

**Unusual Behaviour — Unusual Morphology: Mutualistic Relationships between Ants (Hymenoptera: Formicidae) and *Diaphorina enderleini* (Hemiptera: Psylloidea), Associated with *Vernonia amygdalina* (Asteraceae)**

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**Unusual behaviour – unusual morphology: mutualistic relationships between ants (Hymenoptera: Formicidae) and *Diaphorina enderleini* (Hemiptera: Psylloidea), associated with *Vernonia amygdalina* (Asteraceae)**

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ABSTRACT

*Diaphorina enderleini*, known from Sudan, Tanzania and Yemen, is reported for the first time from Cameroon, Ethiopia and Kenya. Observations and records from Cameroon and Ethiopia show that it develops on *Vernonia amygdalina*. The species is diagnosed and the last instar larva is described and illustrated. The larvae lack a functional circumanal ring and do not produce wax, a feature unusual for psyllids. The lack of wax deprives them of the protection common in various free-living psyllids. Consequently the psyllid is involved in an unusual mutualistic interaction with the ants *Pheidole megacephala* and *Crematogaster striatula*. This association is described and discussed.

**KEY WORDS:** Hemiptera, Psylloidea, Formicidae, *Diaphorina enderleini*, *Pheidole megacephala*, *Crematogaster striatula*, *Myrmecaria opaciventris*, *Camponotus acvapimensis*, *Vernonia amygdalina*, Cameroon, behaviour.

INTRODUCTION

Most Sternorrhyncha and Fulgoromorpha are phloem feeders. Phloem sap provides a highly unbalanced diet, resulting in the excretion of large amounts of honeydew. This carbohydrate-rich liquid provides a valuable food resource for various insects such as ants (Way 1963; Gullan 2001). Ants actively collect honeydew and often live in close association with sap suckers (Way 1963). This mutualistic relationship is beneficial for both partners (Buckley 1987; Delabie 2001; Davidson *et al.* 2003). The ants obtain high quality food and the hemipterans receive protection from predators and parasitoids (Novak 1994). Ant attendance is well known from aphids (Kaplan & Eubanks 2005; Idechiil *et al.* 2007; Grover *et al.* 2008; Nielsen *et al.* 2010) and scale insects (Heckroth *et al.* 1998; Johnson *et al.* 2001; Ueda *et al.* 2008; Ben-Dov 2010) but seems less relevant in psyllids (Novak 1994). Based on observations conducted in Cameroon, we report ant attendance of the widely distributed Afrotropical psyllid *Diaphorina enderleini* Klimaszewski, 1964, developing on *Vernonia* species.

Psyllids or jumping plant-lice are one of the four superfamilies of Sternorrhyncha comprising to date some 3850 described species worldwide. The group is particularly species-rich in the tropics. Psyllids are generally monophagous or narrowly oligophagous with respect to their angiosperm hosts (Hodkinson 1974). Often, related psyllid species develop on related host-plant taxa. A major exception to this high host conservatism is the large Old World genus *Diaphorina* Löw, 1880, which recruits hosts from at least 19 families in 10 plant orders of dicotyledonous angiosperms (Hollis 1987; Burckhardt & van Harten 2006).

*Diaphorina enderleini* was originally described by Enderlein (1910), as *Gonanoplicus guttulatus*, based on material collected from Tanzania. Klimaszewski (1964) transferred

*G. guttulatus* to *Diaphorina* and replaced the name for reasons of homonymy with *D. enderleini*. Loginova (1978) described *Diaphorina siluncula* from Sudan. Burckhardt and Mifsud (1998) finally synonymised *D. siluncula* with *D. enderleini*, provided a detailed morphological description of the adults and reported the species from Yemen. The psyllid is currently known from Tanzania, Sudan and Yemen in the Afrotropical Region (Burckhardt & van Harten 2006). So far nothing has been known about the larvae and host plants of *D. enderleini* apart from the very brief and not diagnostic description by Enderlein (1910).

