

Host-Specific Myrmecophily and Myrmecophagy in the Tropical Coccinellid *Diomus thoracicus* in French Guiana

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ABSTRACT

A variety of arthropods, particularly insects, have developed myrmecophilous interactions with ants to gain access to resources and/or for protection. Among these myrmecophiles, only a few examples have been documented in the Coccinellidae, most of them involving species able to feed on ant-tended Hemiptera. We report here a new case of obligate myrmecophily in the coccinellid *Diomus thoracicus*. Larvae are invariably and exclusively found in the nests of the ant *Wasmannia auropunctata* and seem to rely on ant brood as their only food source. Not only do ant workers show no aggressiveness toward the *D. thoracicus* larvae in their behavioral interactions at the colonial level, but also at the species level; while coccinellid adults are always attacked. The integration of the larvae inside of the ant nests is based on their chemical mimicry of the host's cuticular cues. Therefore, given the presence of the *D. thoracicus* larvae inside of the ant's nest, their predation on *Wasmannia* brood and their chemical mimicry, this species can be considered a specific parasite of *W. auropunctata*. Overall, this new case of myrmecophily not only specifically involves a highly invasive ant species, but also provides insights into the evolution of myrmecophily and myrmecophagy in coccinellids.

Abstract in French is available at <http://www.blackwell-synergy.com/loi/btp>

Key words: ants; ladybird; mimicry; parasite; *Wasmannia auropunctata*.

BECAUSE OF THEIR OMNIPRESENCE, ants are frequently involved in interactions with other organisms. Among the many types of interactions, myrmecophily, in which a variety of species from diverse taxa are associated with ant colonies in or near the ants' nests, is widespread (Hölldobler & Wilson 1990). The vast majority are Hexapoda (including collembolans and thysanurans) and insects mostly from orders as different as the Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera or Orthoptera. Other arthropods (such as myriapods, isopods, acari and spiders), however, have also been recorded (Eickwort 1990, Ito & Takaku 1994, Cushing 1997, Passera & Aron 2005, Dekoninck *et al.* 2007). All of these interactions between myrmecophiles and ants vary from facultative and non-specific to obligate and host-specific associations, and the entire range of interactions from mutualism to parasitism as well as

commensalism has been reported (Hölldobler & Wilson 1990, Pierce *et al.* 2002).

One of the best documented examples of myrmecophily is the trophobiotic interaction between ants and Hemiptera (see the review of aphid–ant interactions in Stadler & Dixon 2005). Through their association with ants, myrmecophilous Hemiptera can gain protection from natural enemies as well as better hygiene and growth in exchange for honeydew, which is rich in carbohydrates and amino acids (Banks 1958, Hölldobler & Wilson 1990, Stadler & Dixon 1999, Völkl *et al.* 1999, Dejean *et al.* 2000, Delabie 2001). The ubiquity and gregariousness of many Hemiptera (*e.g.*, Stenorrhyncha and Auchenorrhyncha) species also make them the favorite or specific prey of many predators and parasites, among them, the Coccinellidae, which are mainly aphidophagous or coccidophagous (Hodek 1996, Dixon 2000). Such predatory interactions place them into competition with ants for resources when larvae or adult ladybirds try to feed on hemipteran colonies tended by ants (Majerus *et al.* 2007). A few cases of myrmecophily do exist

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in coccinellids, however, with some species managing to feed on ant-tended Hemiptera or even being preferentially associated with ants. Such ecological specialization provides myrmecophilous coccinellids protection from predators, parasites and intraguild competitors (Völkl 1992, Liere & Perfecto 2008), as well as the potential to exploit more stable food sources. Indeed, when tended by ants, colonies of Hemiptera – and especially aphids—are larger and more stable (Sloggett & Majerus 2000, Majerus *et al.* 2007). On the contrary, untended colonies are more transient, thus limiting the periods favorable to their exploitation by coccinellids and affecting the life-history traits of the latter, such as oviposition or mating time intervals (Hemprinne *et al.* 1992, Kindlmann & Dixon 1993, Sloggett & Majerus 2000, Majerus *et al.* 2007).

