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First record of a possible predatory collembolan species, *Dicyrtoma fusca* (Collembola: Dicyrtomidae), in New Zealand

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Abstract Specimens of a previously unrecorded collembolan species were found in a field margin of a commercial dairy farm near Christchurch, New Zealand. They were consistently observed apparently feeding on egg batches of the light brown apple moth *Epiphyas postvittana*, which were being used as bait to assess predation rate by potential biocontrol agents. The collembolan specimens were identified as the European species *Dicyrtoma fusca* based on published morphological descriptions of this species. DNA sequence data of the New Zealand specimens clustered with sequence data from GenBank of this species from Norway and England, confirming that *D. fusca* populations in New Zealand originated from Europe. A GenBank sequence had previously identified a collembolan species from Estonia as this species, but its position in the phylogeny indicates that it is a different species. Some morphological variations observed in arrangement of macrochaetae on the head were shown by sequence data to be intraspecific differences only.

Key words cryptic species, genome, morphology, systematic phylogeny.

INTRODUCTION

A catalogue of collembolan species recorded from New Zealand was published in 2012 (Greenslade 2012) and has been kept updated since then (Greenslade, 2015). As a result, 351 species belonging to 101 genera are now known from that country (Bellinger *et al.*, 2015) although it is certain many remain to be discovered and described. For instance, only four species of Dicyrtomidae have been recorded there, although the family worldwide contains nearly 100 described species. The New Zealand species comprise three in the genus *Dicyrtomina* Börner, 1903 (*D. minuta* (Linn.), *D. novazealandica* Salmon and *D. turbotti* Salmon) and one in *Calvatomina* Yosii (*C. superba* (Salmon)). All are currently endemic to the country except for *D. minuta*. There has been no study of the family in New Zealand for over 50 years.

Specimens of a previously unrecorded symphypleonan Collembola were recently collected near Christchurch, New Zealand. They were relatively abundant in weedy vegetation in a field margin between a commercial dairy farm and a railway line. In life, the animals were consistently observed on egg batches of the light brown apple moth *Epiphyas postvittana* Walker, apparently feeding on them (Fig. 3). The egg batches were being used in a project to assess the predatory activity of arthropods in the field margin.

The collembolan species is identified here by using the morphological description of the species provided in Fjellberg (2007) and confirmed by comparing COI sequences with those available on GenBank. A description of the New Zealand specimens is given below.

MATERIALS AND METHODS

The Christchurch region is located on the Canterbury Plains of New Zealand which were once covered in low shrubs, scrub and patchy forest. However, this plant cover was largely destroyed by natural and human-induced fires before Europeans arrived in the early 1800s. The settlers converted the land to agriculture. The invertebrate fauna of this agricultural land has so far received limited research (Brockerhoff *et al.* 2008) although the collembolan fauna was reported by Greenslade *et al.* (2013).

The specimens were collected in pitfall traps set in a field margin consisting of mainly tussocks of perennial grass (Cocksfoot, *Dactylis glomerata* L.), Bracken fern, *Pteridium* sp. (cf *P. esculentum* (G.Forst.) Cockayne 1786), European broom (*Cytisus scoparius* (L.) Link) and scattered Gorse (*Ulex europaeus* L.) (Fig. 1). They were found on six occasions in late winter and early spring 2015 (August and September).

The traps consisted of 350 mL plastic pots, each containing 100 mL of mono-propylene glycol (antifreeze) as a preservative, with a drop of detergent to break the surface tension (Fig. 2). A galvanised steel roof 180 mm × 180 mm with four wire legs was positioned above each pitfall trap to reduce rain and leaves

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Fig. 1. A field margin from where *Dicyrtoma fusca* specimens were collected for morphological examination and COI sequencing (November 2015).

Fig. 2. A pitfall trap surrounded by four baits of eggs of light brown apple moth placed on paper, some of which have been eaten, probably by slugs.

Fig. 3. *Dicyrtoma fusca* on LBAM baits at the collection site.

from entering the trap (Fig. 2). Each trap was baited with four LBAM (*E. postvittana*) egg batches (50–200 eggs per batch) on wax paper around its rim (Fig. 3). Eggs were obtained from Anne Barrington, Plant and Food Research, NZ.

