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ORIGINAL PAPER

Molecular determination of the predator community of a cassava whitefly in Colombia: pest-specific primer development and field validation

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Abstract In South America, the whitefly *Aleurotrachelus socialis* is one of the principal pests of cassava (*Manihot esculenta* Crantz), reaching high population levels throughout the Andean region. Management of this species is primarily based upon the use of insecticides, while biological control has received limited attention. Till present, knowledge of *A. socialis* natural enemies is restricted to occasional records of predators and parasitoids. In this study, we developed PCR primer sets specific for the cassava whitefly, *A. socialis*, to identify their predator community in Colombian cassava. Eleven percent of 586 predator specimens (representing 131 taxa from 29 families) tested positive for cassava whitefly DNA. Of the 21 predator taxa that consumed cassava whiteflies, an unidentified netwing beetle (Lycidae), an unidentified spider species (Araneae), *Harmonia axyridis* (Coleoptera: Coccinellidae), a *Cereaochrysa* sp. (Neuroptera: Chrysopidae), and a *Leucochrysa* sp. (Chrysopidae) were the taxa that consumed cassava whiteflies most frequently under field conditions. Two abundant predators in the system, *Delphastus* sp. (Coccinellidae) and the long-legged fly, *Condylostylus* sp. (Diptera: Dolichopodidae), were both positive for whitefly DNA, but did not have the strongest trophic linkage to the pest relative to other predators. This study shows that a diverse predator community affects cassava whitefly in southern Colombia, and provides the groundwork for the design of cassava production systems with minimal pesticide inputs.

Keywords *Aleurotrachelus socialis* · Biological control · *Harmonia axyridis* · Molecular gut analysis · PCR · Predator

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Introduction

Cassava is a nutritionally important crop plant for subsistence farmers in tropical regions (UNCTAD 2009). It supports a diversity of pests, including a complex of 11 species of whiteflies (Hemiptera: Aleyrodidae) (Bellotti et al. 1999). Dominant species in this complex vary among regions, and include especially *Aleurothrixus aepim* (Goldi), *Bemisia tabaci* (Gennadius), and *Trialeurodes variabilis* (Quaintance) (Thresh et al. 1994; Bellotti et al. 1999; Calvert et al. 2001). In Colombia and northern South America, an important region for cassava production and research, the dominant cassava whitefly is *Aleurotrachelus socialis* Bondani (Bellotti et al. 1999). Whiteflies affect cassava by sucking the phloem of the plant, transmitting viruses to the plant, and by inhibition of photosynthesis through their production of honeydew and associated sooty mold (Bellotti and Arias 2001; Akinbo et al. 2012). Rapid population growth rates and prolonged exposure can decimate local cassava fields, reducing yields substantially (by up to 80 % in some research) (Bellotti et al. 1983; Gold et al. 1989a). The dominant method for controlling whitefly

outbreaks in tropical regions involves the frequent application of insecticides, which leads to environmental and human health risks and the rapid evolution of pest resistance. Developing farm management systems that couple host plant resistance (Bellotti and Arias 2001; Carabalí et al. 2010; Akinbo et al. 2012) or intercropping (Gold et al. 1989a) with the use of natural enemies could offer an alternative to current pesticide-based whitefly management practices (Bellotti et al. 1999). A crucial knowledge gap that challenges the development of this type of system is that the predator community of cassava whitefly remains poorly described.

Generalist predators are an abundant source of biotic pest mortality in cropland, but their identity and role are yet to be characterized in cassava cropping systems. Much of the previous study on cassava whitefly natural enemies has focused on parasitoids that would be amenable to classical biological control releases (Evans and Castillo 1998; Trujillo et al. 2004). Describing important components of the predator community of cassava whiteflies is challenging. Effective predation often leaves no trace of the predation event (unlike parasitism or infection with entomopathogens), and estimating the impact of predators on a pest requires carefully controlled experiments that often disrupt normal predator behaviors. Previous study has identified *Delphastus pusillus* (Le Conte) (Coleoptera: Coccinellidae), = *D. catalinae* (Hoelmer and Pickett 2003), as a commonly encountered predator of cassava whitefly (López-Ávila et al. 2001), with questionable impact on the pest (Gold et al. 1989b; Bellotti et al. 1994; Bento et al. 1999), but efforts to determine relative impacts of various predators on whitefly populations have been cursory at best. Nevertheless, generalist predators are well documented as regulating other pest populations, including a diverse predator community of whiteflies in other crops (Hagler and Naranjo 1994a, b; Gerling et al. 2001; Lin et al. 2008; Hodek and Honěk 2009; Moreno-Ripoll et al. 2012).

