Mitochondrial COI and II provide useful markers for *Wiseana* (Lepidoptera: Hepialidae) species identification

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Abstract

A method for identifying the members of the endemic genus *Wiseana* Viette from New Zealand is described. Seven species have been described in the genus: *W. cervinata* (Walker), *W. copularis* (Meyrick), *W. fuliginea* (Butler), *W. jocosa* (Meyrick), *W. mimica* (Philpott), *W. signata* (Walker) and *W. umbraculata* (Guenée). No morphological characters have been identified to distinguish between the larvae of each species and adult females exhibit high levels of intra and interspecific morphological variation making identification difficult or impossible. Adult males can be distinguished by a combination of scale, antennal and genital characters, but this requires considerable taxonomic experience. Molecular markers were generated via amplification of the cytochrome oxidase subunit I and II (COI and II) of the mitochondrial DNA by the polymerase chain reaction (PCR). Amplified DNA was digested with restriction enzymes to give characteristic fragment patterns. Fourteen restriction enzymes were surveyed and a combination of four of these distinguish all *Wiseana* taxa except *W. fuliginea* and *W. mimica*.

Introduction

Wiseana Viette is one of five endemic genera in the 'Oxyzanus' lineage of hepialid moths (Lepidoptera: Hepialidae) (Dugdale, 1994) from New Zealand. Using a morphological species concept, Dugdale (1994) recognized seven species in the genus: *W. cervinata* (Walker), *W. copularis* (Meyrick), *W. fuliginea* (Butler), *W. jocosa* (Meyrick), *W. mimica* (Philpott), *W. signata* (Walker) and *W. umbraculata* (Guenée).

The larvae of *Wiseana*, commonly referred to as 'porina' (an old, preoccupied generic name), are important pasture pests in New Zealand causing significant economic losses (French, 1973; Barratt *et al.*, 1990; Johnston, 1994; Herbert, 1995). The larvae build vertical tunnels in the soil and feed nocturnally, defoliating pasture grasses, clover and lucerne (Barratt *et al.*, 1990). Barlow (1985) estimated that porina densities of 40–120 m⁻² resulted in 20–80% loss of pasture production. Herbert (1995) found larvae of *W. cervinata* and *W. copularis* in damaged pastures throughout New Zealand

and *W. fuliginea* on South Island. Dugdale (1994) also lists *W. mimica* as a damaging species.

Wiseana taxa are difficult to distinguish using adult morphological characters. Males of all species can be identified but this requires dissection and interpretation of genitalia and experience in interpreting antennal and scale characters (table 1).

Females of all species have been found to have continuous, overlapping external morphological characteristics (MacArthur, 1986; Herbert, 1995) and are only reliably identified using characters from the bursa copulatrix in the female genitalia (Dugdale, 1994). External characters to distinguish between *W. cervinata* and *W. copularis* females are being tested (E.G. White, personal communication). Herbert (1995) found the larvae of each species, reared in the laboratory from positively identified adults, to be morphologically very similar. Further morphological work to distinguish between larvae of the different species is in progress (J. Dugdale, personal communication).

Intra and interspecific character variation has created difficulties in finding stable morphological characters to identify taxa in this genus and has resulted in a history of taxonomic instability (Hudson, 1928; Dumbleton, 1966; Archibald, 1984; Dugdale, 1988). Research on control

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Table 1. Morphological characters from adult males used to distinguish between *Wiseana* taxa.

Character	<i>W. cervinata</i>	<i>W. copularis</i>	<i>W. fuliginea</i>	<i>W. jocosa</i>	<i>W. mimica</i>	<i>W. signata</i>	<i>W. umbraculata</i>
Genitalia							
Twin processes	large, elongate $x=0.4$ mm, $SD=0.04$	large $x=0.33$ mm, $SD=0.04$	small $x=0.07$ mm, $SD=0.02$	small $x=0.18$ mm, $SD=0.06$	small $x=0.12$ mm, $SD=0.05$	small $x=0.05$ mm, $SD=0.03$	large $x=0.44$ mm, $SD=0.04$
Pseudotegumen outer margin	parallel sided	bowed outer margin	bowed outer margin	bowed outer margin	bowed outer margin	bowed outer margin	bowed outer margin
Antennae							
Pectination shape	triangular, apices rounded	long and rectangular	triangular, apices rounded	dark, triangular, apices rounded	triangular, longer than deep	pale	pale
Scales							
White scales, forewing discal cell	tapering, broad truncate (northern populations) tapering, narrow truncate (southern populations)	parallel sided, narrow rounded apex	long, narrow, long sharp apex	very short, truncate	oval in mid-section, tapering abruptly to an acute apex	oval in mid-section, tapering to a rounded apex	oval in mid-section, apiculate

