Research Article

Effect of Habitat Type on Parasitism of *Ectatomma ruidum* by Eucharitid Wasps

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Eucharitidae are parasitoids that use immature stages of ants for their development. *Kapala* Cameron is the genus most frequently collected in the Neotropics, but little is known about the biology and behavior of any of the species of this genus. We aimed to evaluate the effect of habitat type on eucharitid parasitism and to contribute to the knowledge of the host-parasite relationship between *Kapala* sp. and the poneromorph ant *Ectatomma ruidum* (Roger) in Colombia. Twenty *E. ruidum* colonies were extracted from two different habitat types (woodland and grassland), and larvae and cocoons (pupae) were examined in search for parasitoids in different stages of development. Globally, 60% of the colonies were parasitized, with 1.3% of larvae and 4% of pupae parasitized. Planidia (first-instar larvae), pupae, and adults of the parasitoid were observed. All of the pupae and adult parasitoids belonged to *Kapala iridicolor* Cameron. All the colonies collected in the woodlands were parasitized and contained more parasitized larvae (2%) and parasitized cocoons (8%) than those collected in grasslands (4/12 parasitized colonies, 0.5% parasitized larvae, 0.8% parasitized cocoons). The relationship observed between habitat type and parasitism prevalence is a novel aspect of the study of eucharitid impact on ant host populations.

1. Introduction

Several dipteran, strepsipteran, and hymenopteran parasitoids are natural enemies of ants [1–9]. Among the hymenopterans, the Eucharitidae *sensu stricto* is the only monophyletic group, at the family level, where all of its members are parasitoids of ants. They are also one of the largest and most diverse groups attacking social insects [8].

Eucharitidae have a specialized life cycle that includes oviposition away from the host, on or into a host-plant [2]. Although there are more than 400 species of Eucharitidae already described [8], the hosts and host-plants of only a few species are known [10], and knowledge on the life history and ecology of these wasps is even scarcer. In the New World, detailed studies on selected species have only been carried out in a few localities in Mexico, Argentina and North America (e.g., [1, 11–17]). For Colombia, there is no detailed report on the biology of any species of this family.

The impact of eucharitids on their host populations has recently been explored in detail for some Mexican and South American ant populations [12, 17–19]. These, and earlier reports (e.g., [2, 11, 20]), signaled the aggregated nature of eucharitid populations. In fact, prevalence of parasitism by eucharitids varies greatly in time and space [2], with 100% of colonies parasitized at some sites, and other colonies escaping from parasitism (e.g., [18, 19]). Differences in local parasitism, in general, can be attributable to several different factors such as the presence of resources, other than hosts, necessary for maintaining high parasitoid populations locally (e.g., floral and extrafloral nectar, and refuge sites for adults), suitable host-plants, microclimatic differences, and/or dispersal capacity of adult parasitoids [21, 22]. In some cases, for example, parasitoids may be less effective at parasitizing hosts in sites with simpler vegetation [23]. In the case of eucharitids, an aspect not yet studied in detail is the effect of the habitat on the impact of these parasitoids on...
their ant-host populations, though preliminary results of a recent study suggest that differences in management in coffee agroecosystems (i.e., shade, pruning, weed management) might affect parasitism by eucharitids [24].

_Ectatomma ruidum_ (Roger) (Hymenoptera: Formicidae: Ectatommini) is a diurnal, earth-dwelling, Neotropical ant that nests in the soil. This ant is found from southern Mexico to Brazil, from sea level to an altitude of 1500–1600 m [25–27], and is dominant in several ecosystems such as forests [28], or economically important cultivated areas [29, 30]. Two species of _Kapala_ (Eucharitidae) have been reported to parasitize this ant in Mexico [14, 31], and parasitism of _E. ruidum_ by _Kapala_ sp. is also known from Colombia (C. Santamaria and J. Herrera, unpub. data). The purpose of this study is to report observations of the host-parasite relationship between _Kapala_ sp. and _E. ruidum_ in Colombia and to compare the impact of this eucharitid on its ant host population in two different habitat types.

### 2. Materials and Methods

This study was carried out on the grounds of the Melendez Campus at the Universidad del Valle (3° 22’ N, 76° 32’ W), located at the south of the city of Cali, Department of Valle del Cauca, Colombia. The Campus has an area of approximately 100 ha, 8 ha of which are occupied by buildings, 44 ha by woodlands, 46 ha by grasslands, and 1 ha by two ponds. The average elevation is 970 m; mean annual temperature is 24.1°C and average relative humidity 73% [32]. Average annual rainfall is around 1500 mm, with two rainfall peaks, from March to May and from September to November (Instituto de Hidrobiologia, Meteorología y Estudios Ambientales-IDEM, unpublished data, cited by [32]). According to the Holdridge system, the study site is located in an area classified as Tropical Dry Forest (bs-T) [33].

