 2013). These studies also allow the description of interesting behavioral patterns, as reported for wasps. Social wasps seek protein sources to feed themselves and their offspring, and this protein can come from many different preys, but mainly from immature insects (e.g. larvae) (Jeanne, 1972). However, in some cases, they also use decaying vertebrate carcasses as a source of protein (Snelling, 1953), such as the Epiponini wasps from South and Central America (last reviewed by O'Donnell, 1995).

In Central and South America, previous studies have reported species in carcasses distributed in seven genera, with most representatives being part of *Agelaia* Lepeletier, 1836 and *Angiopolybia* Araujo, 1946 (Ducke, 1910; Bertoni, 1912; Richards and Richards, 1951; Cornaby, 1974; Forsyth, 1978; Richards, 1978; O'Donnell, 1995; Silveira et al., 2005), and a few of *Apoica* Lepeletier, 1836 (Simões et al., 2013), *Brachygastra* Perty, 1833 (O'Donnell, 1995),

Parachartergus R. von Ihering, 1904 (O'Donnell, 1995), *Polybia* Lepelletier, 1836 (Bertoni, 1912; Richards and Richards, 1951; Cornaby, 1974; Forsyth, 1978; O'Donnell, 1995), *Protonectarina* Ducke, 1910 and *Synoeca* de Saussure, 1852 (O'Donnell, 1995).

Recently, studies have pointed out the use of wasps in forensic analyses (Gomes et al., 2007a,b; Moretti et al., 2008, 2011). However, there are no published reports on *post-mortem* injuries caused by these wasps, except for *Agelaia fulvofasciata* on domestic pig carcasses in northern Brazil (Barbosa et al., 2015). It is known that carrion is an important source of protein and other nutrients for some wasp's species, usually for *Agelaia* (Cornaby, 1974).

Because of this habit of removing small pieces of decaying flesh, these species are of forensic entomological concern, since they remove tissue mainly from around the cavities of the carcasses, such as the nose, mouth, ear and anus (Gomes et al., 2007a,b). This behavior causes *post-mortem* injuries that alter the skin texture and increase the diameter of the natural cavities of the corpse, as well as causing injuries that occasionally confuse the expert analyzing the corpse (JAR, personal observation), since they resemble injuries caused at, or near, the time of death by puncture-blunt or punch-cutting objects. For ants, some injuries have already been reported and they also produce artifacts that may be misinterpreted as wounds or mutilations on the cadaver, leading to errors in forensic investigations (Moretti and Ribeiro, 2006; Gomes et al., 2007b).

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The flesh is cut from the carcass with the mandibles, macerated, and ingested or transported by the wasp to use as food or to later regurgitate in the nest cells to feed the larvae (O'Donnell, 1995; Gomes et al., 2007a,b). Furthermore, wasps can decrease the fly larvae population on the corpse, since the larvae are also acceptable protein sources for them, and although the effects on the fly population are not clear, they cannot be ignored (Gomes et al., 2007a,b). Another possible consequence, is a change in the dynamics of the entomological succession pattern, since predatory beetles will have reduced food supply, and this order is, generally, the second most abundant in the cadaveric decomposition process (Oliveira-Costa, 2013). This study evaluated the presence of social wasps on rat carcasses and their necrophagous activities during the decomposition stages and discussed the possible implications of our results bring to forensic entomology.

Material and methods

Sampling was carried out in a primary terra firme forest area of the Ducke Reserve, near Manaus, Amazonas, Brazil, using twelve rats, *Rattus norvegicus* wistar lineage (Mammalia: Muridae), as baits for each experimental season. The climate in the area is humid tropical, with a mean annual relative humidity around 80%, mean annual precipitation of 1750–2500 mm and mean annual temperature of 26 °C. The vegetation is lowland tropical rainforest, with a closed canopy and shady understory, characterized by the abundance of palm trees (Baccaro et al., 2008).

The rats were provided by Laboratório Temático Biotério Central in the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil, and have a low corporal mass (~180 g). They were killed in the experimental site, twelve in October 2011 (dry season) and twelve in March 2012 (rainy season), through cervical rupture, followed by puncturing of the jugular for increased blood spill, and laid on their backs to expose the genitalia, thus promoting the release of liquids after death.

Six 2-cm mesh wire cages were suspended around 1 m above the ground and 50 m distant from each other, with two rats placed in each cage. The wasps were actively and regularly collected by tweezers on the carcasses since the time of death, each 4–5 h during daytime, until the carcasses reached the dry remains stage. The carcasses stages follow Oliveira-Costa (2013). The specimens were identified by the first author and compared with the INPA Invertebrate collection material. The voucher wasp specimens were deposited in the same collection. The behavioral activities of the social wasps attacking carcasses was filmed and photographed *in loco* during sampling by a camera Canon G16 and the images were treated in Adobe Photoshop.

