

Eucharitid wasp parasitoids in cocoons of the ponerine ant *Diacamma scalpratum* from Thailand

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Abstract

Different immature stages and adults of the new species *Schizaspidia diacammae* (Chalcidoidea: Eucharitidae) were found inside cocoons of *Diacamma scalpratum* (Formicidae: Ponerinae). Wasp larvae were feeding on ant pupae, while other host cocoons yielded five wasp pupae and both male and female adults. Parasitized cocoons are cut in a distinct manner by the wasps when they exit, and this feature can be used to assess the prevalence of parasitism. Dissection of the ovaries of one recently emerged physogastric female revealed thousands of eggs ready to be laid. These data are used to discuss the life history and reproductive strategy of this parasitoid wasp associated with *Diacamma* brood.

Keywords: *Ponerinae, Eucharitidae, Schizaspidia diacammae, planidia, parasitism.*

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Introduction

Ants are an insect group with very high biomass in most parts of the world, hence an abundant and stable potential food resource for other organisms, but surprisingly few parasitoids are known to attack ant brood. The life histories of the parasitoid wasps associated with ants are poorly understood (Pérez-Lachaud *et al.*, 2012). Among 9 families of wasps parasitic on ants, only Eucharitidae (56 genera) specialize in attacking the immature stages of ants. Unlike most parasitoid wasps, eucharitid females deposit their eggs away from the host, in or on plant tissue (leaves and flower buds). The very active, minute, strongly sclerotized first-instar larvae ('planidia') are transported by foraging ants to their nest where they attach to ant larvae and development proceeds once the latter have spun a cocoon (Clausen, 1940; Heraty and Murray, 2013). The eucharitid parasitoids attacking Ponerinae, Ectatomminae, Myrmeciinae and Formicinae belong to a monophyletic derived lineage of Eucharitidae (Murray *et al.*, 2013).

Diacamma belongs to the early branching ant subfamily Ponerinae, with all 20-30 species characterized by the presence of two minute appendages ('gemmae') on the mesosoma (thorax) of workers. The queen caste is absent and only one of the workers mates and reproduces (termed the 'gamergate') (e.g. André *et al.*, 2001; Baratte *et al.*, 2006; Cuvillier-Hot *et al.*, 2002). Gemmae play a key role in the reproductive division of labour in *Diacamma*, and they are mutilated within hours of emergence (references above). Only the gamergate can retain her gemmae.

Within Ponerinae, *Diacamma* belongs to the *Ponera* genus group (consisting of *Austroponera*, *Cryptopone*, *Ectomomyrmex*, *Emeryopone*, *Ponera* and *Pseudoponera*; Schmidt and Shattuck, 2014). They are solitary hunters on a variety of small invertebrates. *Diacamma scalpratum* (F. Smith) has some of the largest workers in the genus, reaching 16-17mm in length. The species occurs in the hills of northern India, Sikkim, Assam, Myanmar and

northern Thailand (Bingham, 1903). Herein, we report on the first case of brood parasitism for this genus.

Materials and Methods

We excavated 4 complete colonies of *D. scalpratum* outside Thung Salaengluang NP (Phitsanulok province, northern Thailand, 16°34'32"N, 100°53'01"E) during September 2006. Ant colonies were plentiful and easy to find in a small patch of pine forest, and we selected nests that occurred away from the base of trees. Excavation continued to a depth of 90 cm in one colony. Several workers (including one gamergate) had a large mite on the dorsum of the mesosoma. Specimens of *D. scalpratum* workers examined in the British Museum (Natural History), London matched our samples, but proper taxonomic determination needs to be based on male genitalia (W.L. Brown Jr, pers. comm.), and a key is not yet available for *Diacamma*.

In the laboratory, colonies were maintained in plaster nests with a glass roof that allowed observations. Ants were fed with live mealworm pupae or small crickets. All workers were checked for the presence of gemmae, and half a dozen were dissected to assess ovarian activity and presence of sperm in the spermathecae. Cocoons were opened with forceps. Pupae were sexed whenever possible, and different developmental stages of a wasp parasite were collected.

Results

Four colonies of *Diacamma scalpratum* yielded 151±109 workers (mean number ±SD) and 69±38 cocoons (range 47-101). Only one worker had gemmae in each colony, and dissections confirmed that this was the gamergate (i.e. mated and egg-laying). All workers dissected had 16-20 ovarioles, and this number is diagnostic among closely related species (e.g. another species with large workers from Thailand has 8 ovarioles).

A proportion of field-collected cocoons eclosed in the laboratory and yielded workers. Other cocoons had been damaged during excavation and transport, and we cut them open to sex pupae. In addition to ant males and workers at various stages of pigmentation, we

found different immature stages and two adults of the new species *Schizaspidia diacammae* (Chalcidoidea: Eucharitidae: Eucharitinae) (Heraty *et al.*, 2015). Both second-instar and third-instar larvae were feeding on ant pupae (Fig. 1). One cocoon contained two wasp larvae attached to an ant pupa. Five wasp pupae and two adults (male and female) occurred singly in other host cocoons (Fig. 2). Adults (4.7–5.2mm long) are sexually dimorphic (Heraty *et al.*, 2015). In total, we opened more than 50 cocoons of which 9 were parasitized, from 3 out of 4 ant colonies.

