THE EVOLUTIONARY HISTORY OF THE *'OXYCANUS'* LINEAGES OF HEPIALID MOTHS IN NEW ZEALAND

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This thesis attempted to reconstruct the evolutionary history of part of New Zealand's hepialid fauna. Phylogenetic analysis was used to test the monophyly of the informal *'Oxycanus' Cladoxycanus* and *'Oxycanus' s. str.* lineages. Data were morphological characters and molecular characters from the mtDNA COI & II and nrDNA ITS2 regions. Morphological and COI & II phylogenies indicated that the *'Oxycanus' Cladoxycanus* and *'Oxycanus' s. str.* lineages were monophyletic clades. Addition of Australian hepialid exemplars to the morphological data set indicated that *Oxycanus* and *Jeana* taxa were sister group to the monophyletic New Zealand *'Oxycanus'* lineage *s. str.*, while addition to the COI & II data set indicated that *'Oxycanus' Cladoxycanus* was sister group to the *'Oxycanus'* lineage *s. str.*

The morphology and COI & II phylogenies were mostly congruent, with both recovering clades that corresponded to the informal *Aenetus*, *Aoraia*, *'Oxycanus' Cladoxycanus* and *'Oxycanus' s. str.* lineages. The morphological and COI & II phylogenies were not congruent with respect to the placement of the *Dumbletonius* taxa. The morphological data set recovered *Dumbletonius characterifer* in a clade with *D. unimaculatus,* while the COI & II data set recovered *D. characterifer* as the basal taxon in the *'Oxycanus'* lineage *s. str.* and *D. unimaculatus* in a clade with *Wiseana* taxa. Tracing the evolution of morphological characters that supported the monophyly of the genus *Dumbletonius* on to the independently derived COI & II phylogeny indicated that all characters were plesiomorphic. Spectral analysis gave higher support to the *(D. unimaculatus, Wiseana)* clade compared with the *(D. characterifer, D. unimaculatus)* clade.

Morphological characters were mapped on to the COI & II phylogeny. *Aenetus* and *Aoraia* taxa were hypothesised to exhibit ancestral character states, male genitalic characters were less homoplasious compared with other data partitions and the morphological synapomorphies supporting the *'Oxycanus'* lineages and the *'Oxycanus' s. str.* lineage alone, were homologous.

The combined morphology, COI & II and ITS2 data set did not support the monophyly of the *'Oxycanus'* lineages. *Dumbletonius unimaculatus, D. characterifer, Cladoxycanus, Heloxycanus* and *Dioxycanus* taxa were recovered in a clade together. This topology was not recovered from any other data sets. Saturation of nucleotide sites in more divergent taxa and inconsistency in the parsimony method may have contributed to this result. The combined morphology and COI & II data set recovered a phylogeny congruent with that from the morphology alone.

A combined morphology, COI & II, ITS2 and allozyme data set with *Dumbletonius unimaculatus* as outgroup, recovered the most resolved phylogeny for the genus *Wiseana*. The two clades recovered were: (((*Wiseana cervinata* 'southern', *Wiseana cervinata* 'northern'), *W. jocosa), (W. copularis* 'southern', *W. copularis* 'northern'), (*W. fuliginea, W. mimica*)) and ((*W. signata* 'southern', *W. signata* 'northern'), *W. umbraculata*). A diagnostic test was developed for *Wiseana* taxa based on the cleavage of the mtDNA COI & II gene regions with restriction enzymes. All *Wiseana* taxa, apart from *W. fuliginea* and *W. mimica*, were identified by unique restriction fragment length polymorphism patterns.

Key words - hepialid, *'Oxycanus' Cladoxycanus, 'Oxycanus' s. str., Aenetus, Aoraia*, phylogeny, morphology, mtDNA COI & II, nrDNA ITS2, combined analysis, total evidence, taxonomic congruence, character mapping, diagnostic test, restriction fragment length polymorphism, restriction enzymes.

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Chapter 1
General Introduction

Taxonomy, the science of describing and naming taxa and producing classifications, and systematics, the study of relationships of organisms, have been strengthened by the incorporation of evolutionary principles. Recognition that organismal attributes change over time and that organisms exhibit attributes inherited from their ancestors has directed systematists towards recovering groupings of organisms that are hierarchical, exist in nature and originate through a natural process - evolution (Wiley, 1981).

Cladistics overcame the problem that systematists had struggled with for decades, i.e., how to use the similarity seen in morphological characters to recover meaningful groupings of taxa (Sokal and Sneath, 1963; Mayr and Ashlock, 1991). In cladistics (phylogenetic systematics), only shared derived characters are taken to be evidence of descent from a common ancestor and only monophyletic groupings, i.e., those that include all the descendents from the most recent common ancestor, are recognised (Hennig, 1966; Simpson and Cracraft, 1995). Recovery of evolutionary relationships or phylogeny is one of the goals of systematics (Savage, 1995). Phylogeny is always an estimate (Swofford *et al.*, 1996) as we can never know for sure the exact evolutionary path taken to produce the extant species. Each phylogeny is derived from a particular data set and is an hypothesis partially dependent on the quality and quantity of the data and the accuracy of the methods used (Brooks and McLennan, 1991; Penny *et al.*, 1992; Hillis, 1995) but also, and perhaps substantially, influenced by the underlying shape of the tree and the branch lengths (Yang, 1996).

This study focuses on the estimation of evolutionary relationships within the hepialid moth fauna (Lepidoptera: Hepialidae) in New Zealand. Family Hepialidae in New Zealand is part of the Hepialidae *sensu stricto* (Nielsen and Scoble, 1986), one of twelve monophyletic clades within the super family Hepialoidea (Nielsen, 1989). The family Hepialidae in New Zealand comprises 27 endemic species level taxa in the genera *Aenetus*, *Aoraia*, *Cladoxycanus*, *Dioxycanus*, *Dumbletonius*, *Heloxycanus* and *Wiseana*.

A morphological taxonomic revision of the family (Dugdale, 1994) resulted in the delineation of four informal lineages: the *Aenetus* lineage comprising *Aenetus virescens*, the *Aoraia* lineage comprising species level taxa in the genus *Aoraia*, the *'Oxycanus' Cladoxycanus* lineage including just *Cladoxycanus minos* and the *'Oxycanus'* lineage *s. str.* comprising the genera *Dioxycanus*, *Dumbletonius*, *Heloxycanus* and *Wiseana*.