The experiments were authorized and recorded under authorization number no. 035/2018, SEI 01280.000899/2018-61 by the Ethics Committee on Animal Use in Instituto Nacional de Pesquisas da Amazônia (CEUA-INPA), based on the Brazilian Control Committee for Animal Experimentation (CONCEA).

Results

Ten species, in three genera, of social wasps were collected: *Agelaea angulata* (Fabricius, 1804), *Agelaea constructor* (de Saussure, 1854), *Agelaea fulvofasciata* (DeGeer, 1773), *Agelaea pallipes* (Olivier, 1792), *Agelaea testacea* (Fabricius, 1804), *Angiopolybia obidensis* (Ducke, 1904), *Angiopolybia pallens* (Lepelletier, 1836), *Apoica arborea* de Saussure, 1854, *Apoica pallens* (Fabricius, 1804) and *Apoica thoracica* du Buysson, 1906. All species occurred in the dry and rainy seasons.

The wasps were collected only in the first two decomposition stages: 67 specimens in the fresh stage (1–24 h) and 31 specimens

in the bloated stage (24–48 h), the total decomposition time was six to seven days. Five species were collected mainly in the fresh stage of the rat carcasses: *Agelaea angulata*, *Agelaea constructor*, *Agelaea fulvofasciata*, *Agelaea pallipes* and *Angiopolybia pallens*, feeding on flesh (Fig. 1B, C). Five species were collected mainly in the bloated stage: *Agelaea testacea*, *Angiopolybia obidensis*, *Apoica arborea*, *Apoica pallens* and *Apoica thoracica*, feeding on flesh of the rat carcasses and also clearly predating on eggs and first instar larvae of flies of Sarcophagidae and Calliphoridae (first Diptera families to colonize the carcasses).

During the present investigation, 48 specimens of *Agelaea pallipes* were captured in the carcasses, with the most abundant species, representing about 50% of the individuals collected. The wasps lacerated the skin and the musculature of the carcasses in the fresh and bloated stages. The areas most affected were close to the jugular, the mucosa of the nose, eyes, mouth and genitalia, and less frequently in regions with lower hair density, such as the foot and abdomen. The wasps removed musculature, lacerating mainly on natural orifices and in less hairy areas (Fig. 1A–F, Link 1 and Link 2).

Discussion

Among the ten species of wasps collected, *Agelaea fulvofasciata*, *Agelaea pallipes*, and *Angiopolybia pallens* are the most frequently collected in the Ducke Reserve (Somavilla et al., 2014). *Agelaea fulvofasciata* and *Angiopolybia pallens* are possibly the most frequent social wasp species in Amazonian environments, the former being more easily collected in anthropic and disturbed environments, and the latter in preserved environments and *terra-firme* forests.

The Neotropical species of *Agelaea* (33 species) and *Angiopolybia* (four species) are widely distributed in the Neotropical region, extending from northern Argentina to Mexico, and are known for their necrophagous habits and as predators of other insects (O'Donnell, 1995; Gomes et al., 2007b). It was only recently that researchers addressed the potential use of social wasps' presence and injuries caused in the carcasses in forensic analyses (Gomes et al., 2007a,b; Moretti et al., 2008, 2011; Barbosa et al., 2015).

Silveira et al. (2005), in a study in a *terra-firme* forest in Floresta Nacional Caxiuanã, Pará, Brazil, collected six necrophagous species of *Agelaea* and *Angiopolybia* with a trap using putrefied bovine meat as bait. When comparing the fauna of Caxiuanã with the one found in the current study, we found that: Two species were collected only in the Caxiuanã forest (*Agelaea cajennensis* and *Angiopolybia paraensis*), five species only in the Ducke Reserve (*Apoica arborea*, *Apoica pallens*, *Apoica thoracica*, *Agelaea constructor* and *Angiopolybia obidensis*), and five species were common to both localities (*Agelaea angulata*, *Agelaea fulvofasciata*, *Agelaea pallipes*, *Agelaea testacea* and *Angiopolybia pallens*). These results show that species occurring in both localities show necrophagous behavior.

The three *Apoica* species collected in the Ducke Reserve, *Apoica arborea*, *Apoica pallens* and *Apoica thoracica*, are the only polistine wasps that have nocturnal habits and are commonly collected while attracted by artificial light (Somavilla et al., 2014). Their diurnal occurrence on the carcasses suggests a possible daily foraging if have food source that is near the nest and is easily accessible. This is the second record of *Apoica* species consuming carcasses and with necrophagous activities, the first being made by Simões et al. (2013).