Among the aphidophagous coccinellids regularly associated with ants, two species from temperate regions have been studied in detail. Both the larvae and adults of *Coccinella magnifica* Redtenbacher (Coccinellinae) are obligate and host-specific myrmecophiles associated with *Formica rufa*. The low level of aggressiveness by the ants is due to the coccinellids' use of chemical, physical and behavioral defenses, which are adaptations of the general defenses observed in Coccinellidae (Sloggett *et al.* 1998, Sloggett & Majerus 2003). The second species, *Platynaspis luteorubra* Goeze (Chilocorinae), can be found in association with several ant species (*i.e.*, *Lasius niger*, *Myrmica* spp. and *Tetramorium caespitum*) during the larval stages. In this case, the ant's tolerance is the result of a chemical camouflage that provides the larvae with the same cuticular lipids as the aphids attended by the ants and on which the larvae prey (Völkl 1995). In the tropics, only one example of obligate myrmecophily has been described to date. The larvae of *Thalassa saginata* Mulsant (Hyperaspinae) are invariably found inside of *Dolichoderus bidens* colonies; their acceptance depends on the brood's mimicry of their host (Orivel *et al.* 2004). Two other examples of ant predation by coccinellids have also been reported in *Ortalia pallens* Mulsant (Ortaliinae) and *Scymnodes bellus* Pope & Lawrence (Scymninae) larvae, which have been recorded feeding on *Pheidole punctulata* and *Iridomyrmex* sp., respectively (Harris 1921, Pope & Lawrence 1990). Their qualification as obligate myrmecophiles still needs, however, to be studied and more data on their biology are needed to decipher their degree of myrmecophily.

This study was conducted in French Guiana where we investigated the biology of *Diomus thoracicus* Fabricius (Scymninae, Diomini), whose larvae have been found in close association with the little fire ant, *Wasmannia auropunctata*. Our objectives were to understand the basis of its relationships with ants from ecological, behavioral and chemical points of view.

METHODS

STUDY SITE AND SPECIES.—Field observations were conducted in January–February 2007. Ant colonies and ladybirds were collected from two sites in the Sinnamary district: a coffee plantation (5°17'16.0" N, 52°55'04.1" W) and along a forest track near the *Petit Saut* dam (5°03'30.0" N, 52°58'34.56" W). These two sites are ecologically disturbed habitats where large populations of *W. auropunctata*

are present and have previously been studied (Le Breton *et al.* 2003, Fournier *et al.* 2005). When present, the nests of other sympatric ant species found in the leaf litter were also sampled.

QUANTIFYING THE DIET OF LADYBIRD LARVAE.—During preliminary experiments, different kinds of food (*e.g.*, ant brood, prey) as well as ants (in the eventuality of feeding through trophallaxis) were presented to the ladybird larvae, but only brood consumption was observed. To quantify the diet of coccinellid larvae and its impact on the sheltering colony, each larva was isolated in an arena (1.5 cm diam) with ant brood: 20 eggs, 10 larvae and 2 pupae. The mean quantity of brood eaten in 24 h was calculated for 18 *D. thoracicus* larvae, and, among these, 12 were used for a more detailed analysis of the larval stage.

BEHAVIORAL INTERACTIONS BETWEEN LADYBIRDS AND ANTS.—In total, nine *D. thoracicus* larvae were tested individually with three ant workers from (1) the same nest (intranest); (2) another nest in the same colony (internest); and (3) another colony (intercolonial). One-on-one confrontations between the ant workers from the latter nests or colonies were also set up to serve as a control group. Adult coccinellids were confronted with only one ant from the same nest first at emergence (young), and then the day after or on successive days (old). These confrontations were carried out for 10 males and 10 females.

The encounters were set up in a neutral arena (1.5 cm diam) whose walls were coated with Fluon[®] (Asahi Glass Co., Tokyo, Japan) to prevent the individuals from climbing out. During 3 min we scored the interactions between the individuals on a scale from 1 to 4: 1 = touching (physical contact such as antennations, but no aggressive response), 2 = prolonged antennation, 3 = biting or opening the mandibles, and 4 = stinging. For each confrontation, 10 replicates were conducted using different ants each time. The highest score for each replicate was then averaged across trials. For statistical comparisons, the interactions between ladybird larvae and ants were analyzed using Wilcoxon's test while behavioral interactions between adult ladybirds and ants were analyzed using a two-way analysis of variance (ANOVA) for repeated measures. All comparisons were carried out with GraphPad Prism 5.00 software (GraphPad Software, San Diego, CA, U.S.A.).