Dugdale's (1994) lineages were not subjected to an explicit phylogenetic analysis to recover phylogenetic relationships. Therefore, it is unknown whether the taxa represent monophyletic groupings, whether the two *'Oxycanus'* lineages have distinct evolutionary histories or how the four lineages relate to each other. The origins of the New Zealand hepialid fauna are also unknown although, historically, it has been considered that the New Zealand hepialids are most similar to the Australian hepialids (Meyrick, 1890; Dumbleton, 1966; Dugdale, 1989). Several scenarios can be postulated: (i) if the New Zealand fauna originated in Australia and became separated following the breakup of Gondwanaland and the formation of the Tasman Sea, then each lineage may have its nearest relatives in Australia, (ii) if the New Zealand fauna radiated *in situ* following separation from Gondwanaland, then each lineage may have its nearest relatives in New Zealand or (iii) if the New Zealand fauna arrived in several waves of dispersal from the nearest land mass, possibly Australia or New Caledonia (Stevens *et al.*, 1988), then each lineage may have its nearest relatives in Australia or New Caledonia. A phylogenetic study, using New Zealand and Australian hepialid taxa, may indicate which is the more likely scenario.

The phylogeny of the genus *Wiseana* has been estimated by MacArthur (1986) and Herbert (1995). Both used allozyme data sets and recovered two clades (*W. cervinata, W. copularis, W. fuliginea, W. jocosa, W. mimica*) and (*W. signata, W. umbraculata*). *Wiseana mimica* was not included in MacArthur's (1986) study. The larvae of *W. cervinata, W. copularis, W. fuliginea* and *W. mimica* are important improved pasture pests that defoliate vegetation (Dugdale, 1994; Herbert, 1995).

Species concepts

A phylogenetic species concept was considered most appropriate for this current study given that the goal of phylogenetics is to reconstruct the historical, hierarchical pattern of branching or cladogenesis, within a group of organisms (Hoelzer and Melnick, 1994).

Using the phylogenetic species concept, each terminal branch leads to a group of individual entities, e.g., a species, all related to the same most recent ancestor (Ghiselin, 1974), with the individual entities that comprise a species sharing a minimum of one derived character (Cracraft, 1983). The species recovered using the phylogenetic species concept are not only isolated from sister species by the lack of gene flow (the basis of the biological species concept), but also have an explicit evolutionary basis.

Criticisms of the phylogenetic species concept include over-emphasis on shared derived traits causing over-splitting of taxa (Mallet, 1995), lack of direction as to how to recover the history of the organism rather than that of the gene when using genetic data (Avise, 1994), how much genetic divergence between populations identifies the point at which there is no gene flow (Avise, 1994; Packer and Taylor, 1997), and how to recover the order of branching when there were several rapid dichotomous branching events (Hoelzer and Melnick, 1994). Application of the phylogenetic species concept to morphological data may cause an under estimation in the number of species because there may have been a lack of gene flow before the development of any morphological differences between populations (Baverstock and Moritz, 1996).

Characters

An estimate of phylogeny may be displayed in the form of a branching diagram or tree. Tree building methods developed to infer phylogeny may use character/attribute information or character information converted to distances, e.g., neighbourhood joining (Saitou and Nei, 1987) or unweighted pair-group arithmetic average (UPGMA) (Sneath and Sokal, 1973). Character data are considered to have greater informational content and are more testable compared with distance data (Avise, 1994). For example, character state changes can be traced along branches and the topology derived from character data can be checked by reference to the original data set.

Systematists have the choice of many types of characters with which to estimate phylogeny. Morphological, ecological and behavioural characters have been shown to be good indicators of phylogeny (Miller, 1996; McLennan, 1994; Paterson *et al.*, 1995) and technological developments such as gel electrophoresis, restriction enzymes, universal primers, the polymerase chain reaction and DNA nucleotide sequencing allow the use of protein and molecular characters.

Concern that morphological, ecological or behavioural characters would be more homoplasious than molecular ones has been unfounded (Hillis, 1987; Sanderson and Donoghue, 1989; De Queiroz and Wimberger, 1993). Data sets constructed from the various types of characters may be informative at different levels in a phylogeny. For example, phylogenies estimated from molecular characters may provide useful information where a morphological estimate is obscured by convergent evolution and *vice versa* (Brown *et al.*, 1994; Miller *et al.*, 1997). This is known as reciprocal illumination.

The ability to estimate phylogeny from a range of sources has been advantageous for systematists in that independent corroborations of a hypothesis can be made. Congruent phylogenies from independent data sets give confidence in the phylogenetic signal from the data (Hillis, 1987; Swofford, 1991).

Tree building methods

"To oversimplify, there has tended to be three main theoretical positions in relation to reconstructing trees: maximum likelihood devotees, those favouring distance methods, and others preferring parsimony." (Penny *et al.*, 1996).

In this study, a range of tree reconstruction methods are used, rather than preferring one particular method. Parsimony and maximum likelihood methods were applied to the molecular data but only parsimony to the morphological data.

Parsimony - Maximum parsimony methods select phylogenetic trees that have the least number of evolutionary changes and therefore minimise total tree length. In doing so, the amount of homoplasy that has to be invoked to explain the data is minimised and at the same time character congruence is maximised (Scotland, 1992). After analysis, it will be observed that the similarity inherited from the most recent common ancestor covaries with the inferred phylogeny. Some of the similarity that was initially assumed to be indicative of evolutionary relationship (i.e., homologous) may not covary with the phylogeny and is interpreted *post hoc* as being homoplasious (Brooks and McLennan, 1991).

Maximum likelihood - The maximum likelihood criterion (Edwards and Cavalli-Sforza, 1964) may be used to select the most commonly occurring branching pattern as the hypothesis of evolutionary relationships.