measures has previously treated ‘porina’ pests as a one-species problem, principally dealing with the *W. cervinata* ‘complex’ (e.g. Farrell *et al.*, 1974). Failure to accurately identify the taxa under study may have confounded ecological studies (e.g. Dumbleton, 1945; Fenemore & Allen, 1969; French, 1973; Barlow *et al.*, 1986).

Few variable characters were identified for *Wiseana* taxa in a cladistic analysis of a morphological data set (Brown *et al.*, 1999) and relationships within the genus, apart from between *W. signata* and *W. umbraculata*, were not resolved.

Brown *et al.* (in press) identified ten lineages of *Wiseana* from mitochondrial DNA COI and II sequences. These lineages corresponded to the seven currently described species, plus an additional haplotype for *W. cervinata*, *W. copularis* and *W. signata*. Phylogenetic relationships for the genus *Wiseana* were inferred from the mitochondrial DNA sequences (fig. 1). The mtDNA data set produced a more resolved phylogeny for *Wiseana* in comparison to the morphological, but lack of synapomorphies deeper in the clade, a predominance of transitional nucleotide substitutions and the presence of many autapomorphic characters for *Wiseana* haplotypes suggests recent and rapid radiation. This result is consistent with the dearth of morphological features that define the *Wiseana* taxa.

Where morphological characters cannot distinguish between taxa, other methods may be useful. Herbert (1995) resolved the seven recognized *Wiseana* species using isoenzyme electrophoresis and developed a key. The seven species showed unique combinations of fixed differences at the Acp-1, Gp-2, Me-1, 6pg-1 and Pep-1 loci. Additional fixed or frequency differences at other loci also distinguished each species. However, new molecular techniques such as amplification of a fragment of DNA by the polymerase chain reaction (PCR) followed by cleavage with restriction enzymes to produce diagnostic patterns from length and site polymorphisms (RFLPs) offer advantages over allozymes. DNA is less sensitive to

Fig. 1. Strict consensus cladogram of the six most parsimonious trees from the analysis of mtDNA COI and II sequence data for the single species lineages within the genus *Wiseana* (Lepidoptera: Hepialidae) from New Zealand. Branch lengths are proportional to the number of unambiguous nucleotide changes. Decay values are shown above the branches and bootstrap proportions (>50%) below.

degradation, in the short term, allowing specimens to be stored in alcohol before DNA extraction.

Furthermore, any life cycle stage can be used, only a small amount of DNA is required and the restriction patterns are unambiguous and reproducible (Szalanski & Powers, 1996; Armstrong *et al.*, 1997). The technique is also relatively inexpensive in comparison to sequencing, allowing more specimens to be processed.

The mitochondrial genome is relatively small and has a combination of conserved and variable regions (Simon *et al.*, 1994; Mills, 1996) which is useful for the development of diagnostic tests to discriminate between races and sibling species. The polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLPs) of mtDNA have produced diagnostic tests for *Aedes* mosquitoes (Kambhampati & Rai, 1991), *Anopheles* mosquitoes (Diptera: Culicidae) (Mitchell *et al.*, 1992), gypsy moths *Lymantria dispar* (Linnaeus) (Lepidoptera: Lymantriidae) (Bogdanowicz *et al.*, 1993), root knot nematodes *Meloidogyne* spp. (Hugall *et al.*, 1994), ermine moths of the *Yponomeuta padella* (Linnaeus) complex (Lepidoptera: Yponomeutidae) (Sperling *et al.*, 1995), tobacco budworm *Heliothis virescens* (Fabricius) and corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Roehrdanz, 1997).

In this paper we report a method for distinguishing between *Wiseana* taxa using the polymerase chain reaction and restriction fragment length polymorphisms on the mitochondrial DNA cytochrome oxidase I and II (COI and II) regions.