CHEMICAL ANALYSES OF CUTICULAR LIPIDS.—Both solvent extraction and solid phase micro-extraction (SPME) were used. The two methods provide similar results in terms of quality and quantity of extracted substances (Monnin *et al.* 1998, Orivel & Dejean 2000, Tentschert *et al.* 2001); however, SPME, as a non-destructive technique, permits several extractions to be taken from an individual during its development, and avoids having to sacrifice individuals whose numbers are limited.

Solvent extraction was performed on 18 *D. thoracicus* larvae and 5 adults, as well as their corresponding ant colonies. Each ladybird and ant queen was sampled individually whereas the other samples were prepared using the combined cuticular substances of 15–30 individuals for the ant workers, pupae and larvae, and 30–100 for the eggs. Individuals were killed by freezing,

and immersed in 100 μL of hexane for 5 min, except for the eggs, which were placed in 50 μL . The extracts were then evaporated under nitrogen and redissolved in 15 μL of hexane just before analysis. Of this solution, 2 μL were injected into the gas chromatograph.

The SPME extractions were performed on 15 'old' ladybirds and 5 ladybirds both when 'young' and 'old' with a 7 μm polydimethylsiloxane-coated fiber (Supelco, Sigma-Aldrich Co., Saint-Louis, MS, U.S.A.). For each extract, the fiber was rubbed on the coccinellid's dorsal surface during 5 or 10 min for old or young individuals, respectively. This extraction time was determined after preliminary experiments that demonstrated that young individuals had a lower quantity of cuticular compounds and thus needed a longer extraction time. The fiber was then immediately desorbed in the injection port of the gas chromatograph.

All extracts were analyzed with a Hewlett-Packard 5890 series II gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, U.S.A.) equipped with a split-splitless injector, a flame ionization detector, and a HT-5 apolar fused-silica capillary column (5% diphenyl, 95% dimethylpolysiloxane, 25 m \times 0.22 mm ID \times 0.1 μm film thickness). Sample injections were performed in splitless mode (15 s or 5 min for hexane or SPME extracts, respectively) using helium as carrier gas (1 mL/min), with injector and detector temperatures at 300°C and 320°C, respectively. The oven temperature program was as follows: isothermal (100°C) for 5 min, 100–250°C at 15°C/min, 250–300°C at 3°C/min, 300–320°C at 20°C/min, and then isothermal (320°C) for 5 min. Data were processed with Millennium 2.15 software (Waters Corp., Milford, MA, U.S.A.).

MASS SPECTROMETRY ANALYSES.—To ensure a concentration of cuticular compounds sufficient for identification, five solvent extracts from each ant caste and from the coccinellid larvae and adults were pooled together. The gas chromatograph (Thermoelectron Trace Ultra, Finnigan, Waltham, MA, U.S.A.) was equipped with an Rtx[®]-5MS apolar capillary column (5% diphenyl and 95% dimethylpolysiloxane; 30 m \times 0.25 mm \times 0.25 μm) and directly coupled to an ion trap mass spectrometer. The mass spectrums were recorded in electronic impact mode (70 eV) over a mass range of 50–500 units with a frequency of three scans per second. One microliter sample was injected in splitless mode (15 s) at 300°C. Helium Alpha 2 (Air Liquide) was used as gas vector at a flow rate of 1 mL/min, and the temperature of the detector and the GC-MS interface was set at 250°C. The oven temperature was programmed as follows: isothermal (100°C) for 1 min, 100–250°C at 15°C/min, 250–300°C at 2°C/min, and then isothermal (300°C) for 1 min. Data analyses were performed with the Xcalibur data system (version 1.2; ThermoFinnigan, Finnigan) and the identities of the components were verified by comparing their retention times with those of standard compounds. Fatty acids and amides were provisionally identified from their mass spectra (Budzikiewicz *et al.* 1967).