This method can only be used with DNA sequence data, as a probabilistic model of evolution, including pattern of nucleotide substitution, variation in substitution rates across sites and branch lengths, has to be specified. The maximum likelihood method evaluates all tree topologies, and using the criteria from the pre-specified model of evolution, estimates the likelihood of finding the observed base distribution among the taxa at all sites in the data. The method chooses the tree(s) that have the largest value for the likelihood estimate as the optimal tree(s). Several models of evolution have been proposed. The Jukes-Cantor model assumes that the four DNA nucleotides are equally likely to occur, as are transition and transversion substitutions (Jukes and Cantor, 1969). However, this model is now known to be an over simplification (Yang, 1996) as transition substitutions occur more frequently than transversions, especially when closely related taxa are under consideration. The Kimura two-parameter model applied in this study, assumes that the four nucleotides are equally frequent and that there are independent rates of transitions and transversions (Kimura, 1980). More complex models such as the HKY85 (Hasegawa *et al*, 1985) that allow different rates of transitions and transversion and rate heterogeneity among sites are available, but are more computationally intensive, especially with large numbers of taxa.

Accuracy of methods

Congruent phylogenies produced for a data set by different methods give confidence to the accuracy of the methods (Kim, 1993; Hillis, 1995; Miyamoto and Fitch, 1995). For accurate reconstruction of branching relationships, consistency and robustness are important (Hillis, 1995).

A method is said to be consistent if it converges on the correct tree as more data are added. Parsimony is known to become inconsistent when there are unequal rates of evolution along branches (Crozier, 1993; Yang, 1996). Long branches may become paired, i.e., long branch attraction (Hendy and Penny, 1989), because there are more changes along these branches and by chance some of the changes may correspond. These are interpreted as being homologous, but in fact, have occurred independently. Maximum likelihood, however, accounts for branch length and considers changes are more likely on long branches compared to short ones (Swofford *et al.*, 1996).

The robustness of a method to violations of its assumptions affects the accuracy of reconstruction.

When the assumptions associated with parsimony, such as constant rate of substitution between nucleotides and across sites and equal branch lengths among lineages are violated, the method becomes less likely to recover the true topology (Penny *et al.*, 1992; Yang, 1996). Maximum likelihood methods are considered more robust in this regard than parsimony (Yang, 1996).

Congruence between different data sets, generally, implies accuracy (Cunningham, 1997), but it is possible to find improved congruence but not necessarily increased accuracy, if inappropriate methods are used. For example, if transversion parsimony is applied, there may appear to be increased congruence between different data sets, but this may be due to loss of resolution, because transition substitutions are ignored (Allard and Carpenter, 1996; Cunningham, 1997).

Resolving conflict - within tree building methods

Both maximum parsimony and maximum likelihood methods may produce more than one equally likely tree that satisfies the criteria of the method. Information in common on these trees may be summarized on a consensus tree. Strict consensus trees (Sokal and Rohlf, 1981) summarize information found that is common to all trees, while majority rule consensus trees (Margush and McMorris, 1981) summarize information in common to at least 50% of the trees.

Resolving conflict - between tree building methods

Since each extant taxon has only one evolutionary history (Hillis, 1987), estimates of phylogeny from different types of characters should be congruent. However, this is rarely the case (Kluge, 1989; Smith, 1992). The effects of incongruence on phylogenetic accuracy (Huelsenbeck *et al.*, 1996; Cunningham, 1997) and the best method to summarize information are currently major unresolved issues facing systematists. Data sets are either analysed separately and areas of agreement summarized on a consensus tree (taxonomic congruence) (Miyamoto and Fitch, 1995) or all data are combined into one data set and analysed (total evidence) (Kluge, 1989).

Non-tree building methods of inferring phylogeny

Spectral analysis (Hendy and Penny, 1993) allows testing of alternative hypotheses of relationship without necessarily inferring a phylogenetic tree. The 'spectrum' generated by this method though, represents the estimated branch lengths of a phylogenetic tree.

'Spectrum' PPC Version 2.0 (Charleston, 1997) converts nucleotide sequence data into purines and pyrimidines and assesses all possible bipartitions (splits) in the data. The length of a split represents the expected number of character-state changes per site, i.e., the length of a branch. The closer the data match the estimated branch lengths, the higher the support value. An advantage of this method is that support for more than one hypothesis of relationship between particular bipartitions of taxa may be uncovered and this may explain the lack of confidence for a particular node on an inferred tree (Lento *et al.*, 1995).

Testing evolutionary hypotheses

Systematics today has flexible, yet rigorous methods for producing testable hypotheses of evolutionary relationships. Accuracy of assumptions made regarding the evolutionary process (Huelsenbeck and Crandall, 1997) and accuracy of the hypothesis (Hillis, 1995) can be assessed before the application of the phylogeny. For example, an independent test of the homology that was assigned to morphological characters can be made if they are mapped on to a phylogenetic tree derived from a molecular data set (Hillis, 1987). If the morphological characters are indeed homologous, they should covary with the molecular phylogeny.

Estimation of phylogeny is one of the goals of systematics (Miyamoto and Cracraft, 1991; Savage, 1995) and is increasingly recognised as an essential prerequisite for the interpretation of biological systems (Hillis, 1995). A robust and accurate phylogeny can strengthen inferences made in comparative studies (Nee *et al.*, 1996), provide explanations for evolutionary patterns and processes (Brooks and McLennan, 1991) and be used as a basis for 'natural' classifications that have increased predictive value (Cranston *et al.*, 1994; Wheeler, 1995).

The evolutionary history of the *'Oxycanus'* lineages of hepialid moths in New Zealand

Data collected for this study were obtained from individual specimens recognisable using morphological descriptions (Dugdale, 1994). The descriptions were based on a morphological species concept that included consideration of life history, geographical distribution, shared similarities and a detailed knowledge of the South Pacific hepialid fauna. The current study compares a modern classification prepared in a traditional way (Dugdale, 1994), with one produced by phylogenetic methods.

Control of *Wiseana* pest species has been hampered by the confusion over the number, identification and distribution of 'species' (Barratt *et al.*, 1990). The current study has potential to improve our understanding of *Wiseana* systematics, which in turn will enable more intensive ecological studies and improved pest control.

Aims - The aims of this thesis are to produce a robust and accurate phylogeny for the '*Oxycanus*' lineages of hepialid moths from New Zealand and to investigate the relationship of taxa in the genus *Wiseana*.