*Vernonia amygdalina* Delile (Asteraceae) is a perennial shrub native to tropical Africa and the southern tip of the Arabian Peninsula. It is a multipurpose plant used as a medicinal plant throughout its distribution range (CVPSL 2003; Banjo *et al.* 2006; Nzigidahera 2008), or as a vegetable in West Africa (Anonymous 2005; Asawalam & Hassanali 2006). *V. amygdalina* naturally colonises secondary forests and cultivated land. For this reason it can be regarded as a non-timber forest product (NTFP). It is also planted in gardens and as fences around houses. For cultivation it is generally propagated by planting shoots (Shippers 2000), and thus can be regarded as a crop. Some studies highlight its insecticidal or insect repellent properties (Asawalam & Hassanali 2006; Kabeh & Jalingo 2007). These properties, if proved, may be due to the presence of lactone, oxalic acid and hydrocyanic acid (HCN) found in its leaf dust (Kabeh & Jalingo 2007). Other studies have tested its fodder potentials (Bonsi *et al.* 1995; El Hassan *et al.* 2000; Mekonnen *et al.* 2009; Tabuti & Lye 2009). In Cameroon *V. amygdalina* is known as Ndolè, an essential ingredient of the popular meal offered by all local restaurants. The increased demand for this vegetable in cities stimulates the intensification of its production in urban and periurban areas (Anonymous 2005). It is even sold outside Cameroon, as in Nigeria, Equatorial Guinea or Gabon, and sometimes in Europe for niche markets (e.g. Africans in European countries) (Bosch *et al.* 2009).

Several insects, including Hemiptera (Sternorrhyncha, Cicadomorphs, Fulgoromorpha and Heteroptera), Coleoptera (Coccinellidae and Cucurionidae), Lepidoptera and Orthoptera, are reported feeding on leaves of this important crop (Banjo *et al.* 2006; Alene *et al.* unpubl. data) and *D. enderleini* constitutes a potential pest. The present paper aims to provide additional information on the morphology, especially of the fifth instar larva, and to describe the relationships between the psyllids and four associated ant species.

#### MATERIAL AND METHODS

Material of *D. enderleini* from Cameroon collected on *V. amygdalina* was examined from the localities listed in Table 1. The specimens are preserved dry, slide-mounted, or stored in 70 % ethanol, and are deposited in the following institutions: Laboratory of Zoology, University of Yaounde 1, Cameroon (LZUY1); Muséum d'histoire naturelle, Genève, Switzerland (MHNG); Naturhistorisches Museum Basel, Switzerland (NHMB). Additional material examined comes from Ethiopia (*Vernonia amygdalina*, NHMB), as well as Kenya and Yemen (both without host data, MHNG).

The drawings and measurements were made from slide-mounted specimens. The morphological terminology follows Hollis (1976, 1984) and Ossiannilsson (1992). The following abbreviations are used: BL – body length; BW – body width; AL – antenna length; FL – forewing pad length; MTL – metatibia length; CL – caudal plate length; CW – caudal plate width; BL/BW – ratio of body length to body width.

TABLE 1  
Collecting localities of *Diaphorina enderleini* in Cameroon.

Localities	Region	GPS Coordinates	Altitude
Nkolondom	Centre	3°57'07"N 11°29'27"E	645 m
Obala		4°11'02.0"N 11°34'11.4"E	559 m
Okola		4°11'39.0"N 11°23'00.1"E	604 m
Olembé		3°57'49.8"N 11°31'47.7"E	661 m
Yaounde		3°51'26.8"N 11°29'53.6"E	746 m
Koutaba	West	5°39'00.0"N 10°48'23.8"E	1190 m