To estimate the similarity of the cuticular profiles, all of the peaks were inventoried on the basis of their retention time and molecular identity. The similarity among cuticular profiles was estimated by calculating Nei's distances (cosine coefficient) from the relative proportion of each peak. The partitioning of the data was

then conducted through a hierarchical cluster analysis from the distance matrix using the unweighted pair group method with arithmetic mean method. Analysis of similarity (ANOSIM) was used for testing significant differences between the groups obtained based on the Nei's distance. We conducted 10,000 random permutations to assess the statistical significance of *R*. A large positive *R* (up to 1) highlights strong differences between groups. All of these analyses were performed with PAST (version 1.68b; Hammer *et al.* 2001).

RESULTS

BIOLOGY OF *D. THORACICUS* AND INTERACTIONS WITH ANTS.—Aside from first-stage larvae, which were not recorded during our census, all of the *D. thoracicus* larvae were always found inside of the *W. auropunctata* nest. The nests of other sympatric ant species (*i.e.*, *Solenopsis* spp., *Paratrechina* sp., *Pheidole* sp., *Crematogaster* spp. and *Brachymyrmex* sp.) were also inspected and never showed the presence of *D. thoracicus* larvae.

The *D. thoracicus* larvae were flat and < 4 mm long (Fig. 1). There were tubercles on the dorsal face, but their function is still unknown. Overall 18.7 percent (28 of 150) of the nests sheltered *D. thoracicus* larvae with a mean density (\pm SE) of 2.37 ± 0.89 larvae per occupied nest. These larvae were always present in nests containing brood, and most of the time they were located in the brood piles. Pupation also occurred inside of the nest. As we never found any adult *D. thoracicus* inside of a *Wasmannia* nest, it is likely that they leave the nest after emergence. The bifid shape of the adult's mandibles and the fact that all of the Scymninae are carnivorous (Samways *et al.* 1997) suggest that the adults are also probably predators. Unfortunately, details of the adults' life characteristics were unable to be determined and still require further investigation.

Observations and feeding experiments demonstrated that the larvae fed exclusively on ant brood. We recorded neither trophallaxis between ant workers and ladybird larvae nor prey consumption. Moreover, the ants did not tend any Hemiptera inside of their nests. Ant eggs, larvae and pupae were consumed by the *D. thoracicus* larvae. Even if the quantity of food intake increased with larval development and the young ladybird larvae ate ant eggs exclusively (whereas the last stages can also feed on ant larvae and pupae), no significant differences were found in the quantity of brood eaten (Kruskal–Wallis tests: ant pupae: $df = 2$, $P = 0.79$; ant larvae: $df = 2$, $P = 0.12$; ant eggs: $df = 2$, $P = 0.099$; Table 1).

The behavioral interactions between ants and ladybird larvae showed an absence of aggressiveness by *W. auropunctata* workers whatever the origin of the ants (Fig. 2A). The aggressiveness expressed by *W. auropunctata* originating from the same nest or from another nest in the same colony toward the *D. thoracicus* larvae was always low, and these aggressiveness indexes were not significantly different than the ones displayed during confrontations between ants (Wilcoxon's test: intranest $W = 34$, $P = 0.02$; internest $W = -21$, $P = 0.24$). For the confrontations between *D. thoracicus* larvae and *W. auropunctata* workers coming from a different colony, the global aggressiveness index was significantly lower than control interactions between heterocolonial *W. auropunctata* workers,