Five sites on the campus were examined: 2 sites in grasslands dominated by Poaceae and other creeping plants and with no trees, and 3 sites located in woodlands. The sites in the latter habitat had a lesser amount of Poaceae among the creeping vegetation and had, in some cases, abundant litter. Common tree species in these sites were _Pithecellobium dulce_ (Roxb.) Benth., _Samanea saman_ (Jacq.) Merr., and _Calliandra pitiari_ Standl. (Fabaceae); _Mangifera indica_ L. (Anacardiaceae); _Ceciba pentandra_ (L.) Gaertn. (Bombacaceae); _Ficus elastica_ Roxb. (Moraceae); and _Tabebuia chrysanthana_ G. Nicholson, _T. rosea_ (Bertol.) A. DC., and _Spaithodea campanulata_ P. Beav. (Bignoniaceae) [32]. In each of the 5 sites chosen, we determined the number of _E. ruidum_ nests in a plot of 8 × 8 m. One additional plot, placed 50 m from the closest grassland plot and comparable to the others, was censused for nest density evaluation in the grassland area, to get an even sample size. During April, May, and November 2009, 20 nests chosen at random were excavated (8 in woodlands and 12 in grasslands) and transported to the laboratory for examination.

Ant larvae were inspected for planidia (eucharitid first-instar larvae) attached to their cuticle by means of a stereoscopic-microscope (Nikon SZ645). Cocoons were kept in petri dishes at room conditions for 5 days or more and were examined once daily to record emergence of adult eucharitids. At the end of the observation period, all of the cocoons were dissected to look for adults and pupae of dead, or not yet emerged, parasitoids, and to register the caste and sex of ants attacked by the parasitoids. Adult wasps were individually placed in vials covered with cloth mesh, and their survival time was evaluated. No food or water was provided. Pupae and adult eucharitids were identified with available keys [8, 34, 35], and their sex was determined, when possible, based on the dimorphism present in the antennae [8]. The material collected was measured using a stereomicroscope equipped with an ocular micrometer and preserved in 96% alcohol. Voucher specimens of both the ants and the parasitoids have been deposited in the Grupo de Investigación en Ecología de Agroecosistemas y Hábitats Naturales (GEAHNA) collection, at the Museo de Entomología of the Universidad del Valle, Colombia (MEUV), and at the Arthropod Collection of El Colegio de la Frontera Sur, Unidad Chetumal, Mexico (ECO-CH-AR).

A Fisher’s exact test was carried out to establish whether there were significant differences between the proportions of parasitized colonies found in woodlands and in grasslands, and _Z_ tests were used to search for differences in the number of parasitized larvae and parasitized pupae according to habitat. Nest density and colony size according to habitat (woodlands or grasslands), and colony size according to the presence or absence of parasitoids (both habitats), were compared using a Mann-Whitney test. Spearman nonparametric correlation was used to explore the relationship between the size of the colony (adults + brood) and total parasitized brood, between the number of larvae per colony and total parasitized larvae, and between the number of cocoons per colony and total parasitized cocoons. All statistics were calculated using STATISTICA 8.0 (StatSoft, Inc.) and R 2.13.1 (The Foundation for Statistical Computing) programs.

### 3. Results

Of the 20 _E. ruidum_ colonies examined, 12 (60%) were parasitized (Table 1). The global rate of parasitism in the study area was 2.3% (parasitized brood per total ant brood, 27/1162), with 1.3% (9/714) of the larvae and 4.0% (18/448) of the pupae parasitized. In total, 29 eucharitid individuals or their remains were observed, with 2.4 ± 2.6 (mean ± standard deviation; _n = 12_ colonies; range: 1–10) parasitoids per parasitized colony. Parasitoids in 3 different stages of development were found: planidium in 7 colonies (1.6 ± 0.8 parasitized larvae per parasitized colony; range: 1–3), pupae in 3 colonies (3.3 ± 3.2 individuals; range: 1–7), and adults in 5 colonies (1.6 ± 0.9 individuals; range: 1–3). Pupae and adults were identified as belonging to _Kapala iridicolor_ (Cameron).

All of the colonies collected in the woodlands were parasitized (_n = 8_), while in the grasslands only 33.3% (4/12) contained eucharitids (Table 1). Prevalence of parasitism and type of habitat were not independent (Fisher’s two-tailed exact test: _P = 0.0047_), and there was a greater frequency of parasitized nests in the woodlands than in...
the grasslands (Fisher’s one-tailed exact test: $P = 0.039$). Furthermore, the proportion of parasitized pupae differed between both habitats ($Z$-test, $Z = 2.9; P = 0.003$), with a greater number of parasitized pupae in the woodlands than in the grasslands (Figure 1). Although a greater proportion of parasitized larvae was also observed in the woodlands (Figure 1), there was no statistical difference according to habitat ($Z$-test, $Z = 1.79; P = 0.07$). The global rates of parasitism for the woodlands and the grasslands were 4.2% and 0.65%, respectively. The average number of parasitoids per parasitized colony was greater in the woodlands (3 ± 3.1 parasitoids; range: 1–10) than in the grasslands (1.25 ± 0.5 parasitoids; range: 1–2).