An important step in developing predator conservation techniques is establishing which predators have a trophic linkage with the focal pest species. One method that facilitates the establishment of these trophic linkages is PCR-based gut analysis of the predator community (Fournier et al. 2008; Lundgren et al. 2009; King et al. 2010; Sint et al. 2011; Opatovsky et al. 2012). This method implements PCR primer sets that selectively amplify a DNA fragment from a focal pest to search predator stomachs for pest DNA (Sheppard and Harwood 2005; Weber and Lundgren 2009a). While this method does not consistently correlate well with relative impact of the predators on the pest (impact is also related to predator densities, foraging patterns, etc.), it is a very effective method for assessing the strength of the trophic linkage between a predator and pest, and identifying the diversity of predators consuming a pest (Lundgren and Fergen 2011).

The current study is an initial description of the predator community of cassava whitefly (*A. socialis*) near Cali, Colombia. We developed the molecular tools necessary for conducting PCR-based gut analysis to establish trophic connections between a predator community and cassava whitefly, and validated the method by describing which species are consuming whiteflies under field conditions during a short snapshot of the field season. This developmental research can now be used to help develop sustainable pest management plans for cassava whiteflies that target conservation efforts at key predators of the pest.

Methods

Insect collection

Arthropods (non-target herbivores and putative whitefly predators) were collected from a cassava resistance screening experiment at the Cenicaña research station (3.34°, −76.31° latitude and longitude) between April 25 and May 16, 2011. Here, approximately 80 varieties of first year cassava plants are produced on a 4-ha plot; these cassava genotypes display varying levels of whitefly resistance, and are produced without the use of insecticides. To ensure uniformly high whitefly population pressure, experimental blocks were bordered by a row of the highly susceptible cultivar CMC 40, which was artificially infested with *A. socialis* obtained from greenhouse colonies maintained at the International Center for Tropical Agriculture (CIAT). These whiteflies were originally collected at the CIAT research station (3.50°, −76.36°) and kept in culture for over 10 years, with periodic infusions of wild individuals from the same location. At the field site, all life stages of the whitefly were abundant, although the population was patchy in distribution. There were no obvious deterrents to predator establishment at the field site (e.g., specific predators were not seeded at the site, nor were insecticides applied that might shape predator communities), which was surrounded by grassy margins and other crops. An array of adult arthropods (131 herbivorous and predatory taxa from 29 families) was collected from the cassava foliage using sweep nets, or directly from the foliage with aspirators. The goal of the study was to collect a diversity of predators, along with greater numbers of some dominant species, to validate the primer sets. As such, the field was not systematically sampled, some predator groups are over-represented, and others may have been overlooked. Individual predators were isolated in 1.5 µl microcentrifuge tubes, and placed on ice until they

could be frozen in the laboratory in 70 % ethanol at −20 °C. Non-target herbivores were generally left as morphotaxa used in specificity testing of the whitefly primer sets. Predators were identified to the lowest taxonomic level possible. It is important to note that the resulting relative abundances of predators are not actual densities of these species within cassava fields.

Whitefly primer development

Cassava whiteflies were obtained from CIAT's colony (see above). A 709-bp segment of the cytochrome oxidase I (COI) gene and adjacent partial cds genes were sequenced from two populations of this *A. socialis*. The amplified COI region from each whitefly population was cloned into the PGEM®-T Easy vector (Promega Corporation, Madison, WI, USA). Plasmid DNA from *E. coli* used for sequence analysis was purified using a QIAprep spin miniprep kit (QIAGEN; Valencia, CA, USA). DNA inserts in five of these cloned vectors were sequenced in both directions using the Big Dye™ Terminator Cycle Sequencing kit with an Applied Biosystems 377 DNA fragment analyzer by the Cornell University Life Sciences Core Laboratories Center (Ithaca, NY, USA) using vector primers SP6 and T7. The resulting DNA sequences were edited and analyzed using Sequencher® 4.5 (Genes Codes Corporation, Ann Arbor, MI, USA). The COI sequence for *A. socialis* determined in this study has been deposited in GenBank database (Accession # KF059953).

