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PrePub-Article

First report of a gall midge as a parasitoid of weaver ants

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With 2 figures and 1 table

Abstract: Gall midges (Diptera: Cecidomyiidae) comprise a diverse family of small nematoceran flies. While most species are phytophagous, the family also includes predatory species, and endoparasitoids. Endoparasitic species have been reared from aphids and psyllids. We discovered parasitoid-infected weaver ant, *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae) in nests on mango trees in coastal Kenya. Examination of 5 colonies revealed the presence of parasitoids in all of them, with parasitism rates ranging from 2 to 21%. Morphological examination of larval parasitoids indicated that they were gall midges, and molecular data were consistent with the identification. Attempts to rear adults were unsuccessful. This study presents to the best of our knowledge the first report of Cecidomyiidae parasitising Hymenoptera, as well as the first report of a parasitoid of weaver ants. This species should be investigated further because its damage may limit the ability of weaver ants to control pests of cultivated fruits and other insects.

Keywords: Cecidomyiidae, endoparasitoids, biocontrol, *Oecophylla longinoda*, Afrotropical region

1 Introduction

The African weaver ant, *Oecophylla longinoda* (Latreille) has been used successfully as a biological control agent against several insect pests of coconut, cocoa, citrus, mango, cashew and timber trees in most tropical areas of Africa (Van Mele 2008). Van Mele et al. (2007) showed that the percentage of mangoes infested by the fruit flies *Bactrocera dorsalis* and *Ceratitis* spp. (Diptera: Tephritidae) was negatively correlated with the abundance of ant nests in a tree. Twenty-four percent (24%) of mangoes were infested in an ant-free tree compared to only one percent (1%) of mangoes infested in trees with more than eight nests per tree (Van Mele 2008). Weaver ants also reduced the occurrence of diseases caused by viruses and fungi of cacao plantations in Ghana by attacking mirid leaf bugs (Hemiptera: Miridae) (Hölldobler & Wilson 1977).

Oecophylla smaragdina (Fabricius) is distributed throughout tropical Asia, Australasia and some Pacific islands sharing analogous biological and ecological features with the sister species, *O. longinoda* (Sribandit 2008). Besides their importance in controlling pest insects, *Oecophylla* species also give additional service to their host plants. A recent study revealed that colonies of these ants might provide their host plant with a significant amount of nitrogen (Pinkalski et al. 2016). Also, faecal droplets deposited by *O. smaragdina* contain amino acids that are used as foliar fertilizer by *Coffea arabica* (L.) (Pinkalski et al. 2018). Though not widely known, *O. smaragdina* in Asia (Sribandit 2008) and *O. longinoda* in West Africa (Offenberg 2011) are also used as a commercial product, such as traditional medicine, as animal feed and as a human delicacy.

Apart from the hemipteran insects that provide honeydew (Dwomoh et al. 2009) and parasitic caterpillars of moth *Eublemma albifascia* (Noctuidae; Acontiinae), that obtain regurgitations and trophic eggs from ants (Dejean et al. 2016), no insect species have been reported as being associated with *Oecophylla*, probably due to its aggressiveness. This paper reports the discovery of an endoparasitic gall-midge species (Diptera: Cecidomyiidae) of *Oecophylla longinoda*. The prevalence of this parasitoid is briefly discussed.

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2 Material and methods

2.1 Insect rearing

2.1.1 *Oecophylla longinoda*

Parasitised ant colonies by gall midge larvae were collected from mango trees at Muhaka field station (4°28′79″S, 39°56′53″E) of the International Centre of Insect Physiology and Ecology (*icipe*) in Kwale County, Coast Province, Kenya. Ant nests were collected from the same location but from different colonies in November 2018. The nests were collected and transferred to potted mango trees in a greenhouse at *icipe*'s main campus (-1°22′1922″S, 36°89′6639″E) in Nairobi, Kenya. The pots were placed in the centre of a tray filled with soapy water to confine the ants to the host plant and prevent predatory insects from getting access to the ants. Nests were maintained under natural lighting in the screen house. Ants were fed on a 10% sugar solution, and freshly killed fruit flies (*Bactrocera dorsalis* and *Ceratitis cosyra*) twice a week. Parasitised ants were recognised under a microscope by the distinct orange colour of the parasitoid larva inside the host abdomen (Fig. 1). To collect parasitoids, ants were placed in glass vials resting on ice to knock them down. The parasitised ants were then dissected in distilled water under a microscope, and the parasitoids were placed in 70% alcohol for further identification.