These aims are achieved by:
(i) inferring phylogenies from a morphological data set and molecular data sets from the mitochondrial DNA (mtDNA) cytochrome oxidase subunit I and II (COI & II) region and the nuclear ribosomal DNA (nrDNA) internal transcribed spacer-2 (ITS2) region,

(ii) summarizing information from these phylogenies using the taxonomic congruence and total evidence methods,

(iii) hypothesising the evolution of morphological characters by tracing them onto a phylogeny derived from the mtDNA COI & II data set, and

(iv) developing a diagnostic test for taxa in the genus *Wiseana* from restriction fragment length polymorphisms (RFLPs) of polymerase chain reaction (PCR) product from the mtDNA COI & II regions.

Structure of the thesis

This thesis represents work commenced in February 1995 under the supervision of Dr Rowan Emberson and Dr Adrian Paterson. The thesis chapters, each contributing to the overall aims of the thesis, have been written as scientific papers for submission to journals. Stylistic differences between chapters reflects this. The structure of the thesis is as follows:

Chapter 2 - *Phylogeny of New Zealand hepialid moths (Lepidoptera: Hepialidae) inferred from a cladistic analysis of morphological data.* Two data sets are constructed and analysed cladistically using parsimony. The first data set comprises 64 morphological characters, 40 of which are drawn from adult males. The remainder come from adult females, larval and pupal life stages.

The second data set comprises 40 morphological characters from adult males from the New Zealand taxa and the Australian genera *Jeana, Oxycanus* and *Trictena.*

Chapter 3 - *Phylogeny and biogeography of 'Oxycanus' lineages of hepialid moths from New Zealand using sequence variation in the mtDNA COI & II gene regions.* A 527 character molecular data set is constructed with nucleotide sequences from the mtDNA COI & II gene regions for New Zealand taxa and the Australian genera *Jeana, Oxycanus* and *Trictena.* Nucleotide weighting schemes are explored and the data are analysed using parsimony and maximum likelihood methods. A relative rates test is applied to assess the rate of change along each lineage and biogeographical implications are discussed.

Chapter 4 - *Phylogeny of the New Zealand 'Oxycanus' lineages of hepialid moths inferred from the nuclear ribosomal ITS2 region.* A molecular data set of nucleotides from the nrDNA ITS2 region is constructed and analysed using parsimony and maximum likelihood methods. Various data sets are explored including the genus *Wiseana* alone, New Zealand *'Oxycanus'* lineages, all New Zealand taxa and New Zealand and Australian taxa. Results are discussed in relation to findings from the morphological and COI & II data sets.

Chapter 5 - *Phylogenetic relationships of the 'Oxycanus' lineages of Hepialid moths from New Zealand inferred from combined morphological and molecular data sets.* The utility of the taxonomic congruence and total evidence methods is compared and factors affecting the accuracy of phylogenetic hypotheses produced by each method are discussed. Results are compared with those produced from the separate analysis of morphological, COI & II and ITS2 data sets.

Chapter 6 - *Morphological character evolution in hepialid moths (Lepidoptera: Hepialidae) from New Zealand.* In order to trace the evolution of individual morphological characters, they are mapped onto a phylogenetic tree reconstructed from the mtDNA COI & II data set. The Kruskal-Wallis test is implemented to assess whether any particular character partition covaries significantly with phylogeny. Alternative explanations for character state evolution are hypothesised and evaluated.

Chapter 7 - *Identification of Wiseana (Lepidoptera: Hepialidae) from New Zealand by PCR and restriction fragment length polymorphisms (RFLPs).*

A method is described for identifying members of the genus *Wiseana*. PCR product from the mtDNA COI & II region is cleaved with restriction enzymes. The advantages of this methodology and the practical application of the results are discussed.

Chapter 8 - *General Conclusions*. Evidence for general conclusions, from previous chapters, is synthesised and questions arising from this thesis are outlined.

These chapters represent papers co-authored with my supervisors, Drs Rowan Emberson and Adrian Paterson (chapters 2-7) and with John Dugdale (chapter 2). I collected many of the specimens, carried out all of the laboratory work, data collection, analysis and writing reported in these papers. The co-authors provided advice on New Zealand hepialid taxonomy, systematic methods, phylogenetic theory and analysis and writing style.

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Chapter 2

Phylogeny of New Zealand hepialid moths (Lepidoptera: Hepialidae) inferred from a cladistic analysis of morphological data

B. Brown, J.S. Dugdale, R.M. Emberson and A.M. Paterson

Abstract

The phylogeny of the New Zealand hepialid moths was estimated from a cladistic analysis of 64 morphological characters, from all life cycle stages. One hundred and sixteen maximum parsimony trees were produced. The phylogenetic reconstruction indicated that the currently recognised generic concepts and the four informal lineages hypothesised in a previous morphological taxonomic revision, were monophyletic. The relationships of species within the genus *Wiseana* were not fully resolved. Analysis of a data set of 40 characters from adult males from the New Zealand taxa and the Australian genera *Jeana, Oxycanus* and *Trictena*, supported the monophyly of the New Zealand '*Oxycanus*' lineage *s. str.*

Key words - Hepialidae, *'Oxycanus' Cladoxycanus, 'Oxycanus' s. str.*, phylogeny, morphology, parsimony.

Status - Submitted to Systematic Entomology

Introduction

The family Hepialidae in New Zealand comprises 27 endemic species level taxa in the genera *Aenetus*, *Aoraia*, *Cladoxycanus*, *Dioxycanus*, *Dumbletonius*, *Heloxycanus* and *Wiseana*. They form part of the Hepialidae *sensu stricto* (Nielsen and Scoble, 1986), one of 12 monophyletic clades within the superfamily Hepialoidea (Nielsen, 1989). An overall picture of hepialid relationships in the Southern Hemisphere has not yet emerged as only the southern South American fauna (Nielsen and Robinson, 1983), the primitive *Fraus* from Australia (Nielsen and Kristensen, 1989) and the New Zealand fauna (Dugdale, 1994) have been comprehensively examined. Dugdale (1994) carried out a taxonomic revision of the New Zealand family based on morphology. The taxa fell into four distinct groupings with characteristics that he believed indicated possible affiliations with taxa in Australia and further afield (J. Dugdale, pers. comm.). Consequently, he proposed four informal lineages for the New Zealand fauna, the *Aenetus* lineage, *Aoraia*, *'Oxycanus' s. str.* and *'Oxycanus' Cladoxycanus* (Table 1). These hypothesised lineages were not subjected to an explicit cladistic analysis to reconstruct their phylogenetic relationships. Only within the genus *Wiseana*, where larvae of some species defoliate improved pasture, has any phylogenetic analysis been attempted. MacArthur (1986) and Herbert (1995) both reconstructed relationships within *Wiseana* using allozyme data.