The pitfall traps were placed in the centre of a 6.5 m wide field margin between a commercial dairy farm and a railway line. Seven traps were set 5 m apart in a straight line at the collection site and left for two nights. Specimens were also collected using an inverted leaf blower (Macleod *et al.* 1994) with a 0.025 m^2 suction surface area and with a removable cup for trapping specimens. Sampling consisted of continuous suction over *D. glomerata* tussocks for 1 min, four times at 50 cm intervals, near each pitfall trap.

Christchurch has a mean annual rainfall of 648 mm, a mean annual temperature of 12.1 °C (max 41.8 °C, minimum −7.1 °C), a mean of 70 frost days/year and a mean wind speed of 15 km/h (NIWA 2013). Rain in Canterbury comes predominantly from the south and east and is associated with cooler months of winter (NIWA, 2013). The Canterbury region is prone to drought and was declared a drought zone over the summers of 2015/2016 and 2014/2015. The climate is dictated by the Southern Alps, which lie perpendicular to the prevailing westerly air flow. These westerlies create a rain shadow in the east. The soil (soil depth < 45 cm to gravels) is a stony silt loam soil (Chertsey silt loam and Lismore silt loam), which has a low water holding capacity (<80 mm) (Hanson 2009).

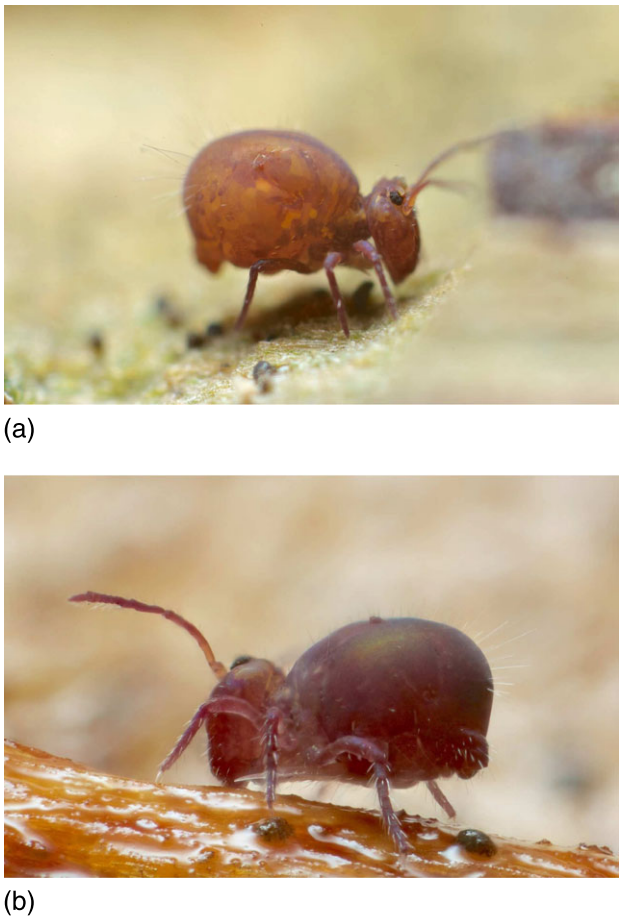
Specimen preparation

Seven of the 10 specimens in alcohol were removed and placed in Nesbitt's solution to clear overnight. Two were then mounted on separate slides in Berlese solution and dissected into head, large abdomen, legs, furca and small abdomen. The parts of each dissected individual were mounted on the same slide but under separate coverslips. The other five specimens were retained whole and mounted on another slide but under separate coverslips.

Molecular analysis

DNA sequencing of the COI barcoding gene was conducted on eight of the symphypleonan specimens to confirm species identification. Four of them had been morphologically identified prior to DNA analyses. Whole specimens were ground in a microcentrifuge tube using a sterile plastic pestle, and DNA extraction was then conducted using the Qiagen DNeasy Blood and Tissue extraction kit following the manufacturer's recommendations. DNA amplification was performed using the GoTaq® Green Master Mix (Promega) and the universal COI primers of Folmer *et al.* (1994) following the protocol described by Lefort *et al.* (2012). Each 10 µL PCR reaction contained 5 µL GoTaq® Green Master Mix, 0.4 µL of forward and reverse primers (10 µM), 0.5 µL of Bovine Serum Albumin, 1 µL of DNA template and 3 µL of nuclease-free water. DNA was sequenced in both directions at Massey Genome Service (Massey University, New Zealand). Sequences were compared to those available on Genbank and the Barcode Of Life Database (BOLD).