Material and methods

Collections

This study is based on adult *Wiseana* males that were collected by light trapping, placed directly into 96% ethanol and stored at 4°C before identification (see table 1) and DNA extraction. *Wiseana* taxa have overlapping distributions within New Zealand with *W. umbraculata* and *W. cervinata* being the most widespread (Dugdale, 1994). Herbert (1995) reported some clinal variation with latitude within populations of *W. cervinata*. Consequently, where possible for each species, adult males from several populations were sampled. Details of collecting sites and numbers of individuals screened are given in table 2.

Voucher specimens are stored at the Entomological Research Museum, Lincoln University, Lincoln, Canterbury, New Zealand.

Preliminary identification of restriction sites

A 527 base pair (bp) region of the COI and II region had been sequenced previously for phylogenetic analysis (Brown *et al.*, in press). Restriction sites in this region were searched for using the range of enzymes listed in DNAMAN (Lynnon Biosoft, 1994–1995), before the amplification of the entire COI and II region.

DNA extraction, PCR and restriction digests

Muscular tissue from the thorax of specimens was homogenized and total DNA extracted using a proteinase-K digestion and high salt precipitation (White *et al.*, 1990). The COI and II gene regions were amplified via the polymerase

Table 2. Locations of populations sampled for each *Wiseana* taxon.

Population	Code ^a	Grid ref ^b	n
<i>W. fuliginea</i>			
Birdlings Flat	MC	M37 860-090	2
Dunedin	DN	I44 133-783	1
<i>W. mimica</i>			
Cass	NC	K34 963-090	2
Mackenzie Country	MK	H38 802-615	1
<i>W. copularis</i> 'northern'			
Opouri Saddle	MB	P27 713-073	2
Greens Beach	WD	I34 118-974	2
Pohara	NN	N25 012-421	5
Cobb Ridge	NN	M26 843-113	2
Queenstown	OL	E41 690-668	3
<i>W. signata</i> 'southern'			
Waimea	NN	N27 182-844	1
Pohara	NN	N25 012-421	2
<i>W. signata</i> 'northern'			
Mangapehi	WO	S17 116-960	2
Woodhill Forest	AK	Q10 367-934	2
Levin	WN	S25 997-496	2
<i>W. cervinata</i> 'southern'			
Birdlings Flat	MC	M37 860-090	5
Nelson	NN	N27 182-844	2
Goulter	MB	N28 288-581	2
Otaio	SC	J39 621-248	2
<i>W. cervinata</i> 'northern'			
Mangapehi	WO	S17 116-960	2
Ohakune	RI	S20 162-958	2
Hawera	TK	Q21 290-790	2
Levin	WN	S25 997-496	1
<i>W. copularis</i> 'southern'			
Christchurch	MC	M35 792-433	2
West Melton	MC	M35 583-416	2
Taitapu	MC	M36 717-266	1
Queenstown	OL	E41 690-668	3
Mackenzie Country	MK	I39 756-447	2
Dunedin	DN	I44 133-783	2
Kaka Point	SL	H46 617-197	1
Ohai	SL	D45 187-627	2
<i>W. jocos</i>			
Cobb Ridge	NN	M26 843-113	1
Franz Josef	WD	H35 816-542	2
Rongahere Gorge	CO	G45 364-675	2
Te Anau	SL	D43 947-163	2
Ohai	SL	D46 234-403	1
Otautau	SL	D45 187-627	2
<i>W. umbraculata</i>			
Mangapehi	WO	S17 116-960	1
Levin	WN	S25 977-496	2
Kaitoke	WN	S26 918-113	1
Christchurch	MC	M35 792-433	2
Mackenzie Country	MK	H38 801-622	1
Otaio	SC	J39 621-248	1
Otatara	SL	E47 470-078	1
Tuatapere	SL	D46 004-387	2

^a System of areas and codes for recording specimen locations in New Zealand (Crosby *et al.*, 1976). ^b Grid references from NZMS 260 series maps. n, number of individuals screened.