One of the goals of biosystematics is to produce stable classifications (Marcus, 1993) that reflect evolutionary history (Miyamoto and Cracraft, 1991). Cladistic analysis of data provides a repeatable and robust method for reconstructing monophyletic groups from independent, homologous characters (Simpson and Cracraft, 1995; Miller, 1996). In this paper, we present a cladistic analysis of morphological data for the New Zealand Hepialidae. We explicitly test the hypotheses of Dugdale (1994) regarding the monophyly of the *'Oxycanus'* lineages with a view to establishing a stable and robust classification. A further aim was to investigate the relationship of taxa in the genus *Wiseana* which has several pest species and a history of taxonomic instability.

Current Classification of the New Zealand genera and species:

(i) The *Aenetus* lineage - There is one species of *Aenetus* in New Zealand, *Aenetus virescens*. Adult males are recognised by their triangular, green-patterned forewings and pale coloured hindwings, while females have more elongate forewings and deeply coloured hindwings. Both sexes have an emarginate termen. The genus occurs in Australia, New Caledonia, New Guinea and the islands of the Banda Arc. The New Zealand species differs from *A. cohici* in New Caledonia and *Aenetus* in Australia in the structure of the labial palpi, male hind tibia, male and female postabdomen, male valvae and female anogenital field. Based on larval, pupal and biological characters, *Zelotypia* from Australia and *Endoclita* from South East Asia are also included in this lineage (Dugdale, 1994).

(ii) The *Aoraia* lineage - This lineage comprises 13 species all belonging to the genus *Aoraia* (Table 1). Individuals are relatively large in size, with shaggy vestiture and intricate wing patterning. Females of several taxa are brachypterous. There is currently no evidence to suggests a close relationship between *Aoraia* and any other members of the Hepialidae *s. str.* Both the *Aenetus* and *Aoraia* lineages have wing venation patterns where the radial veins R_4 and R_5 are on a common stem which is separate from the R_{2+3} stem (Fig. 1A).

(iii) The *'Oxycanus'* lineages - The arrangement of the radial veins in the *'Oxycanus'* lineages (Fig. 1B) differs from that of the *Aenetus* and *Aoraia* lineages. In the *'Oxycanus'* lineages, the radial veins R_4 and R_5 each arise separately from the R_{2+3} stem. *'Oxycanus'* lineages are also present in Australia, New Guinea, Borneo, through to the Orient (Dugdale, 1994) and in South America (Nielsen and Robinson, 1983). Two New Zealand lineages have been hypothesised based on differences in male and female genitalia.

(iiia) The *'Oxycanus'* lineage *s. str.* - The *'Oxycanus'* lineage *s. str.* comprises 12 taxa in the four genera: *Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana* (Table 1). Taxa in this lineage have antennal flagellomeres with basal and apical prominences, dorsal surfaces of the proximal antennal flagellomeres unscaled or sparsely scaled and a dorsally elaborate and well developed male pseudotegumen with twin processes supporting the anal tube. The female anogenital field is higher than wide, with an ovipore at or above mid-field height.

The *corpus bursae* is ovoid with a large or small appendix, and the female tergum 8 caudal margin has a prominent, dense, broad tuft of long hair-like scales.

(iiib) *'Oxycanus'* lineage *Cladoxycanus* - This lineage is represented in New Zealand by *Cladoxycanus minos*. In comparison with the taxa in the *'Oxycanus'* lineage *s. str.*, *Cladoxycanus* has no arolium on the adult pretarsus, no sclerotised bridge between the apices of the pseudotegumen, labial palpus basal segments with rami, and forewing discal cell apex (vein r-m) before half wing length. The female dorsal plate has sclerotised setose lobes fused in dorsal midline, forming a mesal tubercle and larvae are without metathoracic or abdominal sclerites. *Cladoxycanus* has no known close relatives, although *Calada fuegensis* Nielsen and Robinson, from southern South America, also lacks an arolium and has no sclerotised bridge between the apices of the pseudotegumen (Dugdale, 1994).

This is not the first time that a grouping of the New Zealand and Southern Hemisphere hepialids has been proposed. Dumbleton (1966) erected two subfamilies, Hepialinae and Oxycaninae, based on wing venation characters. Hepialinae included *Abantiades* Herrick-Schäffer, *Aenetus* Herrick-Schäffer, *Aoraia* Dumbleton, *Bordaia* Tindale, *Fraus* Walker, *Oncopera* Walker, *Phassodes* Bethune-Baker, *Dalaca* Walker (as *Toenga* Tindale), *Trictena* Meyrick and *Zelotypia* Scott. Oxycaninae included *Cladoxycanus* Dumbleton, *Dioxycanus* Dumbleton, *Elhamma* Walker, *Jeana* Tindale, *Oxycanus* Walker, *Paraoxycanus* Viette, *Trioxycanus* Dumbleton and *Wiseana* Viette. However, it is now considered that both these subfamilies are paraphyletic (Nielsen and Robinson, 1983).

Fig. 1: Wing venation. (A) Wing venation of the *Aenetus* and *Aoraia* lineages of hepialid moths in New Zealand; (B) Wing venation of the *'Oxycanus'* lineages of hepialid moths in New Zealand.

Table 1: Composition of the New Zealand hepialid lineages.