A different group of ants, five ant nests in total, were collected using a plastic container, as mentioned above. The nests were transferred to the laboratory at *icipe*'s main campus in Nairobi, Kenya. The number of ants per nest was counted by putting the insects in a freezer for 48 hours. The parasitised and non-parasitised were separated and counted using a microscope (also sometimes visible even to the naked eye).

2.2 Parasitoids

Three trials were done to establish a population of parasitoids, determine possible pupation sites, and to observe the behaviour of both the parasitised ants and the larvae. First, nests containing parasitised ants were placed in Plexiglass cage (30 cm × 22 cm × 10 cm) with moist sand (washed, sterilized at 120°C for 2 hours and cooled at ambient temperature before use) at the bottom for the last instar larvae to pupate. The sand was checked for the presence of parasitoid pupae weekly for a month. The second approach was to isolate parasitised ants after identifying using a microscope. Those which were parasitised were collected and placed in plastic buckets with fine sand at the bottom. The ants were left undisturbed for several days to allow larvae to complete development. The third method used was to place individual parasitised ants in a petri dish containing different substrates for pupation: filter paper (Whatman International Ltd., Maidstone, Kent, UK), fine sand and soil. When ants were kept alone for more than two weeks, their activity was reduced and eventually died. Observations were made daily between 0900 h and 1100 h for as long as the ants survived (about four weeks), to evaluate the change in behaviour of ants and the emergence of the parasitoid larvae. Room temperature was maintained at approximately 25 ± 2°C and 60 ± 5 RH%.

2.3 Molecular identification

Larvae removed from the abdomen of *Oecophylla longinoda* were sent to CBGP (Centre de Biologie pour la Gestion des Populations, Montpellier, France) CIRAD for identification. DNA was extracted from the whole specimens using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). PCR amplification was carried out using standard primers for barcoding (mitochondrial

Fig. 1. Workers of the parasitised *Oecophylla longinoda* (Photo by Dr Subramanian, Sevgan). Larvae were coming out of the abdomen when workers kept separately from the colony for about two weeks.

cytochrome c oxidase subunit I, *COI*) of invertebrates: LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAA TCA-3' (Folmer et al. 1994). The PCR products were sequenced by Eurofins Genomics (http://www.eurofins-genomics.eu/). Voucher specimens in 96.6% EtOH were deposited at CBGP (CIRAD collection). Barcode sequences were aligned using Codon Code Aligner V.3.7.1. (Codon Code Corporation, Centerville, MA, USA), and checked to identify the presence of pseudogenes using standard detection methods (Haran et al. 2015). In the absence of adult parasitoid specimens for morphological identification, barcode sequences were subjected to a BLAST (implemented in the NCBI platform (https://www.ncbi.nlm.nih.gov/) to relate the species to the closest taxa available in this database.

3 Results

Larvae were identified morphologically as belonging to the gall midge family (Diptera: Cecidomyiidae) based on the presence of a sternal spatula (Fig. 2), a structure unique to this family (Gagné 1994). BLAST analysis showed that the sequences obtained in this study (GenBank accession number MN603974) revealed 92% identity with an unidentified gall midge species of the family Cecidomyiidae (accession number KM993749.1), and 91% identity with three other unidentified species (accession numbers KT100438.1, KT112350.1, and KM988118.1).

From the five nests investigated so far (small nests approximately 300-1100 workers), the parasitism rates ranged from 2 to 21%, combining both major and minor workers (Table 1). Most of the parasitised ants were foragers (major workers). Immature larvae can crawl and survive outside of the host for some time, and the last instar larvae can jump a considerable distance after leaving the host, apparently in search of a suitable pupation site. Mature larvae left their host through the anus of the ant and deceased after some time (Fig. 1). Unfortunately, adult flies never emerged despite using different substrates for pupation, separated parasitised workers from the colony or kept the whole colony together. Multiple parasitisms were observed, with two larvae inhabiting the abdomen of a single worker (data not shown).