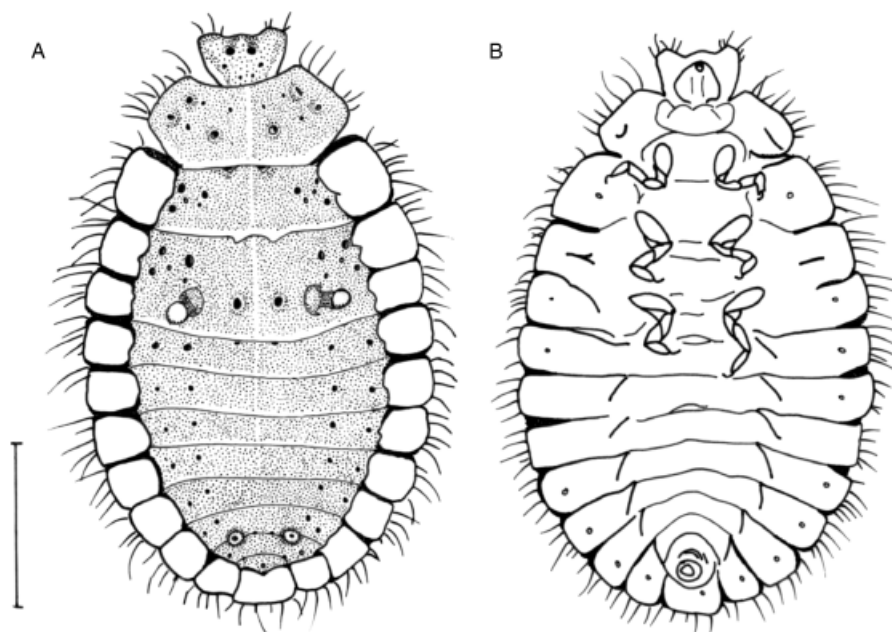


FIGURE 1. Dorsal (A) and ventral (B) faces of the fourth instar larva of *Diomus thoracicus*. The scale indicated equals 1 mm.

highlighting a general tolerance of the ladybird larvae (intercolony $W = -45$, $P = 0.009$).

The same tolerance was recorded during confrontations between young coccinellid adults just after emergence and ant workers from their host colony (Fig. 2B); however, the aggressiveness index toward older individuals was significantly greater, whatever their gender (two-way ANOVA for repeated measures; age factor: $F_{1,18} = 64.5$, $P < 0.0001$; gender factor: $F_{1,18} = 2.29$, $P = 0.15$; interaction: $F_{1,18} = 0.01$, $P = 0.92$).

CHEMICAL ANALYSES.—The analyses of the cuticular profiles of 181 ant and coccinellid individuals enabled us to separate out 44 compounds. Most of them were saturated, unsaturated and branched hydrocarbons shared by both the coccinellid larvae and the ants. Some amides and unsaturated fatty acids were also found; the unsaturated fatty acids were specific to the adult coccinellids (Table S1).

The hierarchical cluster analysis of these cuticular substances enabled us to separate the individuals into two major groups. Adult coccinellids, both at emergence and older, had similar cuticular profiles and aggregated separately from the coccinellid larvae and

the ants (Fig. 3). Within the latter group several clusters can be defined, each represented by members of the *Wasmannia* castes (*i.e.*, workers, queens and two groups of brood) or coccinellid larvae. The cuticular profiles of the *D. thoracicus* larvae were very similar to one of the two clusters of *Wasmannia* brood. Nevertheless, the ANOSIM analysis resulted in significant differences ($R = 0.821$; $P < 0.0001$), and *post-hoc* comparisons revealed that all of the groups were significantly different from each other (Table 2). Coccinellid larvae had their smallest *post-hoc* R values with *W. auropunctata* brood group 1, which could explain their grouping in the cluster analysis. Only adult ladybirds always had high R values in each comparison (> 0.98). From a qualitative point of view, the coccinellid larvae shared all but one compound with the ant brood and only the relative ratios varied (Table S1). The remaining hydrocarbon was present only in traces on the cuticle of the ladybird and *Wasmannia* larvae, and absent from the eggs and the pupae of the host ant.

DISCUSSION

Because they feed on aphids or coccids, most coccinellids have to cope with the characteristics of these food resources (Dixon 2000). Besides being more or less transient (which can limit their exploitation to a restricted time interval), many of the hemipteran colonies are also attended by ants. Predators, such as coccinellids, thus have to compete with ants for access to such resources. Most of the coccinellids have developed defensive mechanisms to face attacks by ants; however, a few myrmecophilous species are tolerated by ants. Majerus *et al.* (2007) mentioned seven facultative or obligate myrmecophilous ladybird species belonging to four subfamilies: the (1) Scymninae (Brachiacanthadini and Hyperaspidini), (2) Ortaliinae (Ortalini), (3) Chilocorinae (Platynaspidini) and (4)

TABLE 1. Mean quantity (\pm SE) of brood eaten during 24-h feeding experiments by the *Diomus thoracicus* larvae according to their larval stage.

