METHODS TO DISTINGUISH BETWEEN THE *MICROCTONUS AETHIOPOIDES* STRAINS THAT PARASITISE *SITONA LEPIDUS* AND *SITONA DISCOIDEUS*

C.B. PHILLIPS[1], I.I. ILINE, C.J. VINK, L.M. WINDER and M.R. MCNEILL

AgResearch, PO Box 60, Gerald Street, Lincoln, New Zealand

Corresponding author: craig.phillips@agresearch.co.nz

ABSTRACT

Two strains of *Microctonus aethiopoides* with differing host preferences have been released in New Zealand. The first was released in 1982 to suppress *Sitona discoideus,* a pest of lucerne, and the second was released in 2006 for biological control of *Sitona lepidus*. This paper describes three genetic approaches (esterase isozymes, aldehyde oxidase allozymes and nucleotide sequences in a 676 bp region of the mitochondrial cytochrome oxidase subunit one gene) for distinguishing between the *M. aethiopoides* strains. A range of research applications for these methods is discussed.
Keywords: isozyme, esterase, aldehyde oxidase, cytochrome oxidase subunit one, intraspecific variation, biotype, clover root weevil, lucerne weevil, biological control.

INTRODUCTION

Two strains of the solitary endoparasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) with differing host preferences have been introduced to New Zealand in separate biological control programmes. The first introduction was made in 1982-83 for management of the lucerne pest, *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae). It involved parasitoids obtained from Australia (Stufkens et al. 1987) that had originally been sourced from Morocco and Greece (Cullen & Hopkins 1982; Aeschlimann 1983b; Aeschlimann 1995). The Moroccan parasitoids readily established in Australia, but establishment of the Greek parasitoids was not confirmed (Aeschlimann 1995). Thus, the *M. aethiopoides* introduced to New Zealand in 1982-83 probably originated from Morocco, although material originating from Greece might also have been present (Aeschlimann 1995). The second introduction of *M. aethiopoides* to New Zealand was made in 2006 for suppression of the clover pest, *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae), and involved parasitoids sourced from Ireland (McNeill et al. 2006).

The second introduction of *M. aethiopoides* was made because, following the discovery of *S. lepidus* in New Zealand in 1996 (Barratt et al. 1996), it was found that the *M. aethiopoides* established to control *S. discoideus* was not an effective parasitoid of *S. lepidus* (Barratt et al. 1997a). Laboratory experiments indicated it would oviposit in *S. lepidus*, but its eggs appeared to succumb to the host's immune system (McNeill et al. 2000). Subsequent searches in the Northern Hemisphere for parasitoids exhibiting greater efficacy against *S. lepidus* identified at least six species (Phillips 2002), including a different strain of *M. aethiopoides* (Goldson et al. 2001; Phillips et al. 2002). The *S. lepidus*-parasitising strain of *M. aethiopoides* is widespread in Europe (Goldson et al. 2004), where it generally reproduces by arrhenotoky (haploid, unfertilised eggs develop into males, diploid fertilised eggs into females), as does the *S. discoideus*-parasitising strain in New Zealand. However, an exception occurs in Ireland where *M. aethiopoides* appears to reproduce only by thelytoky (unfertilised eggs develop into diploid females)

(McNeill et al. 2006). This offered the opportunity to introduce a thelytokous, *S. lepidus*-parasitising strain of *M. aethiopoides* to New Zealand that was less likely to interbreed with the *S. discoideus*-parasitising strain already established here, thus reducing any risk of disruption to biological control of *S. discoideus*.

Previous observations of variations in host range, phenology and morphology of *M. aethiopoides* (Loan 1960; Loan & Holdaway 1961; Adler & Kim 1985; Sundaralingam 1986) supported the hypothesis that *M. aethiopoides* comprises several genetically distinct biotypes (Aeschlimann 1983a). However, direct evidence of genetic differentiation between *M. aethiopoides* populations has only been recently obtained. Phillips et al. (2002) amplified inter simple sequence repeat (ISSR) regions of *M. aethiopoides* DNA and found clear variation between French parasitoids reared from *S. lepidus* and New Zealand parasitoids reared from *S. discoideus*. Iline & Phillips (2003) made an assessment of variation in nine enzyme systems as well as general proteins for *M. aethiopoides* obtained from New Zealand, Australia and nine European countries. Variation was observed at two esterase loci and it is notable that Irish specimens reared from *S. lepidus* were always homozygous for one allele at the esterase 2 locus, while New Zealand specimens reared from *S. discoideus* were always homozygous for another allele at the same locus (Iline & Phillips 2003). Vink et al. (2003) assessed nucleotide sequence variation in four gene regions for *M. aethiopoides* from New Zealand and thirteen other countries and found consistent variation between New Zealand and Irish *M. aethiopoides* (reared from *S. discoideus* and *S. lepidus*, respectively) in nucleotide sequences amplified from the cytochrome oxidase subunit 1 (COI) and 16S mitochondrial genes. Recently obtained nucleotide sequence data have shown that *M. aethiopoides* genetic variation is much more closely correlated with host species than geographic origin (C.B. Phillips, unpubl. data).