There was a significant, positive correlation between the number of parasitized pupae and the number of available pupae (Spearman correlation test, $r = 0.63, P = 0.004, n = 19$ colonies). However, no correlation was found between the following variables: (1) total parasitized brood and colony size (Spearman, $r = 0.39, P = 0.08, n = 20$ colonies), (2) total parasitized larvae and number of available host larvae (Spearman, $r = -0.04, P = 0.86, n = 20$ colonies), and (3) total parasitized brood and number of workers (Spearman, $r = 0.22, P = 0.36, n = 20$ colonies).

Workers and ant larvae were present in all of the *E. ruidum* colonies but there were no cocoons in one of them (Table 1). The global mean size of the colonies (queen, gynes, workers, males, pupae, and larvae) was 82.6 ± 41.7 individuals. Colony size was greater in colonies from the woodlands and in those parasitized (95.1 ± 59.2 and 93.8 ± 47.8 individuals, respectively) than in those from the grasslands (74.3 ± 24.0), or from nonparasitized colonies (66.1 ± 24.7). Nevertheless, there were no significant differences in colony size between parasitized and non-parasitized colonies (Mann-Whitney test, $U = 29, P = 0.14, n_1 = 12, n_2 = 8$), nor according to the habitat from which the colonies came (Mann-Whitney test, $U = 40, P = 0.62, n_1 = 12, n_2 = 8$). A significantly greater density of *E. ruidum* colonies was estimated for the grasslands (3281 colonies/ha), compared to that for the woodlands (1563 colonies/ha) (Mann-Whitney test, $U = 0, P = 0.049, n_1 = n_2 = 3$).

Six planidia attached to the cuticle of *E. ruidum* larvae (Figure 2(a)) were observed. Five were in the interior of a sclerotized ring (Figures 2(b) and 2(c)) while one was not (Figure 2(e)). In 5 cases, sclerotized rings with no planidia were observed (Figure 2(d)). In 2 host larvae from different colonies, 2 planidia (or empty scars) were observed (representing 22% of the parasitized larvae, 2/9). Very small host larvae were found parasitized. The length of parasitized larvae ranged from 2.77 to 10.10 mm ($n = 8$). Planidia were on average 0.086 ± 0.006 mm in length (range: 0.08–0.09 mm, $n = 3$), and the sclerotized rings had a diameter of 0.165 ± 0.072 mm (range: 0.07–0.27 mm, $n = 9$) and a thickness of 0.043 ± 0.026 mm (range: 0.08–0.09 mm, $n = 9$). More male (64.7%, 11/17) than female (35.3%, 6/17)

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| Total       | 1        | 2             | 37    | 450    | 714     | 448    | 1652  | 9 (1.3)   | 18 (4.0)            | 11  | 10 | 8            |

Table 1: Composition of *Ectatomma ruidum* colonies in two different habitat types, percent parasitized brood, and number and stage of development of *Kapala iridicolor* individuals.
eucharitids were observed in six colonies with wasp pupae and adults. Adult *K. iridicolor* males, on the average, lived longer (5.3 ± 2.2 days, range: 2–7 days, *n* = 4) than females (4 ± 0.8 days, range: 3–5 days, *n* = 4) although no significant differences were found (Mann-Whitney test, *U* = 4, *P* = 0.25).

No cases of superparasitism were observed during dissection of cocoons. Empty cocoons from which eucharitid adults emerged had an operculum at the anterior end, opposite to the one with the ant’s meconium, forming a regular circular cut (Figure 3(a)) which is made by the adult wasps with their falcate jaws. From the remains of the host ants found during dissection of the cocoons, it can be stated that male, queen, and worker pupae are attacked (Figure 3(b)). Queen pupae were not completely consumed by the developing wasp.

### 4. Discussion

In this study, a comparison of eucharitid parasitism was made between two contrasting habitats differing in tree cover and associated understory vegetation. This perspective had not previously been considered in detail in studies of the impact of eucharitids on their ant host populations. Although early works (e.g., [2, 11]) indicated variation of parasitism in space, no comparison of parasitism by eucharitids in different habitats involving grasslands is available. However, a recent study also found a greater parasitism of poneromorph ants in forest fragments, compared to the more disturbed areas of Mexican coffee plantations [24].

