Predation by *Ostenia robusta* on *Costelytra zealandica* pupae

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Abstract Soil sampling in a cereal crop near Southbridge, Canterbury, revealed dipteran larvae attacking *Costelytra zealandica* (White) pupae. Approximately 50% of the pupae had larvae associated with them. DNA sequencing analysis of larval specimens indicated they most likely belonged to the family Dolichopodidae. Larval specimens were reared through to adults and were identified as *Ostenia robusta* (Hutton) (Diptera: Dolichopodidae). This is the first record of the association of *O. robusta* larvae with C. *zealandica* pupae. A general description of the adult and larva of this species is provided, along with DNA sequencing data and observations on its association with *C. zealandica* pupae. The potential role of *O. robusta* in regulating populations of C. *zealandica* is briefly considered.

Keywords *Costelytra zealandica*, *Ostenia robusta*, Dolichopodidae, grass grub, predator.

INTRODUCTION

Costelytra zealandica (White) (Coleoptera: Scarabaeidae), commonly known as grass grub, is a widespread native pest of agricultural and horticultural crops in New Zealand, and causes significant impacts on the persistence and productivity of improved pastures (Zydenbos et al. 2011). Although chemical control methods for this pest have dominated in the past, the investigation and development of biological control methods has been the focus in recent decades (e.g. Jackson et al. 2000). *Costelytra zealandica* is attacked by a range of invertebrate predators and parasitoids, and pathogenic micro-organisms (Cameron & Wigley 1989). A number of vertebrate predators also prey opportunistically on *C. zealandica* including various bird species and hedgehog (East et al. 1981). Although many of these enemies occur naturally in the wider environment, few are able to be reliably manipulated to exert consistent levels of biological control on *C. zealandica* with the exception of some bacteria, e.g. *Serratia* spp following the development of commercially useable formulations (Johnson et al. 2001). It is

widely considered that a suite of natural enemies acting at different times on different stages of the life cycle may contribute to population regulation of a pest species and, on occasions, suppress them sufficiently to exert economic control.

This study reports the discovery of a new dipteran larval predator of *C. zealandica* pupae based on morphological and DNA barcode molecular data (Hebert et al. 2003). Of note for the latter, while cytochrome oxidase I (COI) DNA sequence is a good marker for differentiating species in most cases, there is also a significant volume of equivalent data for insects in the Barcode of Life database (BOLD) (http://www.boldsystems.org) and Genbank (http://www.ncbi.nlm.nih.gov/) that is useful for comparison to identify unknown specimens (Armstrong 2010).

MATERIALS AND METHODS

Field sampling and observations

Soil sampling (18 × 18 cm, 25 cm deep) to assess pupal populations of *C. zealandica* was carried out during November 2012 in a winter wheat (cv. 'Oakley') crop at Southbridge, Canterbury (43°49.061S; 172°14.445E). The soil type was a Templeton moderately deep silt loam with an available water holding capacity of 116 mm at 0–100 cm. The crop was direct-drilled in April 2012, treated with a range of herbicides, fungicides and insecticides typical for the region and was harvested in February 2013. A predominantly white clover and wheat crop rotation had been practiced in the paddock for the past 10 years. Pupae from soil samples were collected with a small amount of moist soil, placed individually in cells in a multi-cell tray and held at 4°C until examination in the laboratory.

DNA sequencing

Three live late instar larvae found associated with *C. zealandica* pupae were preserved by immersing in boiling water for 60 s before placing in 99% EtOH. A ~1 mm^3 piece of clear tissue (not containing gut contents) was used for PrepGem (ZyGEM, NZ) DNA extraction according to the manufacturer's instructions, but with incubation time at 75°C increased to 60 min. PCR of the COI barcode region was carried out in 20 µl GoTaq® Polymerase reaction mix (Promega, USA), with 250 nM of each 'universal' primer (Fol A) and (Fol B) plus thermal profile as in Folmer et al. (1994). PCR products were assessed by gel electrophoresis (1% agarose, 0.5× TBE buffer, 500 mA and 80 V, in-gel SYBR® Safe stain). PCR products were bidirectionaly sequenced using the PCR primers and ABI Big Dye (ABI, USA) technology on an ABI PRISM 3130*xl* Genetic Analyzer (ABI, USA). Sequences were edited with Geneious v6.0.3 and submitted to both BOLD and NCBI's BLAST (Basic Local Alignment Search Tool) for species identification.

Identification

Eight mature larvae (10-12 mm) extracted from *C. zealandica* pupae were reared in 50 mm diameter Petri dishes on lightly moistened filter paper and held at room temperature in the dark. Two adults emerged (1 male, 1 female) after ca 3 months at ca 20°C. Specimens were identified using Bickel's (1991) key to genera of Dolichopodidae known from New Zealand. Specimens were subsequently compared with identified material in the Lincoln University Entomology Research Museum. This identification was confirmed by dolichopodid specialist, Renato Capellari, University of São Paulo, Brazil, based on his examination of a photograph of an adult female. Specimen records were collated from the following collections: Auckland Museum, Canterbury Museum, Lincoln University Entomology Research Museum, Museum of New Zealand Te Papa Tongarewa and the New Zealand Arthropod Collection, Landcare Research.

RESULTS

Field sampling and observations

Soil sampling during November within the experimental area in the wheat crop indicated a *C. zealandica* pupal density of ca 100/m^2 with dipteran larvae being associated with 47% of pupae collected (n=70). Inspection in the laboratory showed larvae ranged in length from 5–12 mm. Larvae appeared to have penetrated the body wall of pupae typically with the posterior end of the

Figure 1 *Ostenia robusta* mature larva (body length 11 mm) feeding on *Costelytra zealandica* pupa.