	Ant pupae	Ant larvae	Ant eggs
Larval stage 2	0	0	0.78 ± 0.78
Larval stage 3	0.21 ± 0.21	1.87 ± 0.66	1.87 ± 0.87
Larval stage 4	0	2.99 ± 1.70	3.35 ± 1.30

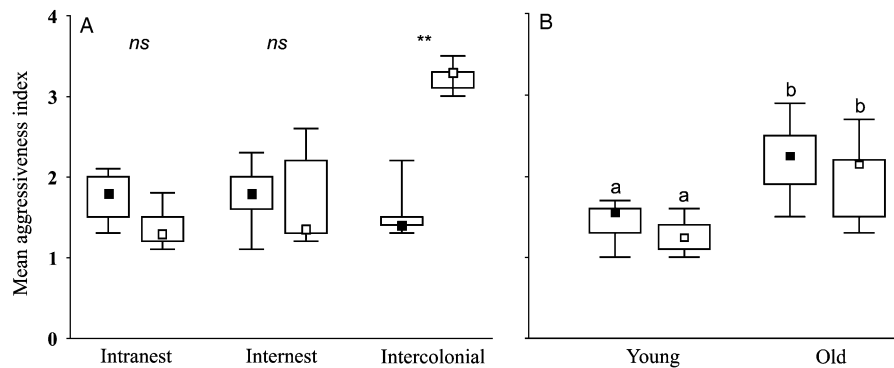


FIGURE 2. Comparisons of the mean aggressiveness index for interactions recorded during confrontations between (A) *Wasmannia auropunctata* and *Diomus thoracicus* larvae (filled squares) and between *W. auropunctata* workers (empty squares) for three types of confrontations; and (B) between *W. auropunctata* and *D. thoracicus* female (filled squares) or male (empty squares) adults when young or old. Filled and empty squares represent median values. Error bars above and below the boxes indicate the 90th and 10th percentiles, and the ends of the boxes indicate the 25th and 75th percentiles. (A) Significant difference at $**P < 0.01$ (Wilcoxon's test with Bonferroni's correction: for intercolonial encounters, $W = -45$, $P = 0.009$). (B) Different letters indicate significant differences (analysis of variance; age factor: $P < 0.0001$; gender factor: ns; interaction: ns).

Coccinellinae (Coccinellini). *Scymnodes bellus* needs to be added to the myrmecophilous Scymninae (Pope & Lawrence 1990), while *D. thoracicus* is the first member of the Diomini tribe found to be associated with ants. Myrmecophily seems, therefore, to be a convergent trait in ladybirds, appearing in different taxa and also several times during the evolution of ladybirds (see Kovár 1996 and Vandenberg 2002 for current knowledge on phylogenetic relationships in the Coccinellidae).

The above-mentioned species have developed adaptive traits to gain access to more stable food resources as well as protection from enemies. In all cases but two, the larvae and sometimes the adults of the myrmecophilous coccinellids feed on ant-tended Hemiptera close to or inside of the nest. The two remaining species—*O. pallens* and *S. bellus*—seem to have shifted their dietary preference to preying on ants; the case of *D. thoracicus* is then of interest and builds on the hypothesis that the appearance of myrmecophagy in coccinellids may not be isolated.

The shape and general morphology of *D. thoracicus* larvae is similar to the larvae of the myrmecophagous *S. bellus*, but also to that of *P. luteorubra*—a myrmecophilous, but not myrmecophagous ladybird. These larvae are ovate and extremely flat with expanded marginal setae and short, stout legs. This body shape, with few exposed extremities, could be considered as a protective type, as has been found in other myrmecophilous Coleoptera (Geiselhardt *et al.* 2007). Furthermore, this body shape is different from that of other closely related species. *Diomus terminatus* (Say), for instance, a species phylogenetically close to *D. thoracicus* (Magro *et al.* 2009), has a campodeiform and a dorso-ventrally flattened body with a prognathus head. That being so, myrmecophagy probably followed rather than preceded myrmecophily. More data regarding the phylogeny of coccinellids are still needed to test this hypothesis.

