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## The ladybird *Thalassa saginata*, an obligatory myrmecophile of *Dolichoderus bidens* ant colonies

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**Abstract** The larvae and pupae of the ladybird *Thalassa saginata* develop inside colonies of the dolichoderine ant *Dolichoderus bidens*. This association is the first specific and obligatory relationship recorded between ants and ladybirds. The ants provide shelter and protection to the larvae but the diet of the latter remains unclear. The integration of *T. saginata* larvae into the ant colonies is achieved by mimicking the cuticular patterns of the ants' brood. Moreover, the larvae secrete substances from their hairs and anal gland that are likely to enhance their attractiveness.

### Introduction

Myrmecophiles are widespread among arthropods and their relationships with ants can vary from facultative to obligate associations and from mutualistic to parasitic. According to Pierce et al. (2002), obligate ant associates are species whose larvae are invariably associated with and dependent on ants for survival; the relationship being either mutualistic or parasitic. On the other hand, facultative myrmecophiles do not need ants for their survival; the associations being casual and nonspecific.

Coleoptera make up a large part of these myrmecophilous species and their relationships with ants reflect

all the possible types (Vander Meer and Wojcik 1982; Hölldobler and Wilson 1990; Navarrete Heredia 2001; Akino 2002). Ladybirds (Coccinellidae) have only rarely been reported as being myrmecophilous. Some ladybird larvae and adults feed on ant-attended aphids and are sometimes tolerated near ants' nests, on ant trails or in shelters where ants attend aphids or coccids (Chapin 1966; Majerus 1989; Hölldobler and Wilson 1990; Völkl 1995; Sloggett et al. 1998). Also, Berti et al. (1983) reported the presence of several workers of the dolichoderine ant *Dolichoderus (Hypoclinea) bidens* L. around two pupae of the ladybird *Thalassa (Monoscelis) saginata* Mulsant (Hyperaspinae) in a carton shelter in French Guinea. The authors suggested a myrmecophilous association, but without providing any evidence. We attempted to verify whether this association is obligate and specific, and to conduct a study on the biology of *T. saginata*. Our research focused on the way in which individuals integrate themselves into the ant colonies by comparing the cuticular compounds of both the ladybirds and the host ants.

### Materials and methods

#### Insects

The arboreal colonies of *D. bidens* (Dolichoderinae) are polydomous, with each nest consisting of a carton cup attached to the underside of a protective leaf. The colonies consist of a few hundred to a few thousand workers and are mainly monogynous, but cases of polygyny have been observed with up to five queens (personal observation). The species is frequent in secondary forests and pioneer vegetal formations but not in the rainforest canopy (Delabie et al. 1991; Dejean et al. 1999, 2000). We sampled all the nests of *D. bidens* that we found along forest edges located in the districts of Sinnamary and Kourou during several field studies conducted between 1994 and 2001. We also systematically inspected a great number ( $n > 500$ ) of nests of other sympatric arboreal ant species in order to verify whether they contained *T. saginata*.

Observations on the behavioral interactions between *T. saginata* larvae and pupae and *D. bidens* were performed in order to obtain data on (1) the life history of *T. saginata* and (2) the reaction of the ants during the artificial implantation of ladybird larvae in a new *D.*

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*bidens* colony. These implantations ( $n=3$ ) were achieved by introducing a *T. saginata* larva taken from a *D. bidens* colony into another colony that did not shelter any *T. saginata* larvae or pupae.

Voucher specimens of the different developmental stages of *T. saginata* were deposited at the Museum National d'Histoire Naturelle, Paris.