Lineage	Species
Aenetus lineage	*Aenetus virescens* Doubleday, 1843
Aoraia lineage	*Aoraia aspina* Dugdale, 1994
	A. aurimaculata (Philpott, 1914)
	A. dinodes (Meyrick, 1890)
	A. ensyii (Butler, 1877)
	A. flavida Dugdale, 1994
	A. hespera Dugdale, 1994
	A. insularis Dugdale, 1994
	A. lenis Dugdale, 1994
	A. macropis Dugdale, 1994
	A. oreobolae Dugdale, 1994
	A. orientalis Dugdale, 1994
	A. rufivena Dugdale, 1994
	A. senex (Hudson, 1908)
'*Oxycanus*' lineage *sensu stricto*	*Dioxycanus fuscus* (Philpott, 1914)
	Dioxycanus oreas (Hudson, 1920)
	Dumbletonius characterifer (Walker, 1865)
	Dumbletonius unimaculatus (Salmon, 1948)
	Heloxycanus patricki Dugdale, 1994
	Wiseana cervinata (Walker, 1865)
	W. copularis (Meyrick, 1922)
	W. fuliginea (Butler, 1879)
	W. jocosa (Meyrick, 1912)
	W. mimica (Philpott, 1923)
	W. signata (Walker, 1856)
	W. umbraculata (Guenée, 1868)
'*Oxycanus*' lineage *Cladoxycanus*	*Cladoxycanus minos* (Hudson, 1905)

Materials and methods

Characters - Morphological characters from adult males formed the basis of this study as males were easily collected at light and could be reliably identified using a combination of antennal, scale and genitalic characters (Dugdale, 1994). Freshly killed, positively-identified specimens were stored in 96% ethanol. The head, prothorax and genitalia of males were treated in 10% potassium hydroxide (KOH) overnight, cleaned, stained with Chlorazol Black E (Comak Chemicals), fixed using a hot KOH solution and stored in 70% ethanol.

Female, larval and pupal characters were taken from Dugdale (1994). All characters were checked using specimens stored in alcohol from the New Zealand Arthropod Collection, Landcare Research, Auckland. Fuller morphological descriptions of taxa are available in Dugdale (1994).

Cladistic Analysis - Cladistic analyses of the morphological data set were undertaken in PAUP 3.1 (Swofford, 1993) with the heuristic search option (10 random addition sequences). The data were tested for significant cladistic signal using the G_1 statistic (Hillis and Huelsenbeck, 1992) in PAUP and bootstrap analyses (Felsenstein, 1985) were performed on the data to examine the robustness of the branches. The Consistency Index (CI) (Kluge and Farris, 1969) was used to measure the level of homoplasy in the data set. The Retention Index (RI) (Farris, 1989) also measured support for trees based on the proportion of similarity due to synapomorphy only.

Terminal taxa and data matrix - The terminal taxa included in this analysis were 17 of the 27 known New Zealand hepialids. Only three of the thirteen taxa from the *Aoraia* lineage were used as exemplars in the data set because the focus of the study was the relationships of the '*Oxycanus*' lineages. Sixty four characters were scored for each taxon. Twenty seven characters were coded as binary, the remainder being multistate. All characters were unordered and equally weighted and are described in Appendix 1. The final data matrix (Appendix 2) consisted of one species of *Aenetus*, three species of *Aoraia*, seven species of *Wiseana*, two species each of *Dumbletonius* and *Dioxycanus* and one species each of *Cladoxycanus* and *Heloxycanus* and 64 characters.

Outgroups - An outgroup (Watrous and Wheeler, 1981) enables the cladogram to be rooted. Outgroup rooting in morphological data is best achieved by including the two closest successive sister groups (Smith, 1994; Baverstock and Moritz, 1996). It is assumed that the remainder of the taxa in the analysis (i.e., the ingroup) are a monophyletic group, sharing synapomorphies. In identifying inclusive synapomorphies, it is important that the characters being compared between the ingroup and the outgroup are in fact equivalent structures (Eldridge and Cracraft, 1980; Page *et al.*, 1995). It may be that identification of inclusive synapomorphies is not possible, in which case information from previous classifications may be used to select outgroups (Hillis, 1985; Nixon and Carpenter, 1993). A rooted cladogram allows character polarity to be determined (Smith, 1994). Character polarity indicates the direction of character state evolution but not the order of character states (Kitching, 1992).

Aenetus virescens, *Aoraia ensyii*, *A. lenis* and *A. rufivena* were selected as outgroups because although they are included in the Hepialidae *s. str.* with the other New Zealand taxa, they have never been considered closely related to the '*Oxycanus*' lineages (Dugdale, 1994; Dumbleton, 1966). An outgroup from Dugdale's '*Oxycanus*' lineages or Dumbleton's Oxycaninae was not selected because of lack of evidence as to what constituted the ingroup.

Results

Parsimony analysis of the morphological data resulted in 116 most parsimonous (MP) cladograms with a length of 170 steps, CI of 0.76 and RI of 0.83. Tree length distribution was left-skewed with a G_1 index of -0.86. The probability associated with this value would indicate strong cladistic signal in the data. The trees are summarized as a majority rule consensus tree in Fig. 2. Synapomorphies provide the main evidence for relationships and bootstrap analysis provides information on the relative support for a clade within a tree (Olmstead and Sweere, 1994). The clades and bootstrap values are shown in Fig. 2 and synapomorphies are shown in Fig. 3. Five hundred bootstrap replications gave support above the 70% level for seven of the 12 internal branches. Although bootstrapping does not measure accuracy, internal branches with this level of support usually indicate true clades (Hillis and Bull, 1993). All of the low bootstrap values occurred within the genus *Wiseana*.

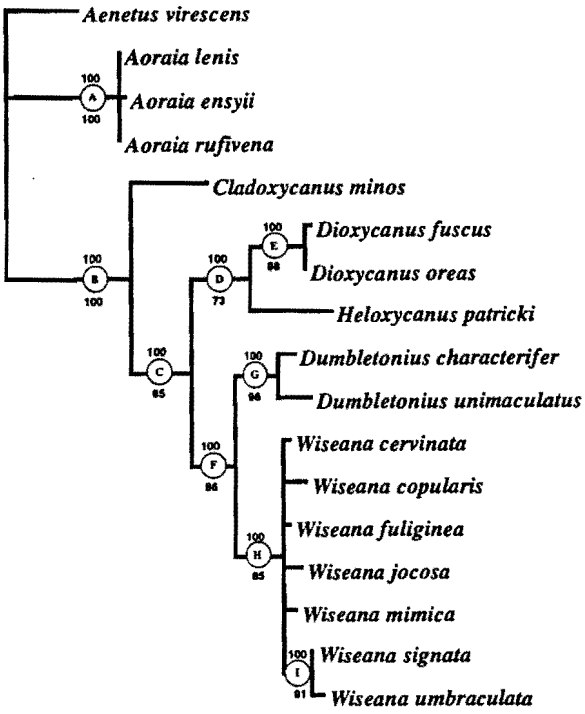

Fig. 2: Majority rule consensus of the 116 most parsimonious trees for the New Zealand Hepialidae. Branch lengths are proportional to morphological character state change. Clades are identified by the letters inside circles, majority rule consensus values are above the circle and bootstrap values are below.