Because the plots in our study (woodlands and grasslands) were interspersed, a site effect is less possible than a “habitat” effect. Our results showed that parasitoids were more prevalent in woodlands than in grasslands with respect to the number of parasitized colonies and parasitized host pupae, although the percentage of parasitized larvae did not differ statistically between both habitats. These findings suggest that the probability of an encounter between eucharitids and their host ant colonies is higher in more complex habitats such as those of the Valle del Cauca dry forest. Alternatively, the survival of eucharitids may be increased in shaded areas. The two habitats are different in vegetation (composition and structure) and environmental characteristics such as temperature and humidity [36], which may affect host and host-plant availability/distribution, and ant foraging strategies.

This study also represents the first detailed record of the interaction between *K. iridicolor* and *E. ruidum* in Colombia, which is the second locality for these species where aspects of the impact of parasitism and other information on the natural history of this eucharitid are known. *Kapala iridicolor* is known to parasitize several species of poneromorph ants in Mexico (*E. ruidum*, *Gnamptogenys regularis* Mayr, *G. sulcata* (Fr. Smith), *G. striatula* Mayr, and *Pachycondyla stigma* (F.) [14]), and it might probably interact with other ants in Colombia. On the Melendez Campus and in the City of Cali, *K. iridicolor* had been reported earlier [37], but its ant host was unknown. In the locality studied, other species of poneromorph ants have also been reported including *Odontomachus bauri* Emery, *O. erythrocephalus* Emery, and *Pachycondyla* sp. [37, 38].

The percentage of *E. ruidum* colonies with *K. iridicolor* parasitoids is very close to that observed for the interaction of *E. ruidum* with *K. iridicolor* and *K. izapa* Carmichael in Mexico [18]. The percentage of immature stages attacked was, however, low compared with the results of some studies that indicate over 16% of the brood parasitized (e.g., [11, 13, 20]). Nevertheless, these figures are within the range observed by Lachaud and Pérez-Lachaud [18] in their year-long study on *E. ruidum* in Mexico.

It is worth noting that a lower density of *E. ruidum* nests was found in the woodlands compared to the grasslands. These observations are consistent with those for the Departments of Valle del Cauca and Cauca [39], but not for Guajira, a drier region in the extreme north of Colombia, where a high abundance of *E. ruidum* nests was found in areas with higher presence of trees [40]. Furthermore, and although not studied in detail, we did note differences in the foraging hours of *E. ruidum* workers in the two habitats sampled. Foragers of this ant species displayed very low activity in the grasslands during the warmest hours (10:00 am to 16:00 pm), while ants were observed foraging during these hours in the woodlands. Nest distribution and nest density, as well as foraging times of ants, are factors that could also contribute to differences in parasitism by eucharitids. *Kapala iridicolor* is known to use a wide range of host plants for oviposition, including species of several plant families (Malvaceae, Boraginaceae, Asteraceae) [14, 34]. The plant(s) used by this eucharitid in our study site remain(s) unknown; however it is likely that differences in understorey vegetation between the woodland and the grassland contribute also to the observed differences in

![Figure 1: Global percentage of *Ectatomma ruidum* immature stages parasitized by *Kapala iridicolor* in two different habitat types (woodland versus grassland). **P < 0.01; N.S.: nonsignificant.](image-url)
Figure 2: First larval stage (planidium) of *Kapala iridicolor* on its *Ectatomma ruidum* larval host. (a) Position of planidia (black dots) and scars (gray dots) on the host larva (modified from [43]). (b) Planidium with scar (sclerotized ring) around it. (c) Extreme back side view of a planidium on the interior of a partially sclerotized ring. (d) Scar without planidium. (e) Planidium joined to the host without formation of a sclerotized ring.

Figure 3: Operculum made by *Kapala iridicolor* wasp on emergence from the host cocoon. (a) View of the cocoon with operculum. (b) Host remains (only the ant cuticle is left).

parasitism of *E. ruidum* by *K. iridicolor*, as has been suggested for differences in eucharitid parasitism between coffee agro-ecosystems and the forest [24]. This issue deserves further study.

A positive correlation was established between the number of parasitized pupae and the number of pupae available in the colonies. This pattern was similar to that reported elsewhere [18, 19]. It was also found that parasitized ant
larvae were quite variable in size and covered almost all sizes of larvae present in the colonies. This observation contrasts with previous reports [11–14, 41] where, in general, only late larval instars were found attacked by planidia, but very young host larvae with planidia have been reported in some cases [19, 42].

In summary, the results of this study showed an effect of habitat type (woodlands versus grasslands) on parasitism of E. ruidum, a widely distributed, dominant poneromorph ant, by K. iridicolor. It also records some aspects of the natural history of this parasitoid in Colombia.

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