Using these sequences from *A. socialis*, a primer set was developed using Primer3 by Simgene (http://simgene.com/Primer3Servlet) that amplified a 117-bp fragment of the sequenced whitefly DNA (fwd 5′-GGGCACTGGTTGAACAGTTT-3′; rev 5′-GCACCTAAAATGGAAGACGC-3′). In silico searches revealed minimal overlap of the primers with sequences available in Genbank, and high fidelity to the target species. Optimal primer concentration and annealing temperatures were determined, and the primer sets were checked for cross reaction against specimens collected in the general predator collections outlined above (wings or legs surface-sterilized in 10 % bleach for 10 s) of 131 herbivorous and predatory arthropod taxa, representing 29 Families and nine Orders found in the Colombian cassava ecosystem (see Table 2 in Appendix 1).

qPCR and gut content analysis

DNA was extracted from whole insect squashes of individual predators using DNEasy® extraction kits for blood and tissue (product #69506, QIAGEN) according to manufacturer instructions. Samples were macerated in ATL buffer using sterilized plastic pestles, and the samples were incubated for 3 h in proteinase K. Whitefly DNA in the predator stomachs was amplified using qPCR reactions containing 12.5 µl 2× Quantitect SYBR Green PCR Master Mix (product #214143, Qiagen), 1 µl fwd primer (150 nM), 1 µl rev primer (150 nM), 1 µl template, and 9.5 µl sterilized water. Reaction conditions run on a Stratagene MX3000P thermocycler (Stratagene, La Jolla, CA, USA) were an initial step of 95 °C for 15 min, followed by 50 cycles at 94 °C for 15 s, 54 °C for 30 s, and 72 °C for 30 s. Fluorescence was measured at 492 nm for the SYBR green dye, and at 582 nm (for ROX dye, which was used as a control to ensure the efficacy of the fluorescence measures) on the annealing step of each PCR cycle. Fluorescence was adjusted manually to bring the baseline-corrected normalized fluorescence (dRn) just above background fluorescence for each plate. A dissociation or melt curve was created for each reaction, and the melting temperature for each positive reaction was measured. Here, samples were heated to 95 °C and cooled to 55 °C at a rate of 0.2 °C/s and fluorescence was measured continuously; whitefly DNA amplified by these primers dissociates at 77.7 °C. On each 96 well plate, five wells were devoted to a positive control series (a DNA extraction from a single whitefly specimen) and three wells were assigned as no-template controls. Each positive sample generated a C_t (the threshold PCR cycle where fluorescence was distinguishable from background), which has an inverse relationship with the initial quantity of prey consumed (Zhang et al. 2007; Weber and Lundgren 2009b). For each predator taxon, the proportion of the population testing positive for whitefly DNA, as well as the mean quantity of prey DNA (represented as $C_t^{-1} \times 100$) was calculated. In subsequent sections of the manuscript, trophic linkage refers to the frequency of detection of the whitefly DNA within the population of a specific predator taxon.

Results

A total of 586 specimens from putatively predatory groups were assayed for whitefly DNA in their stomachs (note, these represent all predatory specimens reported in Table 2 in Appendix 1). Of these 11.09 % of specimens tested positive for whitefly prey DNA. In sum, 24 species of predators consumed cassava whitefly under field conditions. The top five strongest trophic linkages within the predator community included a spider species (Araneae sp. 6), an unknown net-winged beetle (Lycidae sp. 1), the green lacewings *Leucochrysa* sp. 1, and *Ceraeochrysa* sp. 2. Also noteworthy, the invasive multi-colored Asian lady beetle, *Harmonia axyridis* had a strong trophic

Table 1 Frequency of whitefly predation in major predatory arthropod taxa collected, and the quantity of prey DNA ($C_t^{-1} \times 100$) discovered per taxon