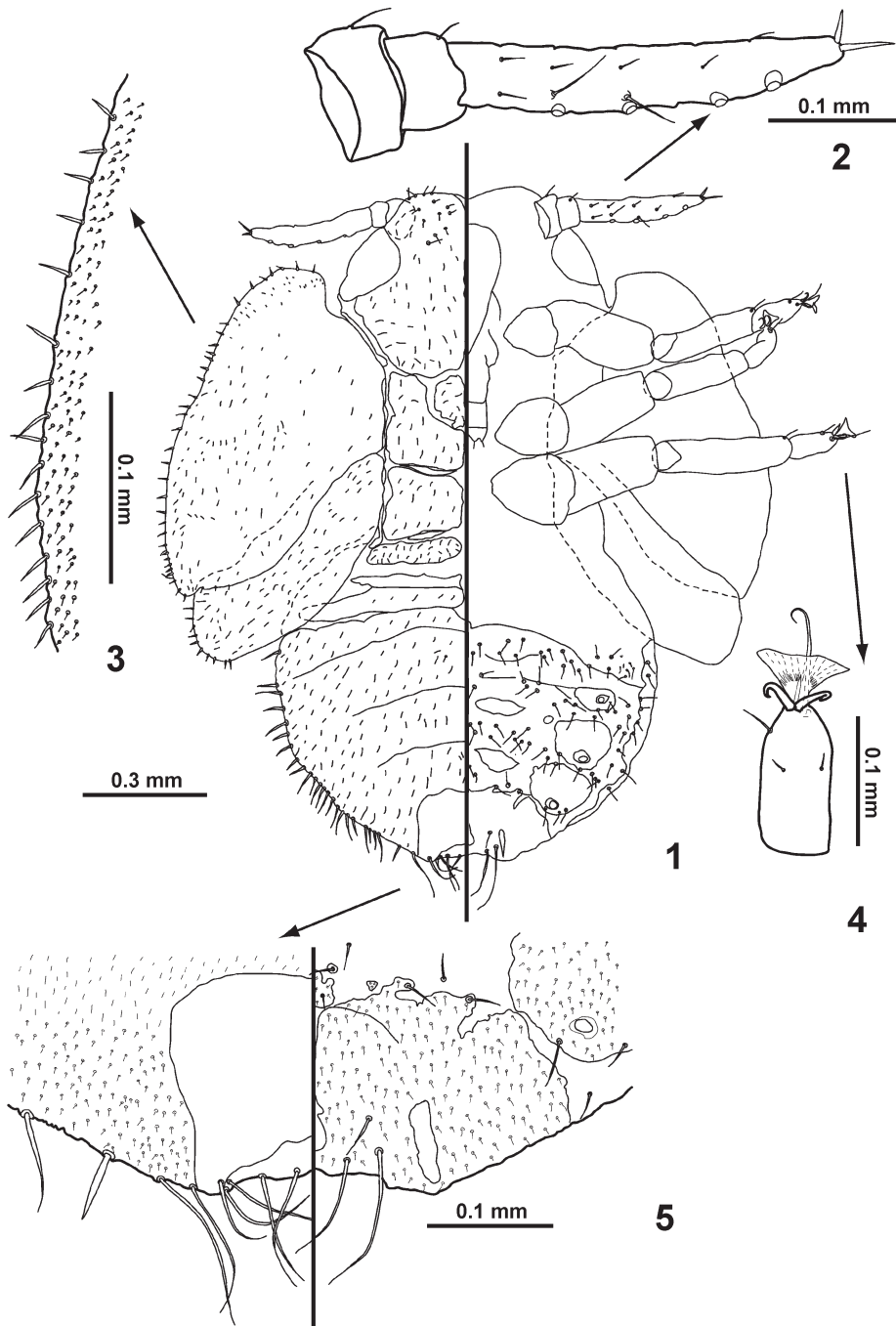
TAXONOMY  
Genus *Diaphorina* Löw, 1880  
*Diaphorina enderleini* Klimaszewski, 1964  
Figs 1–5

*Gonaploicus guttulatus* Enderlein, 1910: 143.  
*Diaphorina enderleini*: Klimaszewski 1964: 59 (replacement name for *Diaphorina guttulata* (Enderlein) nec Lethierry, 1890).  
*Diaphorina siluncula* Loginova, 1978: 77 (synonymised by Burckhardt & Mifsud 1998: 29).

**Description:**  
*Adult.* Described by Burckhardt and Mifsud (1998: 29, figs 48, 55, 56, 82). It is well-defined by its forewing shape and pattern as well as the apically-hooked paramere and the concave dorsal margin of the female proctiger.  
*Fifth instar larva* (Figs 1–5).  
*Colouration.* Sclerites dark brown; membrane beige. Eye reddish pink. Antenna yellow basally, dark brown in apical quarter. Wing pads with dark brown pattern.  
*Morphology.* Body almost as long as wide (Fig. 1). Antenna (Fig. 2) short, 3-segmented, segment 3 (flagellum) bearing 4 rhinaria, apex tapering with two terminal setae, one about 1.5× longer than the other. Dorsal sclerites large and forewing pads well sclerotised; dorsal and ventral sclerites covered in short setae. Forewing pad large and massive, with well-developed humeral lobe, outer margin (Fig. 3) bearing short slender lanceolate setae, posterior apex angled; dorsal surface covered in very short setae. Hindwing pad broad, apex almost rounded, margin and dorsal surface as in forewing pad. Tarsal arolium (Fig. 4) almost twice as long as claws, widened apically with a very short petiole. Caudal plate (Fig. 5) about 1.5× wider than long, rounded posteriorly, weakly indented at apex. Abdominal margin with slender lanceolate setae slightly longer than those on forewing pad margin, setae becoming longer towards apex. Abdominal venter sparsely covered in fairly long setae. Anus terminal, circumanal ring vestigial.  
*Measurements* (in mm) and ratios (11 specimens): BL 1.38–1.75; BW 1.23–1.58; AL 0.30–0.38; FL 0.75–0.88; MTL 0.26–0.30; CL 0.53–0.68; CW 0.85–0.95; BL/BW 1.11–1.12.  
*Distribution.* Cameroon, Ethiopia, Kenya, Sudan, Tanzania, Yemen.