The following abbreviations are used: SAMA—South Australian Museum, Adelaide. Mc—macrochaeta; me—mesochaeta.



(a)

(b)

Fig. 4. Live *Dicyrtoma fusca* from: (a) Nelson, South Island; (b) Christchurch Botanic Garden, South Island. (© A. Murray).

RESULTS

Morphological identification

Dicyrtoma fusca (Lubbock, 1873)

Material examined

New Zealand, Burnham, Aylesbury Road, edge of dairy farm, pitfalls, 17.viii.2015, leg M. W. Shields. Coordinates 43° 32'12.47″S 172°16'43.15″E, 113 m elevation. Mounted 5 females, 2 males and a further 10 specimens in alcohol. All deposited in the SAMA.

Diagnosis

In alcohol, the colour and habitus of the specimens were identical with the figures and descriptions in Lubbock (1873), Stach (1957), Gisin (1960) and Fjellberg (2007) (Figs 4 a,b,c; Fig. 5) except that the legs and antennae were slightly less pigmented. The fine details of chaetotaxy were also identical except that the pattern and number of the frontal spines on the head varied from seven to nine on the New Zealand specimens. This variability had already been noted by Christiansen and Bellinger (1998) and was confirmed by A. Fjellberg (in. litt. 2016). The anterior strong spines on the thorax were the same as the 5+5 quoted by Fjellberg (2007) arranged as 1+1, 2+2 and 2+2 from anterior to posterior, with the most posterior pair in line with bothriothrix A. The dorsal setae on abdominal fused segments V/VI were slightly less strong than in figures by Christiansen and Bellinger (1998) and Gisin (1960). We consider these differences to be intraspecific variations and of no taxonomic importance. Stach (1957) described five colour varieties of the species, but again we consider these differences to be intraspecific and may be because of the temperature at which the animals developed and/or be caused by diet.

Description

A detailed taxonomic description of the New Zealand material is given in Appendix S1 (see Supporting Information).

Remarks

The specimens were identified as belonging to the family Dicyrtomidae because of the short antennal IV segment. The specimens cannot belong to *Ptenothrix* because bothriothrix D is absent. They cannot belong to *Calvatomina* as long spines