4 Discussion

The insect fauna of the Afrotropical region is still poorly known, in particular for small Diptera; and hence, this species could not be identified beyond the family level. The large majority of larvae of Cecidomyiidae species (about 6,590 known species and in 812 genera) are phytophagous (Gagné & Jaschhof 2017). To a lesser extent, some species of this group have been described as predators of aphids such as *Aphidoletes aphidimyza* (Tang et al. 1994; Van Lenteren et al. 1997). Few species are known as endoparasitoids of insects and are grouped in the genera *Endaphis* (11 species, including two species of *Endopsylla* recently synonymised with *Endaphis*) (Gagné & Etienne 2019). They generally develop in aphids, but one *Endaphis* species, *Endaphis psyllophaga* (Abe et al. 2010) is known to develop in psyllids and have been considered as potential agents for the biological control of some aphid pests (Muratori et al. 2009).

This is the first report of a parasitoid from the Cecidomyiidae attacking a hymenopteran, and the first record of a parasitoid of weaver ants. Two hypotheses were drawn that might explain the unsuccessful attempt of rearing adults: (i) when parasitised ants are kept apart from the col-

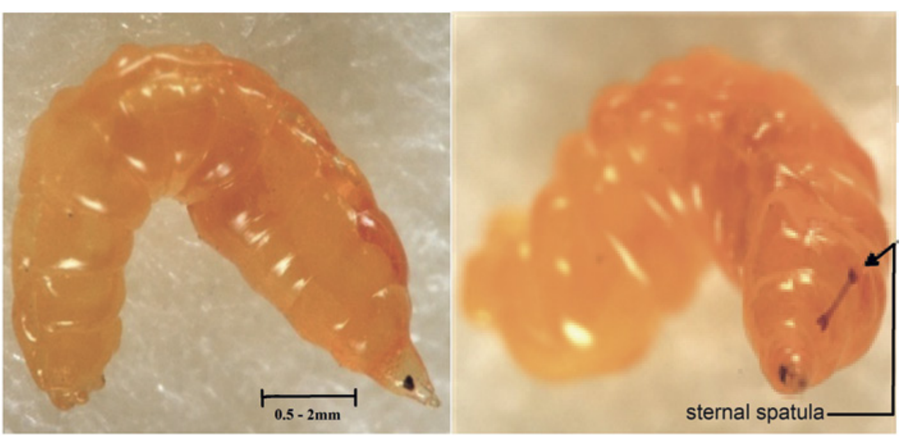

Fig. 2. Larvae of the parasitoid (Diptera: Cecidomyiidae) (Photo by Robert S. Copeland). Note sternal spatula in the image on the right.

Table 1. Percentage of parasitised worker ants from the five different colonies observed.

Colony number	Total number of workers in the nest	Number of parasitised workers	Percent of parasitised ants
1	399	8	2
2	584	123	21
3	639	20	3
4	319	35	11
5	1,013	71	7

ony, their fitness decreases, and parasitoid larvae are forced to leave due to poor host quality; (ii) when kept in the colony, parasitoid larvae are eaten by the ants on leaving the host.

Conservation biological control is considered to have the potential for field use in agriculture (Wyckhuys et al. 2013), and the use of weaver ants as predators of insect pests is an example of this approach. However, in designing a biological control programme, knowledge and understanding of the trophic interaction among species is vital (Sullivan & Völkl 1999). Many ant groups (such as army ants, leaf-cutter ants, fire ants, and carpenter ants) have been reported as hosts of phorid flies (Brown & Feener 1998). For instance, workers of the imported red fire ant, *Solenopsis invicta* are parasitised by *Pseudacteon tricuspis* adults (Henne & Johnson 2007), and this species, is being used to control fire ants (Cônsoli et al. 2001).

However, the use of social insects as biological control agents could be hampered by the presence of parasitoids. Presently, the effect of the Cecidomyiid parasitoid on weaver ant populations remains unknown. The distribution and abundance of this species need to be explored further to estimate its impact on colonies of *O. longinoda* that may pose severe setbacks to the use of weaver ants in biological control programmes. Further research is required to assess biotic and abiotic factors affecting the parasitism rate. Efforts are now geared to finding effective ways of rearing the insect to identify adults at the species level, study its biology and evaluate its effect on the weaver ant population.

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