ANT-TENDING AND SHELTER-BUILDING BEHAVIOUR

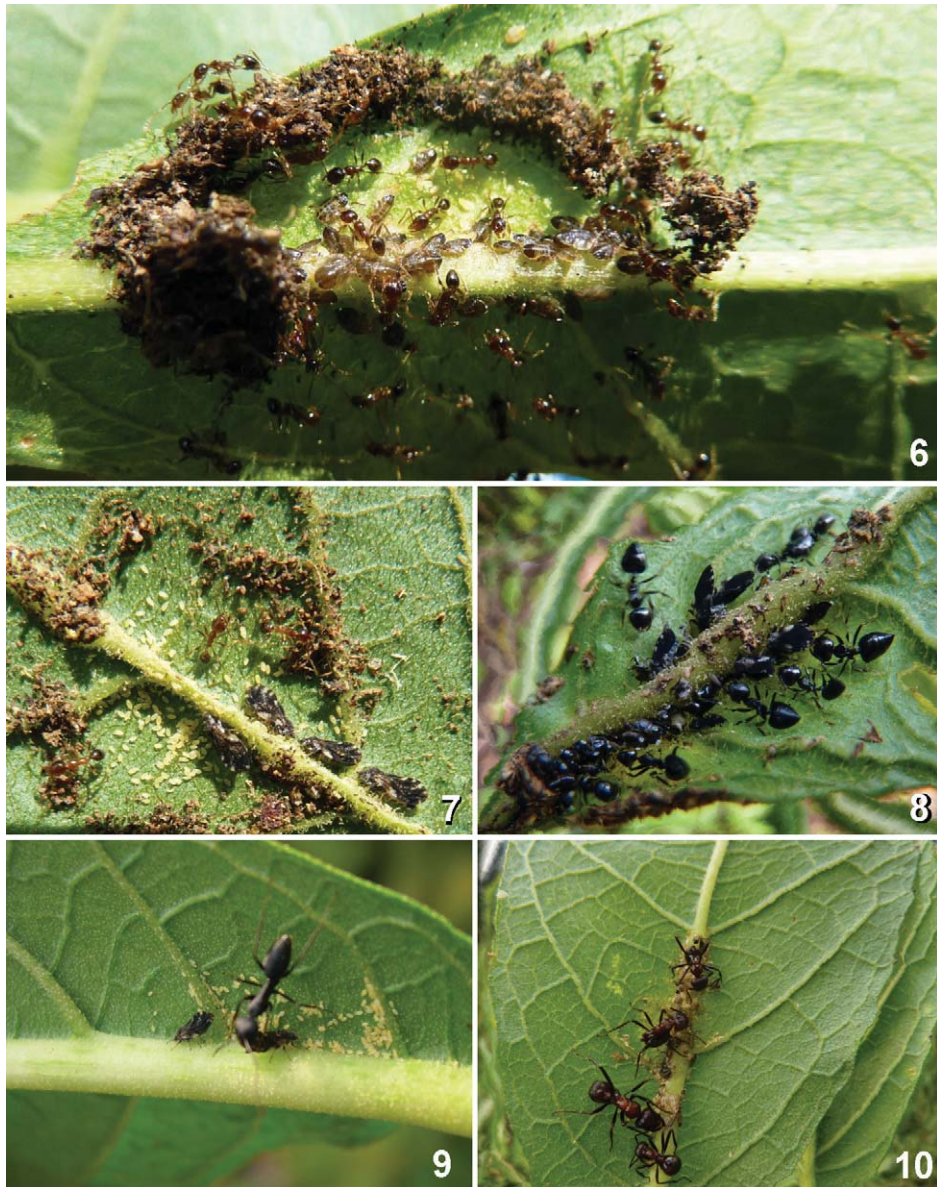
During field studies of *Diaphorina enderleini* on *Vernonia amygdalina*, four ant species (Hymenoptera: Formicidae) widespread in the Afrotropical Region were



Figs 1–5. Fifth instar larva of *Diaphorina enderleini*: (1) habitus, dorsal surface left, ventral surface right; (2) detail of forewing pad, outer margin; (3) antenna; (4) tarsus with claws and tarsal arolium; (5) caudal plate, dorsal surface left, ventral surface right.



encountered with the psyllid colonies: *Pheidole megacephala* (Fabricius, 1793) at Yaounde, Nkolondon, Okola and Obala, *Camponotus acvapimensis* Mayr, 1862, at Olembé and Obala, *Crematogaster striatula* Emery, 1892, at Koutaba, and *Myrmicaria opaciventris* Emery, 1893, at Yaounde.