In this contribution, the *M. aethiopoides* introduced to New Zealand from Australia is referred to as the '*discoideus* strain' and that introduced from Ireland as the '*lepidus* strain'. The results of new DNA and enzyme analyses of variation between the two *M. aethiopoides* strains are presented, the methods available for distinguishing between them are summarised and possible research applications are discussed.

MATERIALS AND METHODS

Isozymes

All four of the thelytokous *M. aethiopoides* lineages originating from Ireland that were released in New Zealand for biological control of *S. lepidus* were studied using vertical polyacrylamide gel electrophoresis. This method was also used for 40 specimens of the *discoideus* strain reared from various host species collected in New Zealand (Table 1), which are additional to those examined by Iline & Phillips (2003). Frozen whole insects were ground in 50-60 µl of 0.1 M Tris-HCl, pH 7.2 extraction buffer containing 0.1% dithiotreitol, 0.1% 2-mercaptoethanol and 12-15% sucrose. Samples were then centrifuged at 14,000 rpm for 5 min. The 7-9% acrylamide gels were loaded with samples (5.5-7.0 µl), run at 80 volts for 40 min at 4-6°C, then for about 3 h at 250-280 volts at 4-6°C. The Ornstein-Davis discontinuous buffer system was used for electrophoresis (Rothe 1994). Esterases were assayed as described by Iline & Phillips (2003) except the gels were pre-incubated for 20-30 min in cold phosphate buffer to enhance esterase activity. Aldehyde oxidase (AOX) was stained following the method of Manchenko (1994) except 0.1M Tris-HCl buffer (pH 8.0) and MTT were used instead of phosphate buffer and NBT, respectively. The most anodally migrating isozyme was designated as A, the next as B, etc.

Mitochondrial cytochrome oxidase subunit one (COI) nucleotide sequences

Nucleotide sequences were obtained from the *M. aethiopoides* COI gene of all lineages of the *lepidus* strain released in New Zealand, as well as additional specimens of the *discoideus* strain (Table 2). DNA was extracted from the legs of each specimen using DNeasyTM Tissue Kits (QIAGEN). The primers LCO1490 (Folmer et al. 1994) and mtD9-aethio (Vink et al. 2003) were used with following thermocycler temperature

profile: 94°C (3 min), then 35 cycles (94°C (30 s), 45°C (30 s), 72°C (1 min)), and a final 72°C (5 min). Purified PCR fragments were sequenced in both directions by the Allan Wilson Centre Genome Service (Massey University).

RESULTS

Esterases

Variation at the EST2 locus was examined in 17 specimens of the *discoideus* strain of *M. aethiopoides*, and 23 specimens of the *lepidus* strain (Table 1). Combining the results of the current study with those of Iline & Phillips (2003) gives totals of 21 specimens of the *discoideus* strain and 28 specimens of the *lepidus* strain (Table 1). As suggested by Iline & Phillips (2003), specimens of the *lepidus* strain appear to be fixed as homozygotes (EST2-BB) at this locus. However, unlike Iline & Phillips (2003), the current study detected the B allele in seven specimens of the *discoideus* strain, all collected at Lincoln, with three of these specimens exhibiting the same EST2-BB genotype as all specimens of the *lepidus* strain. In New Zealand, the *discoideus* strain can therefore be readily identified by the occurrence of either EST2-AA homozygotes or EST2-AB heterozygotes, since these genotypes do not occur in the *lepidus* strain. However, specimens exhibiting the EST2-BB genotype could belong to either strain.