Figure 2
Ostenia robusta adult female (body length 8 mm).

larval abdomen remaining exposed (Figure 1). One dipteran larva was found with each pupa.

DNA sequencing

DNA barcode sequences were obtained for only two of three samples. These were identical, indicating they were likely the same species. No matches were found in BOLD. With NCBI, the closest match at 87% similarity was to three dolichopodid species (Table 1) indicating that the species here was not represented in the database, and possibly neither the genus nor subfamily was represented. Several other dipteran species, including other dolichopodids, were <87% similar.

Identification

The adult specimens reared from larvae were identified as *Ostenia robusta* (Hutton). *Ostenia robusta* is a large dolichopodid species (body length of 6.5–8 mm) with a relatively stout body form (Figure 2). The species has long legs, as typical for the family, which are around 1.2 times the body length when fully extended. The thorax is dark grey-brown and non-metallic in colouration and is covered in long stout, semi-erect bristles. The wings are brown, with the colouration paler at the base, and have a margin of short bristles along the leading edge. The abdomen is glossy and is densely covered with short hairs. Colouration in female specimens is a weak metallic blue-green, whereas in males it is black.

Table 1 Top matches of the DNA barcode sequences against those in Genbank.

Species match of 87% similarity	Specimen reference (BOLD)	Genbank reference
Dolichopodidae sp.	BOLD:AAM6787 voucher BIOUG:TDWG-0816	gb|HQ979127.1|
Asyndetus sp. (Dolichopodidae; Diaphorinae)	SNK-2009 voucher RMBR:103416	gb|FJ808368.1|
Teuchophorus ornatulus (Dolichopodidae; Sympycninae)	RMBR:103493	gb|FJ808415.1|

There are no published descriptions of larvae of this species. The mature larva is maggot-like in form, cylindrical, about 11 mm in length and pale yellow (Figure 1). The head is weakly tapered and the darkened mouthparts are visible. The posterior end is broadly rounded but without any distinct lobes or protrusions. The larval body lacks any readily distinguishable features, making it difficult to identify from the larvae of other fly species. *Ostenia robusta* fly larvae were associated with the adult fly through rearing from field-collected specimens.

DISCUSSION

The family Dolichopodidae, or long-legged flies, is a large group containing around 7,000 species worldwide (Marshall 2012) and 132 species in New Zealand (Bickel 1991). Flies in this family are readily recognised by their slender body form, long legs, often blue-green metallic colouration and long, hair-like arista (distal part of antennae) (Bickel 1991). *Ostenia* is a monotypic genus (i.e. containing one species, *O. robusta*), which is endemic to New Zealand. The species is poorly represented in New Zealand collections, with only 36 identified specimens held. *Ostenia robusta* is present in both the North and South Island. North Island records are from the East Cape (Hicks Bay, Pohutu) and Hawke's Bay (Hastings) regions. South Island records are primarily from Christchurch and Banks Peninsula (Governors Bay, Mount Cavendish and Okuti Valley), with additional records from Marlborough (Blenheim), Kaikoura (Oaro), North Canterbury (Hanmer, Jacks Pass, Mount Grey), South Canterbury (Temuka), the Mackenzie Basin (Grampian Range) and Westland (Croesus Track in the Paparoa Range).

Nothing is known of the biology and ecology of *O. robusta.* Most dolichopodid species inhabit semi-aquatic and terrestrial habitats preferring humid and moist conditions (Pollet & Brooks 2008). The majority of species in the family are predatory, both as adults and larvae (Marshall 2012). Adults typically prey on soft-bodied insects and other small invertebrates (Urlich 2004), while larvae are almost all predators of invertebrates in soil and mud, in plant litter, under bark, in crevices or holes in trees (Dyte 1959). Bickel (1991) noted the often greasy appearance of *O. robusta* adults and commented that in other families this indicates rich larval nutrition, sometimes as parasitoids. Larvae of *O. robusta* had not previously been recorded and the observation of these larvae preying upon *C. zealandica* is novel. Quite remarkable is the occurrence of *O. robusta* larvae 15-20 cm below the soil surface where *C. zealandica* pupae are commonly found. Among a broad range of questions about the biology and ecology of this species, are specific questions about how *O. robusta* larvae located *C. zealandica* pupae, and why the particular site described in this study was apparently favourable to *O. robusta* resulting in nearly 50% of the sampled *C. zealandica* pupae being attacked. It is perhaps a little unusual that this predator has not previously been detected given the intensive research on *C. zealandica* in the past. This may be due to the focus of research typically being on *C. zealandica* larvae, the difficulty in identifying dipteran larvae if they were discovered, or the unique combination of conditions prevailing at this study site that has allowed *O. robusta* to exploit *C. zealandica* pupae as a prey source.

Other Diptera have been recorded as natural enemies of *C. zealandica* including *Proscissio cana* Hutton (Diptera: Tachinidae) a parasitoid of third instar larvae (Thomas 1963), and *Ectenopsis lutulenta* (Hutton) (Diptera: Tabanidae) a predator of third instar larvae (East 1972). East et al. (1981) concluded that both species were likely to be of limited value in the biological control of grass grub because they were not sufficiently abundant in open pasture land. A similar tentative conclusion could be drawn for *O. robusta,* however, further research is required into aspects of its biology and ecology to evaluate its impact on *C. zealandica* abundance.

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