Agelaea fulvofasciata and *A. pallipes* are common species in fish markets in northern Brazil, where it is popularly known as *caba do peixe* (fish's wasp) (O'Donnell, 1995). The experiments in Caxiuanã and the Ducke Reserve demonstrate that it also forages on



Fig. 1. (A) *Rattus norvegicus* nose before and after the wasps; (B, C) *Agelaia pallipes* on the nose of a *Rattus norvegicus* carcass in the fresh stage; (D) *Rattus norvegicus* nose after wasps; (E) *Agelaia pallipes* on the foot of a *Rattus norvegicus* carcass in the fresh stage; (F) *Rattus norvegicus* foot after wasps.
 Link 1. *Agelaia pallipes* on the nose of a *Rattus norvegicus* carcass in the fresh stage.
 Link 2. *Agelaia pallipes* on the foot of a *Rattus norvegicus* carcass in the fresh stage.

mammal carcasses and therefore can be considered an opportunistic species, as already demonstrated for other *Agelaia* species like *Agelaia angulata*, *A. areata*, *A. cajannensis*, *A. hamiltoni*, *A. multipicta*, *A. panamensis*, *A. yepocapa* and *A. testacea* in a wide variety, and diverse sizes, of carcasses by O'Donnell (1995) in Costa Rica, Peru, and Venezuela and Silveira et al. (2005) in Pará, Brazil. The real importance of necrophagy for brood nutrition in social wasps is difficult to assess, but the skills of *Agelaia* sp. at rapidly discovering pieces of carcasses (Cornaby, 1974) may suggest carrion is an important source of protein and other nutrients.

Based on the temporal distribution observed during the experiments, the wasps only visit the carcasses in the first two stages of decay (fresh and bloated). When comparing the species colonizing the fresh stage with the ones in the bloated stage, the higher abundance of wasps in the first than the second stage. During the natural decomposition of proteins of the carcass, production of putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) peak during those two stages (Nelson and Cox, 2014) and probably act as an attractant for the wasps. The presence of the wasps only in the first two stages of decay could be explained because adult wasps lacerate the skin only to access the still irrigated superficial muscle tissue, when there is still adequate water content and protein that

can be consumed and used in the maturation of the wasp's eggs (O'Donnell, 1995) and probably larvae.

According to Gomes et al. (2007a,b) wasps fed on the superficial tissues of the carcasses, as well as preyed on eggs and first instar larvae of Diptera. This behavior has a potential to decrease the larval mass of Diptera on the carcasses and, consequently, reduce the number of older fly larvae, and these older larvae are important to estimate the PMImin. In a place where the wasps are abundantly attacking the carcasses, the PMImin can be influenced by their activities as predators, removing lots of the older flies' larvae and making it more difficult to do any estimative, but once collected and correctly identified, a single older fly larva specimen may be sufficient to generate a precise estimative.

Silveira and dos Santos (2011) reported *Agelaia*, *Angiopolybia* and *Apoica* species with modified structures on the mandible associated with necrophagy (dorsal tooth as long as the mandible and elongated into an acute ridge that spans the length of that surface, which is not so developed in other species of social wasps), suggesting that the adult species of these three genera feed on decaying flesh. For the first two genera, this habit has already been corroborated, since many studies already observed these organisms in animal carcasses (Ducke, 1910; Bertoni, 1912; Richards

and Richards, 1951; Cornaby, 1974; Forsyth, 1978; Richards, 1978; O'Donnell, 1995; Silveira et al., 2005; Gomes et al., 2007a,b; Moretti et al., 2008, 2011; Barbosa et al., 2015). For *Apoica*, due to the well-developed tooth in the mandible, its phylogenetic position (Noll et al., 2004), and the current lack of records about its source of proteins, we suggest that this genus could also be necrophagous, and should therefore be considered opportunistic and important for forensic entomology. The behavior observed in this study seems similar to that which was observed in ants by Patel (1994) and de Sales et al. (2015), who emphasized that the trophic level of ants may vary from predator (when feeding on insect eggs, larvae or pupae) to necrophage (when feeding on body fluids, or on decaying tissues) on animal carcasses.

The process of dehydration, as well as the detachment of pieces of the epidermis after death, influences the appearance of injuries caused by wasps. The type of laceration done by the wasps in the bloated stage made injuries with longer borders, probably due to the ease of extracting tissue parts that had already deteriorated by the decay process, but also because of the powerful oral apparatus of the wasps. Knowledge on the behavior and injuries caused by wasps is important for forensic examiners so as not to mistake the injuries as *pre-mortem*. This is relevant to crimes involving human beings, since the injuries that wasps cause on the corpses during the initial stages of decay (fresh and bloated), and the wasps' occurrence *per se*, can affect the conclusions of forensic investigations. In this way, during the autopsy, all injuries should be adequately understood to avoid compromising the police investigation, therefore, any biotaphonomic event caused by carrion insects must be investigated, especially in criminal cases.

The knowledge on the possible effects of the social wasps as consumers of fresh stage and decaying flesh is still incipient. Further studies with wasps on carrion are required to analyze them as displacement species, consumers of carcasses' resources, and to survey the rates of flesh and fly egg removal, thus assessing their importance to forensic entomology in the Amazon Basin. The records of social wasps associated with rodent carrion in a wet Amazon forest is an important contribution to medicolegal entomology, because Vespidae is one of the most neglected taxa in the cadaveric entomofauna. The actual function of social wasps in carrion decomposition is still not appreciated by crime-scene investigators, and it is about time to do so!

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbe.2018.12.001.

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