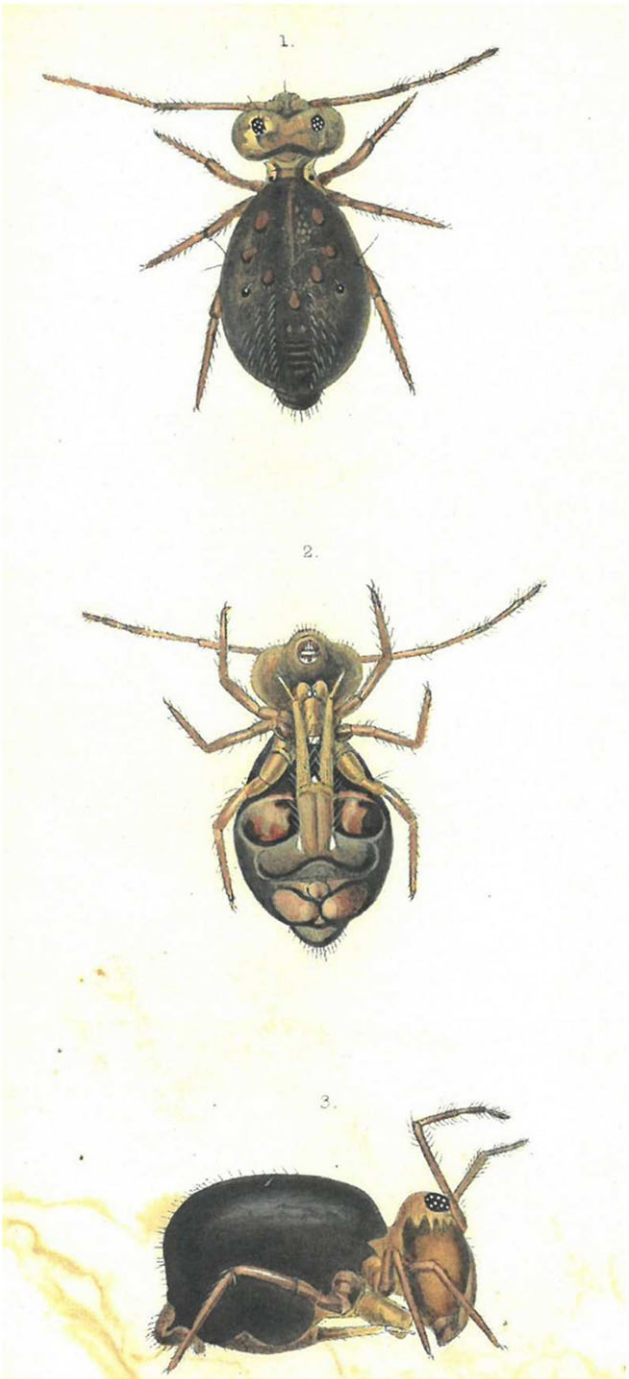


Fig. 5. Colour plate of *Dicyrtoma fusca* from Lubbock, 1873.

are present on the head and anterior abdomen. They cannot belong to *Dicyrtomina* because there is no tunica on the claw and the setae on antennal segment II are not longer than the width of the segment. On the basis of the serrated spines on the dens, the full set of bothriotricha (A, B, C) present but D absent and the numerous short spines on the posterior of the large abdomen, the specimens are assigned to the genus *Dicyrtoma* Bourlet, 1841/2.

No *Dicyrtoma* species have been recorded from New Zealand so far. On the basis of colour and the morphological characters noted above and in the Supplementary data 1, the New Zealand specimens are identified as *D. fusca*. This species has also recently (A. Murray, 2014) been photographed and identified in the Christchurch Botanic Gardens living in leaf litter under exotic trees (A. Murray pers. comm.) (Fig. 4) so the *D. fusca* record here is the first confirmed Southern Hemisphere record.

Biology

In Europe, *D. fusca* has been collected in leaf litter, in dead timber, on fungi and under stones in moist habitats in fields, forests and low heath up to 1500 m and has also been found in caves (Bretfeld 1999). The breeding biology has been studied by Agrell (1941), Hale (1965) and Mayer (1957). Adults have been collected mainly in summer and autumn in Europe.

In the current work, the gut contents of mounted specimens were varied; some fungal hyphae, conidia and chlamydospores, and one conidium of *Alternaria* sp. was observed, supposedly from decomposing plant and animal material (J. Simpson in litt.). Also, a claw that appeared to come from a conspecific was found. There was much brown, amorphous material and strings and groups of small spherical, pale objects with milky cuticle, not yet identified. Any LBAM eggs, which are <1 mm in diameter (Shields pers. obv.) consumed would be rapidly digested and not visible in gut contents. LBAM primers could be used to detect prey DNA in the gut of the collected specimens, but because of the wide variety of food items seen in the gut, it might be difficult to detect. The species seems to be relatively unspecific in food consumed but likely to show a preference for the most highly nutritious items.

Eggs of the codling moth (*Cydia pomonella*) are reported as comprising a mixture of phospholips and free fatty acids, the composition of which changes during development (Forte *et al.* 2002). The chorion or outer coating in the silk moth (*Bombyx mori* L., 1758) is proteinaceous, comprising mainly the amino acids such as glycine (nearly 40%), alanine, valine, leucine and tyrosine (Hamodrakas *et al.* 1982).

Molecular analysis

All sequences from the New Zealand specimens were similar (97–100% similar), and therefore considered to belong to the same species. The best match was a DNA sequence for *D. fusca* (97–98% identity), from the grounds of Roehampton College, near London, collected and identified by Dr Peter Shaw and also to a sequence from a specimen collected and identified from Norway by Dr A. Fjellberg. As the type locality was most probably in Kent (see Discussion below), the specimens from London are here assumed to represent most closely the Lubbock material. As the sequences from these three localities are so similar, they can all be considered to be the same species (Fig. 6).