Diacamma ants routinely discard empty cocoons outside of the nest. In contrast to normal cocoons cut open midway by the ants, parasitised cocoons are cut at one extremity by exiting wasps, and could thus be recognized easily (Fig. 3); at least 3 of these were found. In the laboratory, one cocoon with a cut extremity was carried outside by the ants, and we observed a wasp walking out soon after. This female was strongly physogastric (Fig. 4a). Dissection revealed dozens of ovarioles with thousands of apparently mature (fully chorionated) eggs (Fig. 4b).

Discussion

This is the first record of eucharitid parasitoids in *Diacamma* ants. Adults and brood of *Schizaspidia diacammae* (Heraty *et al.*, 2015) were found together in the same ant nests. Only rarely has more than one eucharitid species ever been found within a single ant colony (Pérez-Lachaud and Lachaud, pers. comm.), and we assume that wasp adults and immatures found in *D. scalpratum* nests all belong to the same species. Immature stages (eggs, first-instar exuvium, second and third-larval instars, pupa) of *Schizaspidia diacammae* are described in Heraty *et al.* (2015) and follow the general morphology of other eucharitids known to attack Ectatomminae and Ponerinae (Pérez-Lachaud *et al.*, 2006).

The different developmental stages of *S. diacammae* extracted from *Diacamma* cocoons indicate that its life history is typical for Eucharitinae. Laboratory observations suggest that, in the field, *Diacamma* workers discard empty cocoons outside the nest, which may facilitate dispersal of the wasps from the nest. In