Aldehyde oxidase

Variation at the aldehyde oxidase (AOX) locus of *M. aethiopoides* was studied in 13 specimens of the *discoideus* strain and 15 specimens of the *lepidus* strain (Table 1). Two isozymes, B and C, became visible in all specimens of the *discoideus* strain after 20-30 min of incubation. However, in the *lepidus* strain only the C isozyme was stained after 20-30 min of incubation, while the A isozyme became faintly stained only after 1.5-2 h of incubation.

TABLE 1: Esterase (EST2) and aldehyde oxidase (AOX) isozymes for Irish and Moroccan strains of *Microctonus aethiopoides* in New Zealand.

Strain	Location	Host species	n	EST2	AOX
discoideus	Christchurch (MC), NZ	*Irenimus aequalis*	2	AA[1]	
discoideus	Lincoln (MC), NZ	*Listronotus bonariensis*	1	AA[1]	
discoideus	Tarras (CO), NZ	*Sitona discoideus*	1	AA[1]	
discoideus	Lincoln (MC), NZ	*Sitona discoideus*	8	AA	
discoideus	Ruakura (WO), NZ	*Sitona discoideus*	2	AA	
discoideus	Lincoln (MC), NZ	*Sitona discoideus*	4	AB	
discoideus	Lincoln (MC), NZ	*Sitona discoideus*	2	BB	
discoideus	Lincoln (MC), NZ	*Sitona discoideus*	1	BB	
lepidus	Athenry, Ireland	*Sitona lepidus*	1	BB[1]	
lepidus	Athenry, Ireland	*Sitona lepidus*	4	BB[1]	
lepidus	Athenry, Ireland	*Sitona lepidus*	10	BB	
lepidus	Crossnacreevy, Ireland	*Sitona lepidus*	7	BB	
lepidus	Oakpark, Ireland	*Sitona lepidus*	5	BB	
lepidus	Solohead, Ireland	*Sitona lepidus*	1	BB	
discoideus	Lincoln (MC), NZ	*Sitona discoideus*	13		B+C
lepidus	Athenry, Ireland	*Sitona lepidus*	8		A+C
lepidus	Crossnacreevy, Ireland	*Sitona lepidus*	5		A+C
lepidus	Oakpark, Ireland	*Sitona lepidus*	1		A+C
lepidus	Solohead, Ireland	*Sitona lepidus*	1		A+C

[1]Data from Iline & Phillips (2003).

Mitochondrial cytochrome oxidase I nucleotide sequence analyses

Segments of COI, each 676 nucleotides long, were PCR amplified and sequenced from 16 *M. aethiopoides* specimens. These specimens had been reared from various host species collected at different New Zealand localities, and also included representatives of the four female lineages introduced from Ireland to New Zealand (Table 2). Sequences from the nine New Zealand specimens all matched COI haplotypes 6 and 7 that have been described by Vink et al. (2003). Of the specimens originating from Ireland, six matched COI haplotype 3 (Vink et al. 2003) and one was a previously unrecorded haplotype, which differed by only one nucleotide from COI haplotype 3.

TABLE 2: Cytochrome oxidase I (COI) haplotypes of Irish and Moroccan *Microctonus aethiopoides* in New Zealand.

Host	Location	n	COI haplotype	GenBank accession no.
Sitona lepidus	Athenry, Ireland	1	3[1]	AY039689
Sitona lepidus	Athenry, Ireland	1	3[1]	AY039689
Sitona lepidus	Athenry, Ireland	3	3[1]	AY039689
Sitona lepidus	Athenry, Ireland	2	3	DQ525079
Sitona lepidus	Crossnacreevy, Ireland	2	3	DQ525079
Sitona lepidus	Solohead, Ireland	2	3	DQ525079
Sitona lepidus	Oakpark, Ireland	1	15[2]	DQ525080
Sitona discoideus	Otago, NZ	2	5[1]	AY039691
Sitona discoideus	Canterbury, NZ	1	6[1]	AY039692
Listronotus bonariensis	Otago, NZ	1	6[1]	AY039692
Listronotus bonariensis	Hawke's Bay, NZ	1	6[1]	AY039692
Listronotus bonariensis	Waikato, NZ	1	6[1]	AY039692
Rhinocyllus conicus	Otago, NZ	3	6	DQ525081
Sitona discoideus	Otago, NZ	1	6[1]	AY039692
Irenimus sp.	Canterbury, NZ	3	6	DQ525081
Listronotus bonariensis	Canterbury, NZ	1	7	DQ525082
Irenimus sp.	Canterbury, NZ	1	7[1]	AY039693
Irenimus sp.	Otago, NZ	1	7[1]	AY039693
Unidentified NZ native	Otago, NZ	1	7	DQ525082
Rhinocyllus conicus	Otago, NZ	1	7	DQ525082

[1]Data from Vink et al. (2003).
[2]Differed by 1/676 nucleotides from COI haplotype 3.