#### Gas chromatography analyses

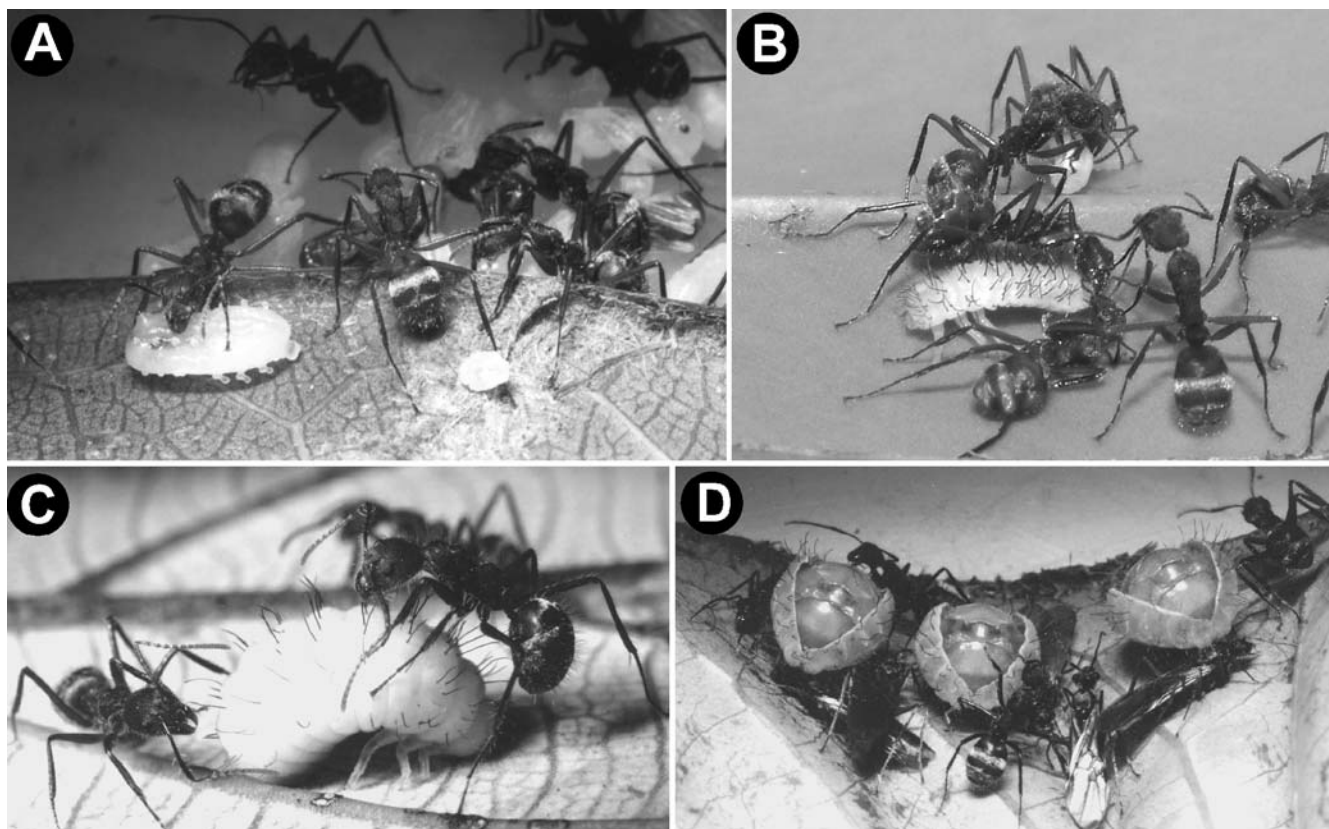
Six colonies of *D. bidens* sheltering a total of nine *T. saginata* larvae, four pupae, and four adults (three young and one old individual) were used for the analysis of cuticular substances. Each ant sample was prepared using the combined cuticular substances from five individuals (workers, larvae, and pupae), while the queens, if present, and the *T. saginata* were extracted individually. Individuals were killed by freezing and immersed in 1 ml of hexane for 5 min. The extracts were then evaporated under nitrogen and redissolved in 200  $\mu$ l of hexane for *D. bidens* workers, 100  $\mu$ l for *T. saginata* pupae, or 50  $\mu$ l for *T. saginata* larvae and adults as well as *D. bidens* larvae and pupae. Of these solutions, a sample of 2  $\mu$ l was analyzed with a Hewlett-Packard 5890 Series II gas chromatograph equipped with a split-splitless injector, a flame ionization detector, and a nonpolar fused-silica capillary column (HT-5, 25m  $\times$  0.22 mm ID  $\times$  0.1  $\mu$ l film thickness). Sample injections were performed in splitless mode using helium as the carrier gas, with injector and detector temperatures at 300°C and 320°C, respectively. The oven temperature program was as follows: 100°C to 180°C at a rate of 15°C/min, increased to 250°C at 5°C/min, increased to 320°C at 3°C/min, and then isothermal (320°C) for 15 min.

Integrations were performed with Millennium 2.15 software (Waters). To estimate the similarities of the profiles, the relative percentage of each peak was calculated. A hierarchical cluster analysis using Ward's method for aggregation (analysis of variance approach to evaluate the distances; Ward 1963) was then conducted with SPAD 3.01 software. Similarities between groups were also estimated by calculating their Nei's distances. Data were converted into binary values (presence/absence) and we used Ochiai's resemblance coefficient, which takes the same value as Nei's distance when binary data are used (Hughes et al. 2001).