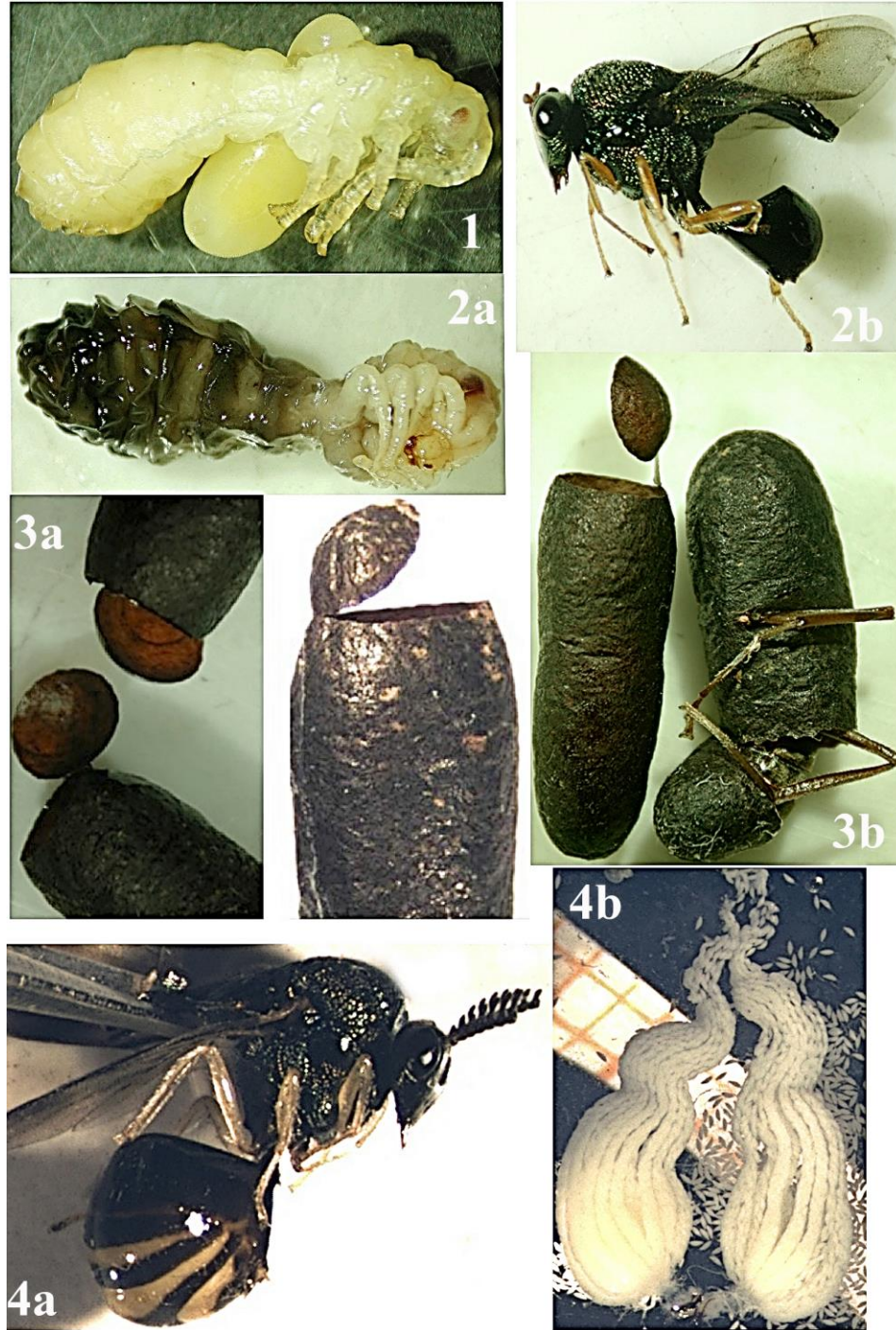


Fig. 1. Larva (second instar) of *Schizaspidia diacammae* feeding on the thorax of an ant pupa. The host cocoon has been removed. 2. Parasitized ant pupa (a) and adult male wasp (b) extracted from *Diacamma* cocoons. 3. *Diacamma* cocoons from which parasitoid wasps exited, showing the distinctive operculum cut at one extremity (a). In contrast, non-parasitized cocoons are cut midway by the ants (b, on right). 4. Physogastric female (a) of *S. diacammae* that exited from a *Diacamma* cocoon. Intersegmental membranes are exposed in dorsum of abdomen. Physogastry was confirmed by dissection (b) more than 10 ovarioles in each ovary, all packed with fully developed oocytes (scale: square = 1mm).

a similar case, a worker of *Dinoponera lucida* was observed to carry out a cocoon that was cut at one extremity (similar to Fig. 3), and a *Kapala* wasp exited from the cocoon in transit (Buys *et al.*, 2010). This contrasts with reports that eucharitid adults exit cocoons inside the ant nests, and are then transported by workers (discussed in Buys *et al.*, 2010).

Dissection of the gravid female of *S. diacammae* indicated that most eggs mature before dispersal (in contrast to oogenesis continuing throughout reproductive life). It would be advantageous for these parasitoids to complete oogenesis in the safety of the ant cocoon before dispersing outside the ant nest. At any rate, it is the parasitoids that cut cocoons open, and presumably they do this only once they are ready to disperse. This physogastric female seemed too heavy to fly, suggesting that she oviposits on a nearby host plant. We assume that males can disperse further, but it is not known where and when mating occurs. It is possible that female wasps lay their eggs on particular plant species. Unlike ectatommine ants, ponerine species are strict carnivores (Peeters, 1997), although *Diacamma* foragers may be attracted to sweet plant secretions. Torr  ns and Heraty (2012) reported that planidia have a propensity to jump, and they may attach to ant foragers walking under vegetation. A planidia of *Schizaspidia nasua* (attached to a larva of *Odontomachus rixosus*) is illustrated in Heraty *et al.* (2015).

Several Ponerinae have been recorded as hosts for Eucharitid (Eucharitinae) wasps (P  rez-Lachaud *et al.*, 2006). The tribe Psilocharitini is linked with *Hypoponera*, while tribe Eucharitini is essentially parasitic on medium to large ponerines (*Dinoponera*, *Neoponera*, *Odontomachus*, *Pachycondyla* sensu stricto and *Pseudoponera*) and ectatommines (*Ectatomma*, *Gnamptogenys*, *Typhlomyrmex* and *Rhytidoponera*), but also myrmeciines (*Myrmecia*) and numerous formicines (*Anoplolepis*, *Calomyrmex*, *Camponotus*, *Cataglyphis*, *Formica*, *Lasius* and *Polyrhachis*). All verified host records for Eucharitinae involve ants that have a cocoon. There are no confirmed records with the naked pupae of myrmicine ants (Lachaud and P  rez-Lachaud, 2012). Another subfamily (Oraseminae) of

Eucharitidae is restricted to myrmicine ants, mostly *Pheidole*, while Gollumiellinae attacks *Nylanderia* (a formicine that lost cocoons) (Murray *et al.*, 2013). Thus, the ability to attack naked ant pupae appears ancestral within Eucharitidae, and the association with ant cocoons is derived (Heraty and Murray, 2013).

The genus *Diacamma* is conducive to the investigation of cocoon parasitoids because researchers open cocoons routinely: (i) newly emerged ant workers are quickly mutilated by nestmates, and workers with intact gemmae are more easily obtained from cocoons (e.g. pheromone or evo-devo studies); (ii) male genitalia are invaluable for taxonomy, and males are occasionally found in cocoons, after which they fly out. The first author of this paper has studied over 10 species of *Diacamma* throughout India, SE Asia and Australia, and has inspected thousands of cocoons, but this is the first discovery of a parasitoid. Five species of *Diacamma* are known from India (*indicum*, *ceylonense*, *cyaneiventre*, *scalpratum*, *sculptum*) (Andr   *et al.*, 2001; Cuvillier-Hot *et al.*, 2002; Viginier *et al.*, 2004), and future fieldwork may reveal more parasitoids.

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