DISCUSSION

The *lepidus* and *discoideus* strains of *M. aethiopoides* are morphologically indistinguishable (Vink et al. 2003), but as previously noted they differ in their modes of reproduction. Moreover, 1-6 larvae of the *lepidus* strain can develop in a single host, but generally only one of the *discoideus* strain (Gerard 2005; M.R. McNeill, unpubl. data). These differing behaviours should assist in distinguishing between the two strains during post-release field monitoring. However, the methods described in this contribution will be useful for confirming such observations, and they also offer opportunities to study some intriguing aspects of the reproduction and population genetics of *M. aethiopoides*. Ongoing testing of additional specimens of the *discoideus* strain collected in New Zealand aims to ensure the methods are completely reliable.

The genetic methods will help to verify the establishment of the *lepidus* strain following its release in New Zealand. Indeed, parasitoids reared from *S. lepidus* collected at a release site in Hawke's Bay in March 2006 have recently been confirmed, on the basis of their COI nucleotide sequences, to belong to the *lepidus* strain of *M. aethiopoides* (C.J. Vink, unpubl. data). These were probably the F1 offspring of parasitoids that had been released there in January 2006 (P.J. Gerard, pers. comm.).

The host range of the *discoideus* strain of *M. aethiopoides* in the field has been comprehensively studied in New Zealand (Barratt et al. 1997b), and quarantine-based tests have indicated that the *lepidus* strain should have a similar host range (Gerard 2005). The methods described in this contribution will assist in identifying the *M. aethiopoides* strain responsible for parasitism of target and non-target species, and will thus facilitate field research to validate quarantine-based predictions.

Laboratory testing of the *discoideus* strain showed it is not an effective parasitoid of *S. lepidus* (Barratt et al. 1997a; McNeill et al. 2000). This has been supported by observations of minimal parasitism of *S. lepidus* by the *discoideus* strain in the field in New Zealand. However, the *discoideus* strain does successfully parasitise *S. lepidus* at low (0-6%) rates in the laboratory (Barratt et al. 1997a). This suggests natural selection operating in the field could favour representatives of the *discoideus* strain that are able to parasitise *S. lepidus*. Moreover, this selection could become more intense as *S. lepidus* disperses to dryer parts of New Zealand where more lucerne is grown and where the *discoideus* strain of *M. aethiopoides* is more abundant. Similarly, the *lepidus* strain of *M. aethiopoides* can sometimes successfully parasitise *S. discoideus* in the laboratory (Gerard 2005), thus raising the possibility selection could favour individuals of the *lepidus* strain that have greater efficacy against *S. discoideus* once this newly introduced strain becomes widely established in New Zealand. The genetic methods described here will enable any response of the *M. aethiopoides* strains to such hypothetical selection pressures to be measured.

The *discoideus* strain of *M. aethiopoides* in New Zealand reproduces by arrhenotoky, as do many European populations of *S. lepidus*-parasitising *M. aethiopoides*, but the *lepidus* strain of *M. aethiopoides* introduced to New Zealand from Ireland reproduces by thelytoky (Goldson et al. 2001). Thelytoky is widespread in many species and appears to have independently evolved numerous times, but its reversal to arrhenotoky is considered unlikely because, over time, asexually reproducing lineages seem to lose their ability to reproduce sexually (see refs in Schneider et al. 2002). Nevertheless, the cytological mechanism that enables the *lepidus* strain of *M. aethiopoides* to reproduce by thelytoky remains unknown, and its stability under New Zealand conditions is clearly of interest (Gerard 2005). The methods described here will facilitate recognition of any change in reproductive mode by either of the *M. aethiopoides* strains in New Zealand.