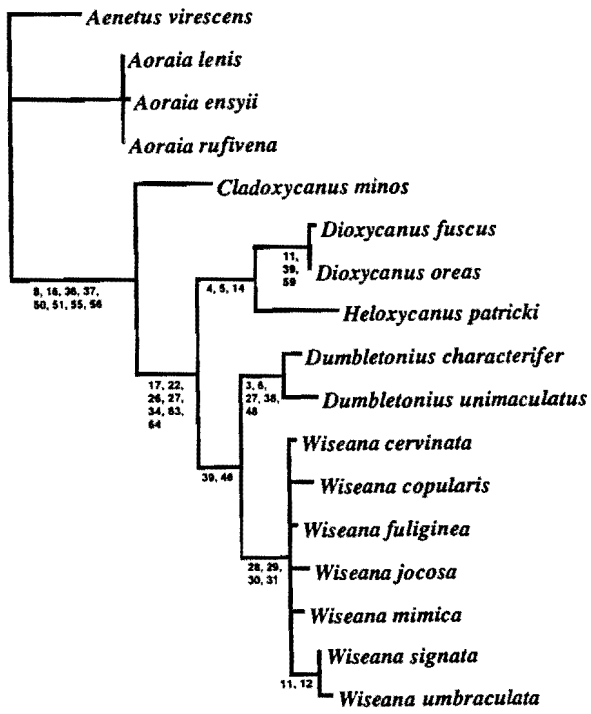

Fig. 3: Majority rule consensus phylogram of the 116 most parsimonious trees for the New Zealand Hepialidae indicating unambiguous synapomorphies. Branch lengths are proportional to morphological character state change.

Discussion

Character choice for this analysis was driven by two factors: (i) the need for reliable and quick identification of specimens, especially within the genus *Wiseana*, hence adult males were collected and the majority of characters came from male specimens, and (ii) the need to find characters that might indicate how taxa within *Wiseana* relate to each other. Adult female, larval and pupal characters from Dugdale (1994) were added in an attempt to improve the resolution of interior nodes.

The 116 MP trees recovered in this analysis showed congruent topologies apart from within the genus *Wiseana*. The analysis indicated that the generic concepts in the current classification are monophyletic. Clade A (*Aoraia*), Clade E (*Dioxycanus*), Clade G (*Dumbletonius*) and Clade H (*Wiseana*) were supported by synapomorphies and high bootstrap proportions (Figs. 2, 3). The monotypic genera *Aenetus*, *Cladoxycanus* and *Heloxycanus* were supported by autapomorphic states.

***Aenetus* and *Aoraia* lineages** - *Aenetus* and *Aoraia* have many synapomorphies that support their monophyly. It is not known if the synapomorphies for *Aenetus* will apply to the genus outside New Zealand, but a revision of Australian *Aenetus* is underway (J. Dugdale, pers. comm.). In *Aenetus*, the synapomorphies are from adult males, adult females, larval and pupal stages and in *Aoraia* are from all life stages except the pupal (Table 2). This analysis supports the current classification with *Aenetus* and *Aoraia* as separate lineages.

Table 2: Synapomorphies that support the New Zealand Hepialid genera *Aenetus* and *Aoraia*.

Lineage	Character Number	Character State Description ( ) character state number
Aenetus	7	(0) contiguous labial palps
	10	(0) clypeus higher than wide
	11	(0) moniliform antennae with flagellomeres having no rami
	14	(1) complete and uniform covering of microsensillae
	15	(0) no sensilla chaetica on the central dorsal surface of flagellomeres.
	18	(1) hindwing Sc and R_1 veins curved towards the costa
	21	(0) green hindwings
	22	(0) triangular episternal tooth not reaching the ventral base of the laterocervicale
	25	(0) tergum 9 strongly sclerotized
	28	(0) parallel sided pseudotegumen
	36	(0) no strongly defined trulleum sclerite
	51	(0) larval with head stemmata in two parallel rows
	55	(2) large prosternum on larval abdominal segments A3-6, not fused to V_1 pinacula
	57	(2) antennal scape and pedicel form a crenulate carina
	59	(0) plane frons

Table 2 continued.

Lineage	Character Number	Character State Description ( ) character state number
Aoraia	1	(1) distinct mound-like labral sclerite
	2	(1) mandibles reduced to plate on epicranial wall
	11	(3) long, ovate rami posteriorly placed on antennal flagellomeres
	12	(1) rami not as wide as flagellomere shaft
	14	(0) no microsensilla on ventral surface of antennae
	22	(1) slender episternal tooth not reaching the ventral base of laterocervicale
	31	(3) long, slender, subspinose-acuminate ventral processes on pseudotegumen
	33	(2) short, thumb-like supraphallic papilla
	36	(1) trulleum lightly sclerotized with mesal conical process
	40	(1) widely separated S7 & 8 in females
	41	(1) female spiracle 8 anteroventral to anterolateral corner of T8
	42	(1) female S9 triangular
	44	(1) weak sclerotization of female dorsal plate midline
	51	(1) ventral row of stemmata on larval head capsule curved round antennal base
	55	(0) larval A3-6 with prosternum fused to V_1 pinacula

The *'Oxycanus'* lineages - Clade B identified as a monophyletic grouping, corresponds to Dugdale's *'Oxycanus'* lineages. This clade is supported by eight synapomorphies and 100% bootstrap support. All adult males have palpi inserted directly into the prelabium (Character 8), (Fig. 4 A2), oxycanus wing venation (Character 16), (Fig. 1B), strongly sclerotized trulleum sclerite (Character 36) and unarmed valvae (Character 37), (Fig. 4 B1). Adult females have a long tuft of hair-like scales on T8 (Character 50). Larvae have stemmata in two parallel arcs around the antennal base (Character 51), a prosternum separate from the V_1 pinaculum on the abdominal segments (Character 55) and the metathoracic seta L_3 not on pinaculum SD_1 or SD_2 (Character 56).