Figs 6–10. Ants attending *D. enderleini* on *V. amygdalina* leaves: (6) shelter enclosing psyllid larvae and *Pheidole megacephala* workers; (7) psyllid females and eggs with *P. megacephala* workers; (8) psyllid females and larvae with *Crematogaster striatula* workers and carton shelter's remains; (9) psyllid females and eggs with *Camponotus acvapimensis* workers; (10) psyllid larvae with *Myrmicaria opaciventris* workers.

Among these ants, a very unusual behaviour of *P. megacephala* and *C. striatula* was observed. While female psyllids are laying eggs on the lower leaf surface, workers of *P. megacephala* quickly pile up detritus combined with soil around and partly above the psyllids, resulting in a shelter-like structure (Figs 6, 7). Those of *C. striatula* build carton shelters (Fig. 8). During this procedure the adult female psyllids hardly move away from their eggs and larvae. The psyllid larvae are arranged in tight rows along the leaf veins, especially the principal one, and sometimes at the base of very young branches. The larvae are mostly sedentary and only move, slowly and steadily, when disturbed. They do not produce wax, as can be expected from the almost complete lack of the circumanal ring. Females and larvae excrete large drops of honeydew, which are immediately recovered by ants.

In *C. acvapimensis* and *M. opaciventris* the workers did not build any structure around the psyllids but they were observed taking honeydew from them (Figs 9, 10).

#### DAMAGE TO THE HOST

Adults and larvae of *Diaphorina enderleini* feed by sucking the plant sap. Feeding by the larvae is especially injurious due to their large number and their immobility (they feed at the same spot for a long time). A noticeable symptom is the appearance of translucent aureoles on the leaves, apparently resulting from the toxicity of the saliva injected by the psyllids.

#### DISCUSSION AND CONCLUSION

The larvae of *Diaphorina enderleini* share a series of morphological characters with other *Diaphorina* spp., such as the three-segmented antenna with four rhinaria on segment 3, the presence of a humeral lobe on the forewing pad and the marginal lanceolate setae on the wing pads and caudal plate. However, while a circumanal ring of wax-producing pores is well-developed in the larvae of the other known species of *Diaphorina* (Burckhardt 1985, 1986; Burckhardt & Mifsud 1998; Burckhardt & van Harten 2006), it is almost completely lacking in *D. enderleini*, and is not functional. In fact, the larvae do not produce wax. This habit probably exposes them to environmental constraints and natural enemies.

Among psyllids, different strategies for the protection of larvae are realised. In several free-living psyllids, such as *Mesohomotoma* and *Psylla* spp., the larvae are completely covered in abundant wax produced by wax glands which are connected to the circumanal ring and to additional pore fields on the caudal plate (Hodkinson 1974). In *Diclidoplebia xuani* Messi, 1998, and *Diclidoplebia harrisoni* Osisanya, 1969, the larvae covered in wax induce leaf roll galls in which they live (Burckhardt *et al.* 2006; Aléné *et al.* 2008). Many larvae of the Australian Spondylaspidini form lerps, i.e. protecting shields mostly composed of starch under which they live (Hollis 2004). The New World *Euphalerus* spp. associated with *Lonchocarpus* spp. live either in galls or under lerps (Hollis & Martin 1997), whereas many other taxa live in closed galls, such as the Afrotropical *Phytolyma* spp. on Moraceae (Hollis 1973), some Afrotropical *Pseudopharopteron* spp. (Malenovský *et al.* 2007; Malenovský & Burckhardt 2009) or members of the South American *Calophya rubra* (Blanchard, 1852) group on *Schinus* spp. (Burckhardt & Basset 2000).

As the larvae of *D. enderleini* neither induce gall-like structures nor produce wax, they need an extrinsic protection which may be provided by the attendance of ants. The latter expect to be recompensed by honeydew excreted by the psyllids. Two ant species, *P. megacephala* and *C. striatula*, appear as good partners in this interaction since they build shelters to protect the psyllids from environmental constraints and natural enemies.

The shelter is probably also a means for the ants to protect the source of honeydew from competitors such as other hymenopterans and dipterans. Similar relationships are widely recorded in several groups of Sternorrhyncha and Auchenorrhyncha (Delabie 2001; Naskrecki & Nishid 2007; Verheggen *et al.* 2009) and a few groups of Heteroptera (Pfeiffer & Linsenmair 2000; Gibernau & Dejean 2001). There are very few records of this type of trophobiosis in psyllids (Novak 1994).

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