In laboratory experiments, the offspring of crosses between arrhenotokous forms of the *discoideus* and *lepidus* strains of *M. aethiopoides* exhibited evidence of reduced efficacy in parasitising *S. discoideus*, though not *S. lepidus* (Goldson et al. 2003). Therefore, thelytokous *M. aethiopoides* from Ireland was chosen for introduction to New Zealand to reduce the probability the two strains would interbreed in this country (Gerard 2005). However, there is still uncertainty that differences in reproductive mode will maintain genetic isolation between the strains in the field. For example, arrhenotokous and thelytokous populations of the solitary parasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae) have been shown to interbreed both in the laboratory and field (Schneider et al. 2002; Schneider et al. 2003). After being mated by arrhenotokous males, thelytokous *V. canescens* females transmitted some of the males' genes to their predominantly female offspring (Schneider et al. 2002).

The methods described in this contribution should be suitable for detecting crosses between the *M. aethiopoides* strains in New Zealand. The currently available data (Table 1) suggest the 'A' allele for AOX is unique to the *lepidus* strain, while the 'B' allele is unique to the *discoideus* strain. Therefore, any *M. aethiopoides* exhibiting an 'A+B' genotype for AOX should be a descendent of an inter-strain cross. Alternatively, a combination of mitochondrial COI nucleotide sequencing and enzyme analyses could be used to identify parasitoids descended from such crosses. Mitochondrial DNA is inherited almost exclusively from the female parent (Brown 1985, but see Meusel & Moritz 1993), thus any *M. aethiopoides* arising from crosses between males of the *discoideus* strain and females of the *lepidus* strain should only exhibit one of the two COI haplotypes observed in the *lepidus* strain (haplotypes 3 and 15; Table 2). In contrast, the genes

coding for the variable EST and AOX proteins have a nuclear origin, thus females arising from an inter-strain cross should inherit these alleles from both parents. Interbreeding between the *lepidus* and *discoideus* strains would therefore be revealed by specimens which exhibited one of the two COI haplotypes of the *lepidus* strain, and at least one of the enzymes that is unique to the *discoideus* strain (i.e. either the 'A' allele for EST2 or the 'B' allele for AOX; Table 1).

ACKNOWLEDGEMENTS

We are grateful to Pip Gerard, Scott Hardwick and Derrick Wilson (AgResearch, Ruakura) for providing many of the *M. aethiopoides* specimens from the North Island and to Barbara Barratt, Colin Ferguson and Kim Crook (AgResearch, Invermay) for providing many of the specimens from Otago. Dr S.A. Forgie (HortResearch, Auckland) provided helpful suggestions to improve an earlier version of this manuscript.

REFERENCES

Adler PH, Kim KC 1985. Morphological and morphometric analyses of European and Moroccan biotypes of *Microctonus aethiopoides* (Hymenoptera: Braconidae). Annals of the Entomological Society of America 78: 279-283.

Aeschlimann J-P 1983a. Notes on the variability of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae: Euphorinae). Contributions of the American Entomological Institute 20: 329-335.

Aeschlimann J-P 1983b. Sources of importation, establishment and spread in Australia of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), a parasitoid of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae). Journal of the Australian Entomological Society 22: 325-331.

Aeschlimann JP 1995. Chapter 7: Lessons from post-release investigations in classical biological control: the case of *Microctonus aethiopoides* Loan (Hym., Braconidae) introduced into Australia and New Zealand for the biological control of *Sitona discoideus* Gyllenhal (Col., Curculionidae). In: Hokkanen HMT, Lynch JM ed. Biological Control: Benefits and Risks. Cambridge University Press, New York. Pp. 75-83.

Barratt BIP, Barker GM, Addison PJ 1996. *Sitona lepidus* (Coleoptera: Curculionidae), a potential clover pest new to New Zealand. New Zealand Entomologist 19: 23-30.

Barratt BIP, Evans AA, Ferguson CM 1997a. Potential for biocontrol of *Sitona lepidus* Gyllenhal by *Microctonus* spp. Proceedings of the 50th New Zealand Plant Protection Conference: 37-40.

Barratt BIP, Evans AA, Ferguson CM, Barker G, McNeill MR, Phillips CB 1997b. Laboratory nontarget host range of the introduced parasitoids *Microctonus aethiopoides* and *M. hyperodae* (Hymenoptera: Braconidae) compared with field parasitism in New Zealand. Environmental Entomology 26: 694-702.

Brown WM 1985. The mitochondrial genome of animals. In: MacIntyre RJ ed. Molecular Evolutionary Genetics. Plenum Press, New York. Pp. 95-130.

Cullen JM, Hopkins DC 1982. Rearing, release and recovery of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) imported for the control of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) in south eastern Australia. Journal of the Australian Entomological Society 21: 279-284.