Diomus thoracicus larvae seem to be specialized parasites of *W. auropunctata* brood, a food resource available year around and

which allows them to vie with enemies or competitors. Even if we did not test every type of food resource, the healthy development of the ladybird larvae through the consumption of ant brood indicates that it probably constitutes an essential food source for this species. As stated by Hodek (1996) 'essential food enables larval development with low mortality.' The larval mortality under laboratory conditions was around 50 percent, and so not far from the one observed for ladybird larvae from common species usually kept in the laboratory (mortality around 40%; A. Magro, pers. comm.). Where does this niche specialization come from? In coccinellids, larvae and adults usually rely on the same food resources (Hodek 1996); however, even if the *D. thoracicus* adults' diet remains unknown, it is unlikely that they feed on the same resources because it is probable that they leave the nest soon after emergence and are attacked by ants. Furthermore, *Diomus* species are known to prey on mealy bugs, scale insects, aphids and white flies (Ślipiński 2007). *Diomus thoracicus* is the only known species in the genus to be myrmecophagous. Consequently, myrmecophagy most probably appeared after a dietary shift from ant-tended Hemiptera to ant brood (see Sloggett 1998).

This specialized parasitism requires that the larvae are accepted into the ant nests. The integration of myrmecophilous parasites is often optimized by behavioral strategies or by chemical ones like the use of attractive substances or a specific chemical signature (Vandermeer & Wojcik 1982, Vandermeer *et al.* 1989, Völkl 1995, Sloggett *et al.* 1998, Akino *et al.* 1999, Akino 2002, Allan *et al.* 2002, Elgar & Allan 2006, Lohman *et al.* 2006, Nash *et al.* 2008). In the case of *D. thoracicus*, behavioral assays have showed the absence of aggressiveness by *W. auropunctata* workers toward the ladybird larvae. Whether they originated from the same colony or not, the level of aggressiveness was always low; that is to say, the acceptance of the ladybird larvae by *W. auropunctata* workers was not only true at the colony level, but also at the species level. Analyses of cuticular substances corroborate these results. Indeed, the