chain reaction (PCR) (Saiki *et al.*, 1988) using the primers mtDNA 4 (5'TACAATTTATCGCCTAAACTTCAGCC) (Sperling & Hickey, 1994) and mtDNA 18 (5'CCA-CAAATTTCTGAACATTGACCA) (Simon *et al.*, 1994). A single product band of approximately 2200 bp was produced for all taxa. 25 µl reactions comprised 2.5 µl of 10× *Taq* buffer (Boehringer Mannheim), 3.75 µl of 1 mM dNTPs, 0.625 µl of 20 mM magnesium, 2.5 µl of 2 µM mtDNA 4 and mtDNA 18 primers, 0.2 µl of 5 u µl⁻¹ *Taq* DNA polymerase (Boehringer Mannheim) and 0.9 µl of 20 ng µl⁻¹ DNA. A Perkin-Elmer 2400, thermal cycler was used with a cycling profile of 94°C for 2 min pre-PCR followed by 93°C for 20 sec, 52°C for 40 sec and 72°C for 1 min for 36 cycles with a 5 min extension at 72°C after the final cycle. The resulting double stranded PCR product was run on a 2% agarose LE (low electroendosmosis) gel (Boehringer Mannheim) in an ethidium bromide buffer at 80 volts for 1 h, with a 100 bp ladder (Gibco BRL), before being visualized under UV.

Subsequently, the PCR product from the entire COI and II region was digested in a 10 µl reaction with restriction enzymes in a water bath for 2 h at temperatures according to the manufacturer's (Boehringer Mannheim) recommendations.

Reactions with 10 u µl⁻¹ enzymes comprised: 1 µl of 10× manufacturer's buffer, 0.35 µl of 10 u µl⁻¹ enzyme, 3 µl PCR product and 5.65 µl deionized water. Reactions with enzymes at concentrations other than 10 u µl⁻¹ comprised: 1 µl of 10× buffer, 3.5 units of enzyme, 0.1 µl 100× bovine serum albumin (BSA), 3 µl PCR product and deionized water to make up to a total volume of 10 µl. The digest product for each enzyme was initially run on a 2% gel in ethidium bromide buffer for 2–3 h at 135 v, with a 100 bp ladder (Gibco BRL) and visualized under UV. To improve resolution of the fragments, the *AsnI* and *TaqI* products were run on 3% metaphor gels at 100 v for 5 h and 4 h respectively. Fragment sizes were estimated by comparison with the 100 bp ladder on each gel.

Results

A mtDNA fragment approximately 2200 bp long was amplified with the primers mtD4 and mtD18 for all the *Wiseana* taxa. The fragment was digested with 14 commercially available restriction enzymes that were selected after screening the 527 bp COI and II sequence data. There were no restriction sites for *Hind* III. The enzymes *Pst*I, *Hinf*I, *Bgl*III, and *Tru*91 cleaved the product but no polymorphisms were evident. *Sau*IIIa, *Alu*I, *Dra*I, *Rsa*I and *Mae*III cleaved the product and polymorphisms were evident in a small number of taxa. Four enzymes produced diagnostic patterns to distinguish between *Wiseana* taxa (tables 3 and 4), including the additional haplotypes for *W. cervinata*, *W. copularis* and *W. signata*. The enzymes, *Asn*I, *Taq*I, *Hind*II and *Hae*III cleaved the product and exhibited polymorphisms that were consistent for individuals within and between populations of *Wiseana* taxa. No intraspecific

Table 3. Diagnostic key for *Wiseana* taxa following the amplification of COI and II mtDNA with primers mtD4 and mtD18 and digestion with *Asn*I, *Taq*I, *Hind*II or *Hae*III.

	<i>Asn</i> I	<i>Taq</i> I	<i>Hind</i> II	<i>Hae</i> III
<i>W. copularis</i> 'northern'	✓	—	✓	—
<i>W. copularis</i> 'southern'	✓	✓	—	—
<i>W. cervinata</i> 'northern'	✓	—	—	—
<i>W. cervinata</i> 'southern'	✓	✓	—	—
<i>W. jocosa</i>	✓	—	✓	—
<i>W. umbraculata</i>	✓	■	—	—
<i>W. signata</i> 'northern'	■	■	—	✓
<i>W. signata</i> 'southern'	■	■	—	—
<i>W. fuliginea</i>	—	—	—	—
<i>W. mimica</i>	—	—	—	—

✓, Unique pattern; ■, —, shared pattern.

Table 4. Expected fragment sizes following the amplification of *Wiseana* COI and II mtDNA with primers mtD4 and mtD18 and digestion with *Asn*I, *Taq*I, *Hind*II or *Hae*III.