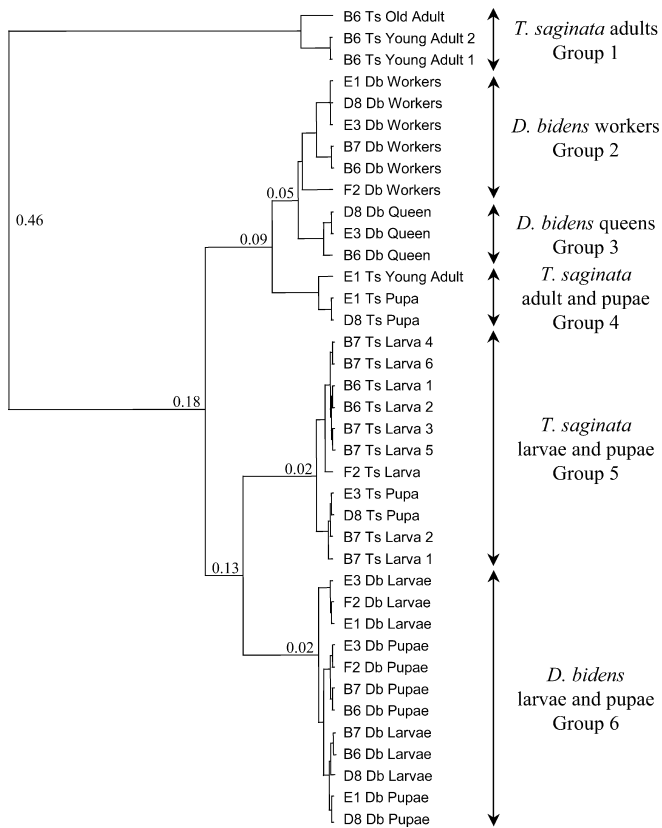
## Results

The systematic inspection of arboreal ant colonies during the 8-year field study did not reveal the presence of *T. saginata* in nests other than those of *D. bidens*. We noted 26 colonies of *D. bidens* out of the 103 inspected (25.2%) that sheltered one of the developmental stages of *T. saginata* or exuviae (shed exoskeleton of pupae). In total, we found 110 larvae, 74 pupae, 25 freshly emerged adults, and 180 pupal exuviae of *T. saginata*. This corresponded to  $4.23 \pm 10.89$  larvae,  $2.85 \pm 5.94$  pupae,  $0.96 \pm 3.75$  adults, and  $6.92 \pm 14.98$  exuviae per host colony (mean  $\pm$  SD), the total being  $14.96 \pm 34.46$  individuals or exuviae per host colony. Moreover, we also observed three times an adult of *T. saginata* near a nest of *D. bidens*.

*Thalassa saginata* larvae (Fig. 1a, b) were highly attractive to *D. bidens* workers, which constantly licked



**Fig. 1** a Young *Thalassa saginata* larva. b *Dolichoderus bidens* workers licking the anal gland secretions of a *T. saginata* larva. c *T. saginata* prepupa groomed by *D. bidens* workers. d *T. saginata* pupae



**Fig. 2** Hierarchical cluster analysis of the cuticular profiles of *Thalassa saginata* (larvae, pupae, and adults) and *Dolichoderus bidens* (larvae, pupae, workers, and queens). Node values are expressed in terms of dissimilarity. The dissimilarity index varies from 0 (identical profiles) to 1. The number of each extract refers to the *D. bidens* colony

their stiff hairs and the secretions of an anal gland. Larvae always remained in the ants' brood pile mainly containing prepupae and pupae and were surrounded by *D. bidens* workers. When disturbed, the *D. bidens* workers transported them like their own brood. Pupation occurred inside the ants' nest (Fig. 1c, d). At emergence, the adult ladybirds avoided ant aggressiveness by remaining in the burst pupal exuvia until their exoskeleton was fully hardened. They later left the *D. bidens* nests and were

immediately attacked if encountered by *D. bidens* workers.

The hierarchical cluster analysis of the cuticular substances of *T. saginata* and *D. bidens* larvae, pupae, and adults (workers and queens for *D. bidens*) enabled their separation into six groups (Fig. 2). The cuticular profiles of the *D. bidens* larvae and pupae were very similar (see Table 1; group 6 in Fig. 2). The same degree of similarity occurred between the larvae and pupae of *T. saginata* (Table 1), even if two pupae were not included in this group (see groups 4 and 5). The cuticular profiles of the larvae and pupae of both *D. bidens* and *T. saginata* were closely related (Table 1; Fig. 2, groups 5 and 6), while those of *D. bidens* workers and queens aggregated separately (groups 2 and 3). Adult *T. saginata* had their own cuticular profile (group 1) but one adult had a cuticular profile related to *D. bidens* workers and queens (group 4, also including the two above-mentioned pupae). Tests performed with the larvae of three *T. saginata* and three *D. bidens* colonies resulted, in each case, in the immediate peaceful adoption of the larvae. The ant workers immediately licked the stiff hairs of the *T. saginata* larvae without displaying any aggressive behavior. These larvae then moved or were transported by the *D. bidens* workers close to the brood pile.