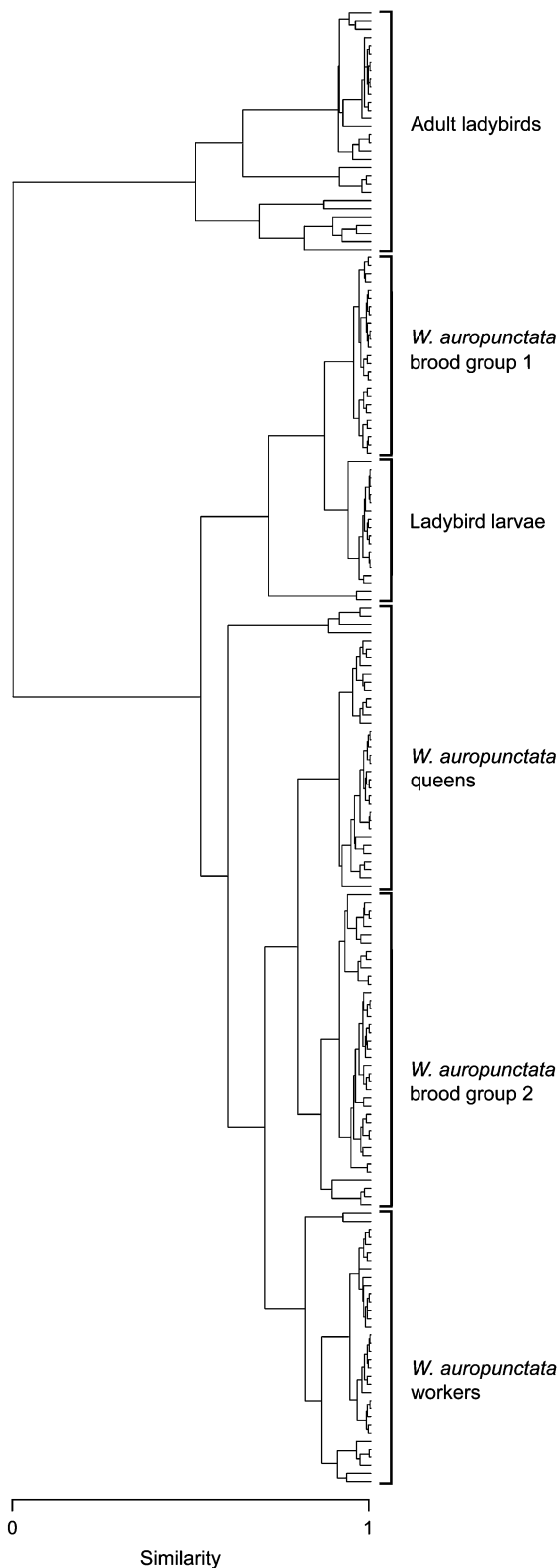


FIGURE 3. Hierarchical cluster analysis of the cuticular profiles of *Diomus thoracicus* (adults and larvae) and *Wasmannia auropunctata* (eggs, larvae, pupae, workers and queens). The similarity index is based on Nei's distances and varies from 0 (different) to 1 (identical).

TABLE 2. Results from an analysis of similarities illustrating the post-hoc results from pairwise comparisons of the different groups obtained in the cluster analysis. R-values are given; all of the tests were statistically significant $P < 0.05$.

	<i>Wasmannia auropunctata</i>		Ladybirds			
	Queens	Workers	Brood group 1	Brood group 2	Larvae	Adults
Queens	–	0.834	0.884	0.535	0.980	0.994
Workers	0.834	–	0.997	0.836	0.999	0.987
Brood group 1	0.884	0.997	–	0.639	0.86	0.993
Brood group 2	0.535	0.836	0.639	–	0.974	0.994
Larvae	0.980	0.999	0.86	0.974	–	0.996
Adults	0.994	0.987	0.993	0.994	0.996	–

cuticular profiles of the ladybird larvae are closest to the ant brood that they eat, allowing this parasite to be integrated into the *W. auropunctata* colonies.

Cases of chemical-based integration are frequent in myrmecophiles; *e.g.*, in Lycaenidae (Fiedler 1991, Akino *et al.* 1999) and in arachnids (Allan *et al.* 2002, Elgar & Allan 2004). There are two ways to obtain chemical congruency with one's host. Either the myrmecophiles synthesize the host cues through chemical mimicry, or they get the cues from the ants through active or passive acquisition *via* camouflage (Dettner & Liepert 1994, Lenoir *et al.* 2001). Our results suggest that the integration of the *D. thoracicus* larvae seems to depend on chemical mimicry rather than camouflage. In the case of chemical camouflage, one would expect chemical congruency at the colonial level, and, thus, aggressiveness during encounters with ants from another colony – contrarily to what was observed. Nevertheless, we cannot exclude the existence of pacifying volatile substances or secretions in the integration of the larvae.

From a chemical point of view, amides are relatively rare compounds in the cuticular lipids of insects. They were already found in a booklouse for which they prevent the adhesion of some entomopathogenic fungi (Howard & Lord 2003, Lord & Howard 2004) and on the cuticle of an *Acromyrmex* leaf-cutting ant, where they seem to be inherited from their fungal symbionts (Richard *et al.* 2007). Adult coccinellids were the only stage in *D. thoracicus* showing a high level of unsaturated fatty acids in their cuticular lipids. These unsaturated fatty acids may act as an ant repellent, and provide protection to young adults before they leave the ant nest. Indeed, Dani *et al.* (1996) and Dani (2006) have already shown that *Polistes* wasps use some unsaturated fatty acids as an ant repellent on their nest pedicel, and Howard *et al.* (1982) suggested that this could be a widespread phenomenon among insects.

Diomus thoracicus can therefore be considered as a myrmecophagous and most probably a host-specific myrmecophile whose larvae specifically parasitize *W. auropunctata* – possibly one of the most harmful of all invasive ant species (Holway *et al.* 2002, Le Breton *et al.* 2007). Our study may have further importance because the identification of the factors that regulate invasive ant populations in their native ranges constitutes a central key to

understanding their success in introduced areas (Holway *et al.* 2002). Even if this parasite does not have a significant impact on the populations of *W. auropunctata* because of the low level of predation on the brood, further studies would be of interest toward determining the potential existence of chemical and behavioral mechanisms underlying this specificity.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

TABLE S1. *Identification and relative percentage of the cuticular compounds of the different castes of Wasmannia auropunctata and of the larvae and adults of Diomus thoracicus.*

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