Order: Family	Species	Percent positive (*N*)	Prey DNA ($C_t^{-1} \times 100$; mean ± SEM)
Araneae	Unknown sp. 6	*42.86 (7)*	3.26 ± 0.07
Neuroptera			
Chrysopidae	*Ceraeochrysa* sp. 1	17.65 (17)	3.47 ± 0.19
Chrysopidae	*Ceraeochrysa* sp. 2	*33.33 (6)*	3.48 ± 0.36
Chrysopidae	*Leucochrysa* sp. 1	*40.00 (5)*	3.15 ± 0.12
Coleoptera			
Coccinellidae	*Delphastus* sp.	13.64 (22)	3.13 ± 0.11
Coccinellidae	*Harmonia axyridis* (Pallas)	*35.00 (40)*	3.55 ± 0.11
Coccinellidae	*Cycloneda sanguinea* (L.)	0 (5)	
Lycidae	Unknown sp. 1	*50.00 (4)*	2.98 ± 0.04
Diptera			
Dolichopodidae	*Condylostylus* sp. 1	21.28 (47)	3.40 ± 0.13
Dolichopodidae	*Condylostylus* sp. 2	0 (6)	0
Empididae	*Drapetis* sp.	0.62 (162)	3.22
Empididae	*Elaphropeza* sp.	2.98 (168)	3.78 ± 0.24
Hemiptera			
Reduviidae	Unknown sp. 2	25.00 (8)	3.44 ± 0.01
Reduviidae	Unknown sp. 3	16.67 (6)	3.57
Reduviidae	Unknown sp. 4	0 (4)	
Reduviidae	Unknown sp. 8	0 (5)	

The top five highest frequencies of consumption are in italics. All predators were adults

linkage with cassava whitefly. This report constitutes an early record of this species in Colombia published in the peer review literature (see Kondo et al. 2013 for an earlier observation of this species in Colombia). Frequency of consumption by the putative whitefly predator, *Delphastus* sp., was intermediate (13 %) relative to other predators. Notably weak trophic linkages to whitefly prey were found in *Cycloneda sanguinea* (Coccinellidae), several species of assassin bugs (Reduviidae) and the predatory flies in the Empididae and Dolichopodidae families. An exception was *Condylostylus* sp. 1 (Dolichopodidae), of which 21 % of 47 specimens tested positive for whitefly prey (Table 1). The known herbivorous lace bug, *Amblystira machalana* (Drake) (Hemiptera: Tingidae) (Bellotti et al. 1999) ($n = 16$) was not omnivorous on whiteflies.

There were a number of predatory taxa that were minor components of the community (sample sizes <4 specimens; not included in Table 1), but their gut contents were analyzed, and some tested positive. There were seven additional spider taxa that tested positive for whitefly DNA in their guts (unknown species 13, 14, 16, 18, 19, 22, and 33). Of the three additional Chrysopidae morphospecies tested, one taxon (Chrysopidae sp. 2) tested positive. Finally, one cricket tested positive for whitefly prey (Gryllidae unknown sp. 1).

Discussion

This study clearly shows that a diverse predator community consumes the cassava whitefly, *A. socialis*, within Colombian cassava fields. 24 species of predators consumed whiteflies in this field, some of these trophic linkages to the pest were surprisingly strong; >25 % of the specimens of some taxa consumed the pest DNA within a few hours before collection. Of the species examined, spiders, *H. axyridis*, and lacewings consumed the pest most frequently. It is important to note that the strength of these trophic interactions is only one component of the impact that specific taxa will have on the pest. Relative predator abundance, whitefly population dynamics and physiological status, pest age (and thus quantity of DNA), and availability of alternative resources all affect the impact that a given species or group of predators ultimately has on a target pest. Also, the field validation of the whitefly primers was focused over a 21-day period in April on mature plants; thus other predators occurring at other times during the season or during the plant's development may also be important components of the predator community. Additional research that identifies the relative impacts and constraints of these predators, as well as ways to manage the cassava system to best conserve and promote natural control of these species, is necessary before conservation biological control can be a reality for producers.

