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

R.J. Chynoweth¹, J.W.M. Marris², K.F. Armstrong³, A. Chomic³, J. Linton¹ and R.B. Chapman⁴

¹Foundation for Arable Research, PO Box 23133, Templeton, Christchurch, New Zealand

²Ecology Department, Lincoln University, PO Box 85084, Lincoln University, 7647, New Zealand

³Bio-Protection Research Centre, Lincoln University, PO Box 85084, Lincoln University, 7647, New Zealand

⁴Insect Science Solutions, 830 Selwyn Road, RD 8, Christchurch, 7678, New Zealand
Corresponding author: insectsciencenz@gmail.com

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 mm³ 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 bidirectionally sequenced using the PCR primers and ABI Big Dye (ABI, USA) technology on an ABI PRISM 3130xl 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/m² 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.

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.

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

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
<i>Asyndetus</i> sp. (Dolichopodidae; Diaphorinae)	SNK-2009 voucher RMBR:103416	gb FJ808368.1
<i>Teuchophorus ornatulus</i> (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 (Ulrich 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.

ACKNOWLEDGEMENTS

We gratefully acknowledge the funding provided by the Sustainable Farming Fund (12-013), and the cooperation of Phoenix Park Farm,

Southbridge, for access to the property for experimental work. We also thank Renato Capellari, University of São Paulo, Brazil, and Dan Bickel Australian Museum, Sydney, Australia, for taxonomic advice. We are grateful to the following curators for providing details of *Ostenia* specimen records: John Early, Auckland Museum; Grace Hall, New Zealand Arthropod Collection, Landcare Research; Phil Sirvid, Museum of New Zealand Te Papa Tongarewa; and Cor Vink, Canterbury Museum.

REFERENCES

- Armstrong K 2010. DNA barcoding for plant biosecurity: a new module in New Zealand's diagnostic toolbox. EPPO Bulletin 40(1): 91-100.
- Bickel DJ 1992. Sciapodinae, Medeterinae (Insecta: Diptera) with a generic review of the Dolichopodidae. Fauna of New Zealand 23. 74 p.
- Cameron PJ, Wigley PJ 1989. *Costelytra zealandica* (White), grass grub (Coleoptera: Scarabaeidae). In: Cameron PJ, Hill RL, Bain J, Thomas WP ed. A review of Biological Control of Invertebrate Pests and Weeds in New Zealand 1874-1987. CAB International, Wallingford, UK and DSIR Auckland, New Zealand. Pp. 9-16.
- Dyte CE 1959. Some interesting habitats of larval Dolichopodidae (Diptera). The Entomologist's Monthly Magazine 95:139-143.
- East R, King PD, Watson RN 1981. Population studies of grass grub (*Costelytra zealandica*) and black beetle (*Heteronychus arator*) (Coleoptera: Scarabaeidae). New Zealand Journal of Ecology 4: 56-64.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294-299.
- Hebert PDN, Cywinska A, Ball SL & deWaard JR 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B 270: 313-321.
- Jackson TA, Alves SB, Pereira R 2000. Success in biological control of soil-dwelling insects with pathogens and nematodes. In: Vincent C, Goettel MS, Lazarovits G ed. Biological Control: a global perspective. CAB Publishing, Wallingford, UK. Pp. 160-168.
- Johnson VW, Pearson JF, Jackson TA 2001. Formulation of *Serratia entomophila* for biological control of grass grub. New Zealand Plant Protection 54: 125-127.
- Marshall SA 2012. Flies: the natural history and diversity of Diptera. Firefly Books, Canada. 616 p.
- Pollet MAA, Brooks SE 2008. Long-legged flies (Diptera: Dolichopodidae). In: Capinera JL ed. Encyclopaedia of Entomology. 2nd Edition. Springer, The Netherlands. Pp. 2232-2241.
- Ulrich H 2004. Predation by adult Dolichopodidae (Diptera); a review of literature with an annotated prey-predator list. Studia Dipterologica 11: 369-403.
- Zydenbos SM, Barratt BIP, Bell NL, Ferguson CM, Gerard PJ, McNeill MR, Phillips CB, Townsend RJ, Jackson TA 2011. The impact of invertebrate pests on pasture persistence and their interrelationships with biotic and abiotic factors. Pasture Persistence – Grassland Research and Practice Series 15: 109-118.