A search for additional *D. fusca* sequences revealed a discrepancy in the existing databases, with a second group of sequences also identified as *D. fusca* but with 78–83% similarity to the first group (Fig. 6). Such a genetic distance is much larger than the 2% threshold commonly used to distinguish animal species, but is characteristic of interspecific distances between congeneric species of Collembola (Porco *et al.* 2013;

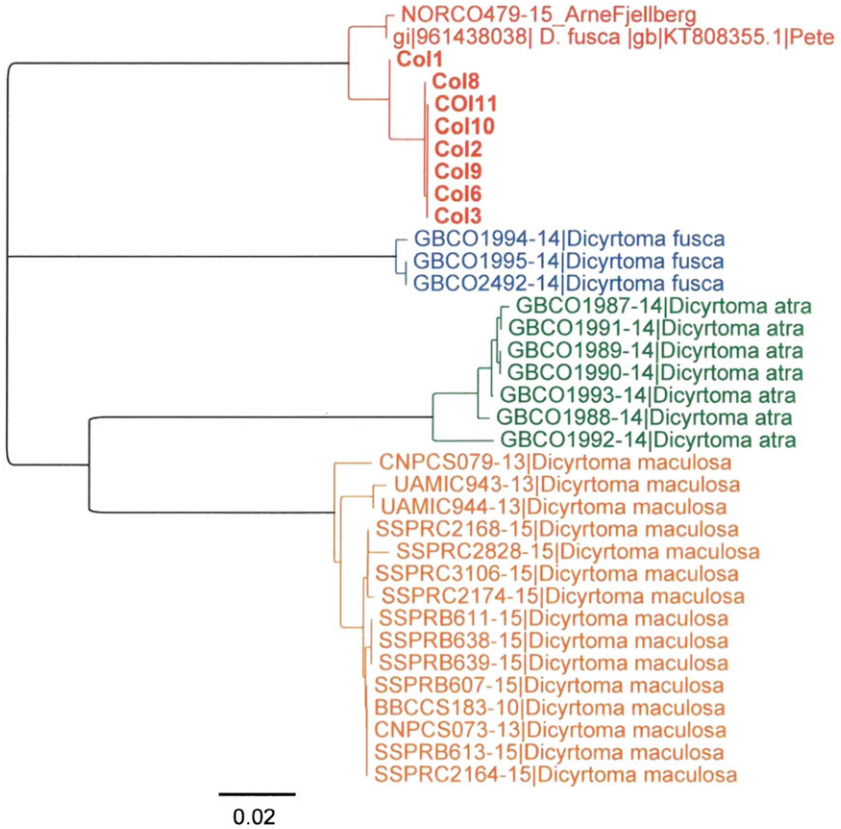


Fig. 6. Results of sequencing specimens of *Dicyrtoma fusca* from Christchurch and other specimens of Dicyrtomidae from Genbank and BOLD (Table 1). Neighbour-Joining tree based on the COI sequences (555 bp) for 34 individual Collembola from the *Dicyrtoma* genus. Eight sequences are from specimens collected as part of this study (specimen names in bold); other sequences were retrieved from Genbank and BOLD. The tree is drawn to scale, with horizontal branch lengths corresponding to percentage differences (see scale for 2%). Specimens linked by the same coloured lines correspond to individuals of the same species (based on a conservative 3% similarity threshold).

Table 1 Collection data of specimens barcoded and those from GenBank

Barcode reference	Name of taxon	Locality	Collector	Identifier
CO1,2,3,,6,8,9,10,11	*Dicyrtoma fusca*	Field margin, Canterbury, August 2015, South Island, New Zealand	Morgan Shields	Penelope Greenslade
NORC479-15	*Dicyrtoma fusca*	Norway	Arne Fjellberg	Arne Fjellberg
Gi,961438038,gb, KT808355.1	*Dicyrtoma fusca*	England, Digby Stuart College, Roehampton University, London, 4 January 2013 TQ21956-74654	Peter Shaw	Peter Shaw
GBCO1994m 1995 m 2492	'*Dicyrtoma fusca*'	Tähtvere,*Vaccinium myrtillus* type, *Pinus sylvestris* forest; Kardla, *Oxalis* type, *Picea abies* forest, Estonia	Sten Anslan	Edite Jucevisa

Deharveng *et al.* 2015). Therefore, the second group of sequences clearly belongs to a different species. The corresponding specimens were collected from Estonia but have not yet been examined in detail morphologically.