Folmer OM, Black W, Hoeh R, 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.

Gerard PJ 2005. Conditional release from containment of the Irish strain of the parasitic wasp *Microctonus aethiopoides* for biological control of *Sitona lepidus* (clover root weevil). Application code NOR05001, Environmental Risk Management Authority, Wellington, New Zealand. 75 pp.

Goldson SL, McNeill MR, Gerard PJ, Proffitt JR, Phillips CB, Cane RC, Murray PJ 2004. British-based search for natural enemies of the clover root weevil, *Sitona lepidus*, in Northwest Europe. New Zealand Journal of Zoology 31: 233–240.

Goldson SL, McNeill MR, Proffitt JR 2003. Negative effects of strain hybridisation on the biocontrol agent *Microctonus aethiopoides*. New Zealand Plant Protection 56: 138-142.

Goldson SL, Phillips CB, McNeill MR, Proffitt JR, Cane RP 2001. Importation to New Zealand quarantine of a candidate biological control agent of clover root weevil. New Zealand Plant Protection 54: 147-151.

Iline II, Phillips CB 2003. Allozyme variation between European and New Zealand populations of *Microctonus aethiopoides*. New Zealand Plant Protection 56: 133-137.

Loan CC 1960. The biology of insect parasitoids of the genus *Sitona* Germar (Coleoptera: Curculionidae). PhD, University of Minnesota, Minnesota, USA. 156 pp.

Loan CC, Holdaway FG 1961. *Microctonus aethiops* (Nees) auctt. and *Perilitus rutilus* (Nees) (Hymenoptera: Braconidae), European parasites of *Sitona* weevils (Coleoptera: Curculionidae). The Canadian Entomologist XCIII(12): 1057-1078.

Manchenko GP 1994. Handbook of Detection of Enzymes on Electrophoretic Gels. CRC Press, Boca Raton, FL., USA. 341 pp.

McNeill MR, Barratt BIP, Evans AA 2000. Behavioural acceptability of *Sitona lepidus* (Coleoptera: Curculionidae) to the parasitoid *Microctonus aethiopoides* (Hymenoptera: Braconidae) using the pathogenic bacterium *Serratia marcescens* Bizio. Biocontrol Science and Technology 10: 205-213.

McNeill MR, Proffitt JR, Gerard PJ, Goldson SL 2006. Collections of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) from Ireland. New Zealand Plant Protection 59: 290-296.

Meusel MS, Moritz FA 1993. Transfer of paternal mitochondrial DNA during fertilization of honeybee (*Apis mellifera* L.) eggs. Current Genetics 24: 539-543.

Phillips CB 2002. A search for biological control agents of *Sitona lepidus* (syn. *flavescens*) Gyllenhal (Coleoptera: Curculionidae). First International Symposium on Biological Control of Arthropods. p. 500.

Phillips CB, Cane RP, Mee J, Chapman HM, Hoelmer KA, Coutinot D 2002. Intraspecific variation in the ability of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) to parasitise *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae). New Zealand Journal of Agricultural Research 45: 295-303.

Rothe GM 1994. Electrophoresis of enzymes: Laboratory methods. Springer-Verlag, Berlin. 307 pp.

Schneider MV, Beukeboom LW, Driessen G, Lapchin L, Bernstein C, van Alphen JJM 2002. Geographical distribution and genetic relatedness of sympatrical thelytokous and arrhenotokous populations of the parasitoid *Venturia canescens* (Hymenoptera). Journal of Evolutionary Biology 15: 191-200.

Schneider MV, Driessen G, Beukeboom LW, Boll R, van Eunen K, Selzner A, Talsma J, Lapchin L 2003. Gene flow between arrhenotokous and thelytokous populations of *Venturia canescens* (Hymenoptera). Heredity 260-267.

Stufkens MW, Farrell JA, Goldson SL 1987. Establishment of *Microctonus aethiopoides,* a parasitoid of the *Sitona* weevil in New Zealand. Proceedings of the 40th New Zealand Weed and Pest Control Conference: 31-32.

Sundaralingam S 1986. Biological, morphological and morphometric analyses of populations of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae). M.Sc., Pennsylvania State University, USA. 80 pp.

Vink CJ, Phillips CB, Mitchell AD, Winder LM, Cane RP 2003. Genetic variation in *Microctonus aethiopoides* (Hymenoptera: Braconidae). Biological Control 28: 251-264.