The diversity of predators that consume cassava whitefly under field conditions makes the case for conserving entire predator communities in and near cassava production areas. Much of the study done previously on whitefly predators were attempts to find species that were specific for a target pest and that could be released in classical biological control programs. This revealed *Delphastus* sp. as a common predator with limited value as classical biological control agent. The lady beetle requires too many prey to reproduce, and thus pest populations must be large in order to elicit a functional response to the pest (Gold et al. 1989b; Hoelmer et al. 1993). Indeed, our study showed that *Delphastus* had a fairly weak trophic linkage with cassava whitefly (13 % of adults tested positive), relative to other taxa (Table 1), and probably is not the most effective predator of whiteflies on its own. Other predators found in our study have been previously found to consume whitefly pests (not necessarily *A. socialis*), including lacewing adults (Dean and Schuster 1995; Cortéz-Madrigal et al. 2008; Lin et al. 2008), spiders (Zhang et al. 2007), *H. axyridis* (Zhang et al. 2007), and others (Gerling et al. 2001). Small predatory flies (Empididae and Dolichopodidae) were a particular focus of this study, as they are some of the most abundant putative predators associated with cassava whitefly in Colombia. Our results echo those of Hagler (2002), who found that *Drapetis* nr. *divergens* (Diptera: Empididae) was a specialist on adult whiteflies that had minimal impacts on the pest by themselves. However, many of these species such as *Delphastus* spp. or empidid flies have a role to play as part of a greater community response to cassava whitefly establishment and proliferation, and are worthy of conservation within cassava production systems. A final result worthy of note is that a literature review indicates that this is one of few if any reports which show that adult Lycidae are predatory; most previous studies focus on this family as nectarivorous as adults. Clearly, we have much to learn regarding the trophic position of many insects within the cassava agroecosystem.

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Appendix

See Table 2.

Table 2 Morphotaxa collected from cassava fields and tested for cross-reactivity with cassava whitefly-specific primer sets

Order: Family	Species (*N*)
Myriapoda	
Myriapoda	Unknown sp. 1 (1)
Myriapoda	Unknown sp. 2 (1)
Araneae	
Araneae	Unknown sp. 1 (1)
Araneae	Unknown sp. 2 (2)
Araneae	Unknown sp. 3 (1)
Araneae	Unknown sp. 4 (3)
Araneae	Unknown sp. 5 (1)
Araneae	Unknown sp. 6 (7)
Araneae	Unknown sp. 7 (1)
Araneae	Unknown sp. 8 (1)
Araneae	Unknown sp. 9 (3)
Araneae	Unknown sp. 10 (1)
Araneae	Unknown sp. 11 (2)
Araneae	Unknown sp. 12 (1)
Araneae	Unknown sp. 13 (3)
Araneae	Unknown sp. 14 (1)
Araneae	Unknown sp. 15 (1)
Araneae	Unknown sp. 16 (2)
Araneae	Unknown sp. 17 (2)
Araneae	Unknown sp. 18 (1)
Araneae	Unknown sp. 19 (3)
Araneae	Unknown sp. 20 (1)
Araneae	Unknown sp. 21 (1)
Araneae	Unknown sp. 22 (1)
Araneae	Unknown sp. 23 (1)
Araneae	Unknown sp. 24 (1)
Araneae	Unknown sp. 25 (1)
Araneae	Unknown sp. 26 (1)
Araneae	Unknown sp. 27 (1)
Araneae	Unknown sp. 28 (1)
Araneae	Unknown sp. 29 (1)
Araneae	Unknown sp. 30 (1)
Araneae	Unknown sp. 31 (1)
Araneae	Unknown sp. 32 (1)
Araneae	Unknown sp. 33 (1)
Blattodea	
Blattodea	Unknown sp. 1 (1)
Orthoptera	
Gryllidae	Unknown sp. 1 (1)
Hemiptera	
Aphididae	Unknown sp. 1 (1)
Cicadellidae	Unknown sp. 1 (1)
Lygaeidae	*Neopamera* sp. (1)
Lygaeidae	Unknown sp. 1 (1)
Lygaeidae	Unknown sp. 2 (1)
Lygaeidae	Unknown sp. 3 (2)