Restriction enzyme	Taxa	Predicted size of resultant fragment (bp)
<i>Asn</i> I	<i>W. fuliginea</i> and <i>W. mimica</i>	50, 90, 140, 185, 200, 210, 220, 300, 310, 500
	<i>W. copularis</i> 'northern'	50, 90, 140, 150, 180, 195, 210, 300, 350, 500
	<i>W. signata</i> 'southern' and 'northern'	50, 80, 90, 140, 150, 180, 190, 220, 260, 310, 500
	<i>W. cervinata</i> 'southern'	50, 90, 140, 180, 190, 220, 230, 260, 315, 500
	<i>W. cervinata</i> 'northern'	50, 90, 140, 180, 190, 210, 220, 230, 315, 500
	<i>W. copularis</i> 'southern'	50, 90, 140, 150, 180, 190, 210, 300, 320, 500
	<i>W. jocosa</i>	50, 80, 90, 135, 195, 200, 230, 280, 310, 680
	<i>W. umbraculata</i>	50, 80, 90, 135, 180, 190, 210, 230, 250, 310, 500
	<i>W. fuliginea</i> , <i>W. mimica</i> , <i>W. cervinata</i> 'northern', <i>W. copularis</i> 'northern', <i>W. jocosa</i>	50, 125, 150, 250, 270, 400, 460, 540
	<i>W. signata</i> 'southern' and 'northern', <i>W. umbraculata</i>	50, 125, 150, 240, 270, 530, 890
<i>Taq</i> I	<i>W. cervinata</i> 'southern'	50, 125, 150, 250, 450, 530, 690
	<i>W. copularis</i> 'southern'	50, 125, 150, 250, 260, 400, 450, 530
	<i>W. jocosa</i>	790, 1400
	<i>W. copularis</i> 'northern'	1000, 1200
<i>Hind</i> II	<i>W. cervinata</i> 'southern' and 'northern', <i>W. copularis</i> 'southern', <i>W. fuliginea</i> , <i>W. mimica</i> , <i>W. signata</i> 'southern' and 'northern', <i>W. umbraculata</i>	2200
	<i>W. signata</i> 'northern'	420, 1770
	<i>W. cervinata</i> 'southern' and 'northern', <i>W. copularis</i> 'southern' and 'northern', <i>W. fuliginea</i> , <i>W. jocosa</i> , <i>W. mimica</i> , <i>W. signata</i> 'southern', <i>W. umbraculata</i>	2200
	<i>W. signata</i> 'southern', <i>W. umbraculata</i>

Fig. 2. *AsnI* restriction patterns for *Wiseana* taxa, from 2200 bp amplified mtDNA COI and II (mw, molecular weight marker).

polymorphisms were observed with these enzymes. The enzyme *AsnI* produced a diagnostic pattern for all *Wiseana* taxa apart from *W. fuliginea*, *W. mimica*, *W. signata* 'southern' and *W. signata* 'northern' (fig. 2). The enzyme *TaqI* produced four patterns (fig. 3). *Wiseana cervinata* 'southern' and *W. copularis* 'southern' exhibited unique patterns. A further pattern was shared by *W. signata* 'southern', *W. signata* 'northern' and *W. umbraculata* and the final pattern was shared by the remaining taxa. *Hind* II produced unique patterns for *W. jocosu* and *W. copularis* 'northern', while the enzyme did not cleave the remaining taxa (fig. 4). *Hae* III only cleaved *W. signata* 'northern' (fig. 5).

No enzymes produced unique patterns for *W. mimica* or *W. fuliginea* in the DNAMAN search of the 527 bp section of COI and II and neither did the enzymes digested with the complete COI and II region.

Discussion

There are difficulties distinguishing between *Wiseana* taxa using morphological characters, for all life stages. Other barriers to rapid and accurate identification are overlapping distributions and adult emergence times (Dugdale, 1994; Herbert, 1995). Lack of variability in morphological characters resulted in a poorly resolved phylogeny for this genus (Brown *et al.*, 1999).