## Discussion

These results clearly demonstrate that the association between *T. saginata* and *D. bidens* is species-specific, as no ladybird larvae or pupae have ever been found in the presence of other ant species. The development of the *T. saginata* larvae takes place inside the *D. bidens* nests. As with any parasite of a social insect, its adoption in the host society depends on the congruency of the cuticular profiles (Lenoir et al. 2001). The integration of *T. saginata* into the *D. bidens* colonies is achieved by the congruent cuticular patterns of the ant brood and ladybird larvae. This is probably the result of chemical mimicry of the host cues rather than chemical camouflage. Chemical mimicry is the result of an active biosynthesis, while chemical camouflage is achieved thanks to a passive or active acquisition of cuticular substances (Dettner and Liepert 1994; Lenoir et al. 2001). Indeed, ladybird larvae

**Table 1** Average Nei's distances (mean  $\pm$  standard error) as a measure of similarity in the cuticular profiles between *D. bidens* and *T. saginata* larvae, pupae, and adults (workers and queens for

*D. bidens*). Nei's distances were calculated from the binary values (presence/absence) of each recorded peak and vary between 0 (totally different) and 1 (identical)

	<i>D. bidens</i>				<i>T. saginata</i>		
	Larvae	Pupae	Workers	Queens	Larvae	Pupae	Adults
<i>D. bidens</i> larvae	0.98 $\pm$ 0.01	0.94 $\pm$ 0.01	0.90 $\pm$ 0.02	0.83 $\pm$ 0.02	0.93 $\pm$ 0.01	0.92 $\pm$ 0.02	0.76 $\pm$ 0.07
<i>D. bidens</i> pupae	–	0.99 $\pm$ 0.01	0.87 $\pm$ 0.01	0.83 $\pm$ 0.01	0.95 $\pm$ 0.01	0.91 $\pm$ 0.01	0.77 $\pm$ 0.08
<i>D. bidens</i> workers	–	–	0.92 $\pm$ 0.07	0.79 $\pm$ 0.02	0.89 $\pm$ 0.01	0.88 $\pm$ 0.02	0.76 $\pm$ 0.07
<i>D. bidens</i> queens	–	–	–	0.96 $\pm$ 0.03	0.83 $\pm$ 0.01	0.84 $\pm$ 0.03	0.75 $\pm$ 0.06
<i>T. saginata</i> larvae	–	–	–	–	0.99 $\pm$ 0.01	0.94 $\pm$ 0.02	0.89 $\pm$ 0.01
<i>T. saginata</i> pupae	–	–	–	–	–	0.97 $\pm$ 0.01	0.83 $\pm$ 0.08
<i>T. saginata</i> adults	–	–	–	–	–	–	0.87 $\pm$ 0.09



and pupae remain closer to each other than to the ant brood, and adoption tests highlighted their immediate integration into alien *D. bidens* colonies. Moreover, *T. saginata* larvae and pupae are continuously attractive to and groomed by the ants, so that their chemical mimicry seems to be reinforced by the secretion of attractive substances. However, further work is required in order to demonstrate a real biosynthesis of the host cuticular compounds. The chemical mimicry of an ant host colony's odor has been demonstrated in the *Maculinea rebeli* butterfly caterpillar and in the syrphid fly larvae of *Microdon piperi* (Howard et al. 1990; Akino et al. 1999). The *T. saginata*-*D. bidens* association also resembles these cases in some other aspects. The social status of *T. saginata* larvae (treated as ant brood), the carrying of the larvae by the ants, the existence of trophallaxis (even if rare), and the attractive anal gland secretions are reminiscent of *Maculinea*-*Myrmica* associations (Fiedler 1998). The attacking of the adult ladybirds based on their different cuticular profile is similar to the *Microdon*-*Camponotus* association (Howard et al. 1990).

However, we were unable to determine the diet of the *T. saginata* larvae. Hyperaspinae, like most ladybirds, are generally aphido-coccidophagous but we never found honeydew-producing insects inside the nests nor in their proximity, as *D. bidens* workers generally forage relatively far from their nest (Delabie et al. 1991). Occasional trophallaxis cannot be the main food source of the *T. saginata* larvae, which, however, were never observed preying on the ant brood. Therefore the biology of *T. saginata* differs greatly from other cases of myrmecophily in coccinellids that feed on ant-attended aphids and are ignored by the ants (Majerus 1989; Völkl 1995).

We conclude that the association between *T. saginata* and *D. bidens* is the first specific obligatory association known between ants and ladybirds. The ants provide shelter to the ladybird larvae and pupae, resulting in a parasitic relationship rather than mutualism. However, further investigations are needed to show whether and to what extent *T. saginata* larvae receive food from the ants.

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