Table 2 continued

Order: Family	Species (*N*)
Miridae	Unknown sp. 1 (1)
Miridae	Unknown sp. 2 (1)
Pentatomidae	Unknown sp. 1 (1)
Reduviidae	Unknown sp. 1 (1)
Reduviidae	Unknown sp. 2 (8)
Reduviidae	Unknown sp. 3 (6)
Reduviidae	Unknown sp. 4 (4)
Reduviidae	Unknown sp. 5 (5)
Reduviidae	Unknown sp. 6 (1)
Reduviidae	Unknown sp. 7 (1)
Reduviidae	Unknown sp. 8 (5)
Reduviidae	Unknown sp. 9 (1)
Tingidae	*Amblystira machalana* (16)
Tingidae	Unknown sp. 1 (4)
Tingidae	Unknown sp. 2 (1)
Tingidae	Unknown sp. 3 (1)
Hemiptera	Unknown sp. 1 (1)
Hemiptera	Unknown sp. 2 (1)
Hemiptera	Unknown sp. 3 (1)
Hemiptera	Unknown sp. 4 (1)
Coleoptera	
Cantharidae	Unknown sp. 1 (1)
Carabidae: Cicindelinae	Unknown sp. 1 (1)
Cerambycidae	Unknown sp. 1 (1)
Chrysomelidae	*Colaspis* sp. (1)
Chrysomelidae	*Disonycha* sp. (1)
Chrysomelidae	Unknown sp. 1 (1)
Chrysomelidae	Unknown sp. 2 (1)
Chrysomelidae	Unknown sp. 3 (1)
Chrysomelidae	Unknown sp. 4 (1)
Coccinellidae	*Cycloneda sanguinea* (5)
Coccinellidae	*Delphastus* sp. (22)
Coccinellidae	*Harmonia axyridis* (40)
Coccinellidae	Unknown sp. 1 (1)
Coccinellidae	Unknown sp. 2 (1)
Coccinellidae	Unknown sp. 3 (1)
Lycidae	Unknown sp. 1 (4)
Lycidae	Unknown sp. 2 (1)
Nitidulidae	Unknown sp. 1 (1)
Staphylinidae	Unknown sp. 1 (1)
Coleoptera	Unknown sp. 1 (1)
Coleoptera	Unknown sp. 2 (1)
Coleoptera	Unknown sp. 3 (1)
Coleoptera	Unknown sp. 4 (1)
Neuroptera	
Chrysopidae	*Ceraeochrysa* sp. 1 (15)
Chrysopidae	*Ceraeochrysa* sp. 2 (6)
Chrysopidae	*Leucochrysa* sp. 1 (5)

Table 2 continued

Order: Family	Species (*N*)
Chrysopidae	Unknown sp. 1 (2)
Chrysopidae	Unknown sp. 2 (1)
Chrysopidae	Unknown sp. 3 (1)
Lepidoptera	
Lepidoptera	Unknown sp. 1 (1)
Lepidoptera	Unknown sp. 2 (1)
Diptera	
Culicidae	Unknown sp. 1 (1)
Dolichopodidae	*Condylostylus* sp. 1 (41)
Dolichopodidae	*Condylostylus* sp. 2 (6)
Dolichopodidae	Unknown sp. 1 (1)
Empididae	*Drapetis* sp. 1 (169)
Empididae	*Elaphropeza* sp. 1 (165)
Otitidae	Unknown sp. 1 (1)
Syrphidae	Unknown sp. 1 (5)
Syrphidae	Unknown sp. 2 (1)
Tephritidae	*Anastrepha* sp. 1 (1)
Tipulidae	Unknown sp. 1 (1)
Tipulidae	Unknown sp. 2 (1)
Diptera	Unknown sp. 1 (1)
Diptera	Unknown sp. 2 (2)
Diptera	Unknown sp. 3 (1)
Diptera	Unknown sp. 4 (1)
Diptera	Unknown sp. 5 (1)
Diptera	Unknown sp. 6 (1)
Diptera	Unknown sp. 7 (1)
Diptera	Unknown sp. 8 (1)
Diptera	Unknown sp. 9 (1)
Diptera	Unknown sp. 10 (1)
Hymenoptera	
Braconidae	Unknown sp. 1 (1)
Formicidae	Unknown sp. 1 (1)
Formicidae	Unknown sp. 2 (1)
Formicidae	Unknown sp. 3 (1)
Formicidae	Unknown sp. 4 (1)
Formicidae	Unknown sp. 5 (1)
Formicidae	Unknown sp. 6 (1)
Formicidae	Unknown sp. 7 (1)
Vespidae	Unknown sp. 1 (1)

Wings or legs were removed and washed in 10 % bleach from individual specimens of each taxon prior to DNA extraction. All predators were adults

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