DNA methods have not previously been used to identify *Wiseana* taxa. This technique produced diagnostic markers for *W. cervinata* 'southern', and *W. cervinata* 'northern', *W. copularis* 'southern' and *W. copularis* 'northern', *W. jocosu*,

Fig. 3. *TaqI* restriction patterns for *Wiseana* taxa, from 2200 bp amplified mtDNA COI and II (mw, molecular weight marker).

W. signata 'northern' and *W. umbraculata*. *Wiseana signata* 'southern' can be identified by a process of elimination.

No unique RFLP pattern was produced for *W. fuliginea* or *W. mimica*. This result is not unexpected considering that their COI and II sequence only differed by one nucleotide and corrected divergences show that these taxa differ by only 0.19% (Brown *et al.*, in press). The sequence from the ITS2 region for these taxa was identical (B. Brown *et al.*, in press, b).

A 200 bp variable region from the mitochondrial 16S rRNA gene, which is thought to be evolving as rapidly as the control region, (Parker & Kornfield, 1996) was sequenced for *W. fuliginea* and *W. mimica*, but again both produced an identical sequence (B. Brown, unpublished data).

Mitochondrial DNA COI and II sequences identified additional haplotypes for *W. cervinata*, *W. copularis* and *W. signata* (Brown *et al.*, in press, a). Cleavage of the entire COI and II region by restriction enzymes produced diagnostic RFLPs consistent with each additional haplotype. *Wiseana cervinata* 'southern' can be identified by the unique RFLPs produced when cleaved by *TaqI* and *AsnI*. *Wiseana cervinata* 'northern' can be identified using *AsnI*. The differentiation of *W. cervinata* into 'southern' and 'northern' groupings, based on morphological, biological and molecular characteristics, adds further evidence that *W. cervinata* as currently described is two species. *Wiseana cervinata* populations south of latitude 40°S have an adult emergence

Fig. 4. *Hind*II restriction patterns for *Wiseana* taxa, from 2200 bp amplified mtDNA COI and II (mw, molecular weight marker).

time from September until December, while populations north of this latitude emerge from September until March (Dugdale, 1994). Herbert (1995) reported allozyme differences in *W. cervinata* populations consistent with a geographical cline and differing responses to pesticide application were attributed to possible differences in susceptibility between 'southern' and 'northern' populations. In this study, the *W. cervinata* 'northern' RFLP pattern was recovered from populations at Mangapehi (WO), Ohakune (RI), Hawera (TK) and Levin (WN) all on North Island.

Levin is approximately 100 km south of the expected 'northern' *W. cervinata* boundary given in Dugdale (1994) and may represent an expansion of the range of *W. cervinata* 'northern'. The phylogeny of *Wiseana* taxa recovered from mtDNA COI and II sequence data (Brown *et al.*, in press) could not resolve the *W. cervinata* 'southern' – *W. cervinata* 'northern' relationship.

The biological significance and the geographical boundaries of the southern and northern forms of *W. copularis* and *W. signata* have not yet been established, but each can be distinguished by this technique. *Wiseana copularis* 'southern' produces a unique pattern with *Asn*I and *Taq*I, while *W. copularis* 'northern' can be distinguished using *Hind*II. These taxa were always recovered in a clade together in the mtDNA phylogeny (fig. 1). *Wiseana signata* 'northern' can be distinguished using *Hae*III. *Wiseana signata* 'southern' produces a pattern identical to those of *W. signata* 'northern' (*Asn*I) or *W. signata* 'northern' and *W. umbraculata* (*Taq*I). However, both *W. signata* 'northern' and *W. umbraculata* can be identified by unique patterns produced by *Hae*III and

Fig. 5. *Hae*III restriction patterns for *Wiseana* taxa, from 2200 bp amplified mtDNA COI and II (mw, molecular weight marker).

*Asn*I respectively. Both the morphological and mtDNA phylogenies recovered *W. signata* and *W. umbraculata* as sister taxa and the mtDNA supports a *W. signata* 'southern', *W. signata* 'northern' clade.

The technique reported in this paper offers economical, rapid and accurate identification of *Wiseana* taxa. In conjunction with morphological characters (for adult males), this method will allow the distribution of species, as well as species composition within one pasture, to be determined. Accurate identification will facilitate improved understanding of the life history, behaviour and ecology of non-pest and pest species. For pest species, an improved understanding of host plant preference, behavioural patterns and susceptibility to disease and insecticide should contribute to more efficient and effective pest management practices.

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