As *D. fusca* has been identified from the Christchurch Botanic Gardens, New Zealand, it is likely that it was introduced via imported plants for the garden from the Northern Hemisphere, perhaps in the 19th or early 20th Century. The species has not been found in Australia despite searches in several southern botanic gardens. The published records of the species are from throughout Northern Europe, Canada (Nova Scotia) and Japan (Salmon, 1964).

DISCUSSION

The taxonomic history of *D. fusca* is complex, mainly because it was not possible, from the very early literature in which the name was first used, to correctly identify the species referred to, as these descriptions were too brief. Ellis and Bellinger (1973) fixed the published description of the species to be that of Lubbock (1873) (later Lord Avebury) who then became the authority of the species because he was the first to provide an adequately detailed morphological description (Fig. 5). They formalised the species as the type species of *Dicyrtoma* Bourlet, 1841/2 by application to the Commission for Zoological Nomenclature.

Lubbock's (1873) description includes three hand coloured paintings of the species (Fig. 5). As was common at the time, he gave no locality for the specimens he examined but, from a perusal of his records (1873), and his autobiography, it is likely to be in Downe, Kent, where he lived most of his life in a house called High Elms. Charles Darwin's house, Down House, was an adjacent property and, as a boy from aged eight, John Lubbock frequented the Darwin garden learning from the great man himself, who instructed him in natural history during their walks the woods along his 'Sand Walk' and in dissections. From the time of Darwin's settlement in Downe House in 1842, he gave the boy encouragement and direction, beginning a friendship that continued for 40 years (Pumphrey 1958; Somkin, 1962). Later, Lubbock certainly made collections there (P. N. Lawrence, pers. comm.). It is probable, therefore, that the original description of *D. fusca* was made on specimens collected from Down House grounds.

Although Lubbock was 26 years younger than Darwin, their friendship continued throughout Darwin's life. Lubbock has another taxonomic connection to New Zealand as he was the first to describe a species of Collembola from the country, the charismatic *Holacanthella spinosa* (Lubbock, 1899), formerly *Anoura spinosa*.

The new record of *D. fusca* for New Zealand, which is a species widely distributed in the Northern Hemisphere, is of significance because, first, it has not been found in the Southern Hemisphere before, and second because of its possible unusual feeding habits, being apparently predatory here on moth eggs. This has yet to be confirmed in culture and with chemical gut analyses (M. Shields in prep). What is also of significance is that the sequence data have also been able to confirm that the observed morphological variability in the number and arrangement of spine-like setae on the front of the head, which might be considered diagnostic at species level, are only intraspecific variations.

The genetic confirmation of the species as identical, at over 95%, with European specimens is also significant as three other so-called cosmopolitan species (*Ceratophysella denticulata* (Bagnall), *Parisotoma notabilis* (Schäffer), *Heteromurus major* (*M*oniez)), have been shown to comprise a number of genetically different lineages (Porco *et al.* 2012a,b). This is the first time that an exotic, introduced collembolan species in New Zealand is confirmed as genetically identical to specimens from Europe, and this result is strengthened by the fact that the European specimens came from two distant localities.

Finally, the ecosystem services, if any, that *D. fusca* provides to agriculture remain unknown. This is important in the context of extensive population declines in native and endemic invertebrate groups, especially from landscapes modified by human activities, and their replacement with introduced exotic species (Greenslade *et al.* 2013). The result is a homogenisation of invertebrate faunas in New Zealand, as well as in Australia (Greenslade 2007) with those in Europe. The ecological functions of this introduced fauna may not be well suited in all respects to New Zealand's soils and climates.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Appendix S1 Taxonomic description of *Dicyrtoma fusca* (Lubbock, 1873) from New Zealand.