This analysis supports a monophyletic clade C, that corresponds to Dugdale's *'Oxycanus'* lineage *s. str.* Clade C is supported by seven synapomorphies and 65% bootstrap support. Unfused hindwing Sc and R_1 veins (Character 17), (Fig. 4 C1), slender episternal tooth reaching the distal margin of the laterocervicale (Character 22), (Fig. 4 D3), dorsal hood present (Character 26), twin processes present (Character 27), (Fig. 4 E) and a flange on the posterior margin of the saccus (Character 34), (Fig. 4 F) are all present in the *'Oxycanus' s. str.* adult males. Only D_1 seta are present on the pupal abdominal segments (Character 63) and a short carina is present on the pupal segments A4-6 (Character 64).

Dugdale (1994) identified *Cladoxycanus* as being morphologically distinct from the *Heloxycanus, Dioxycanus, Dumbletonius* and *Wiseana* taxa, placing it in a separate informal lineage. In this data set, *Cladoxycanus* has 12 autapomorphic character states supporting its morphological distinctiveness and does not form part of clade C described above. Is this sufficient evidence to support *Cladoxycanus* as a terminal taxon and a separate lineage rather than a primitive member of the *'Oxycanus'* lineage *s. str.*? To explore this we employed mid-point rooting (Farris, 1972), where the root is placed midway between the most divergent taxa. Mid-point rooting enables trees to be rooted in cases where the outgroup is unknown. *Cladoxycanus* grouped with *Aenetus* and *Aoraia*, indicating that it was closer to them than the members of the *'Oxycanus'* lineage *s. str.* (Figure 5). However, this would only hold true if the assumptions behind mid-point rooting, i.e., that the rate of change between the most divergent taxa was equal, had not been violated (Hillis, 1985).

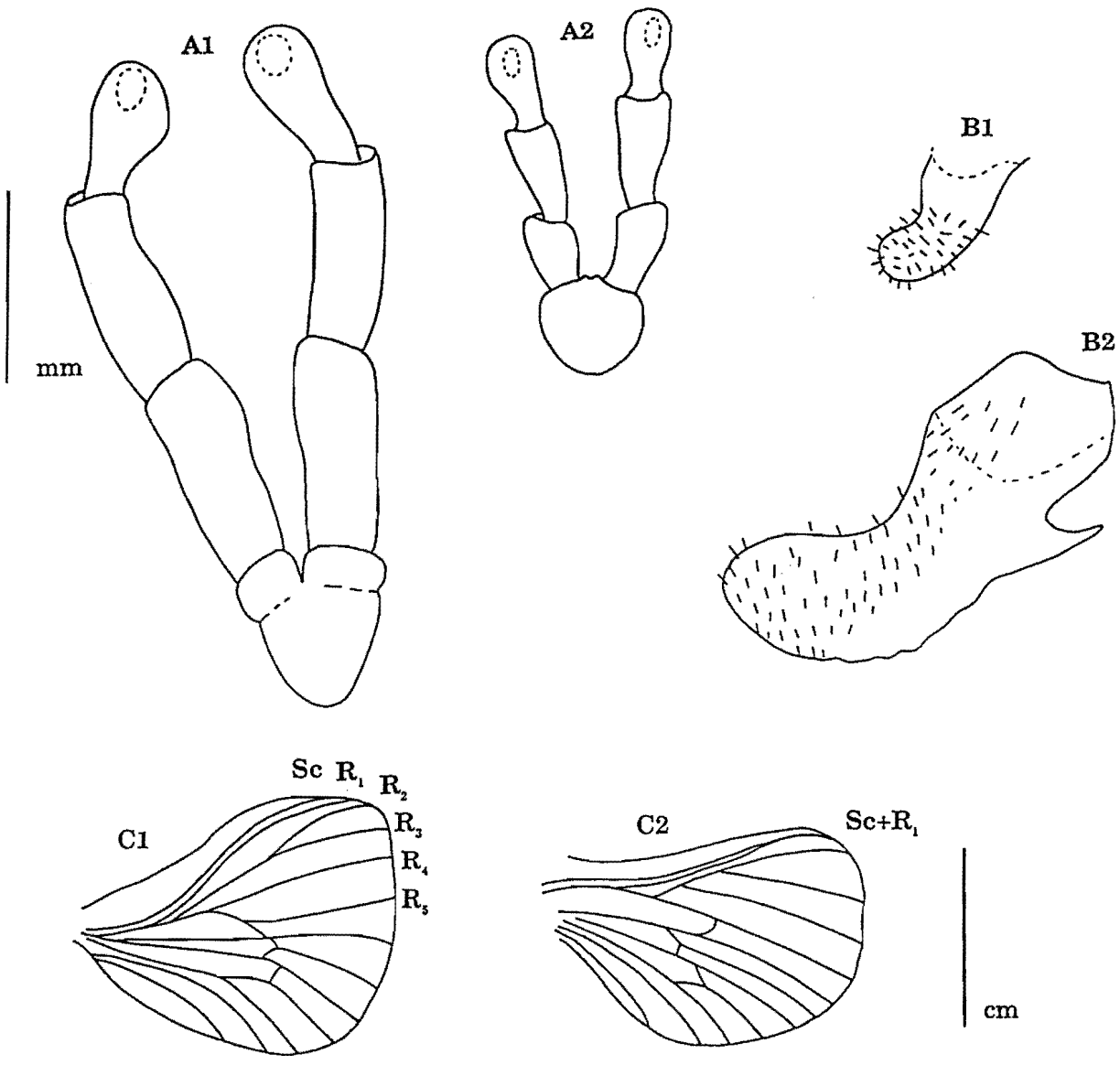

Fig. 4: Taxonomic features of New Zealand hepialids. (A) Labial palpi insertions: A1, raised; A2, not raised. (B) Valvae: B1, unarmed; B2, armed. (C) Hindwing Sc and R_1 veins: C1, not fused apically; C2, fused apically.

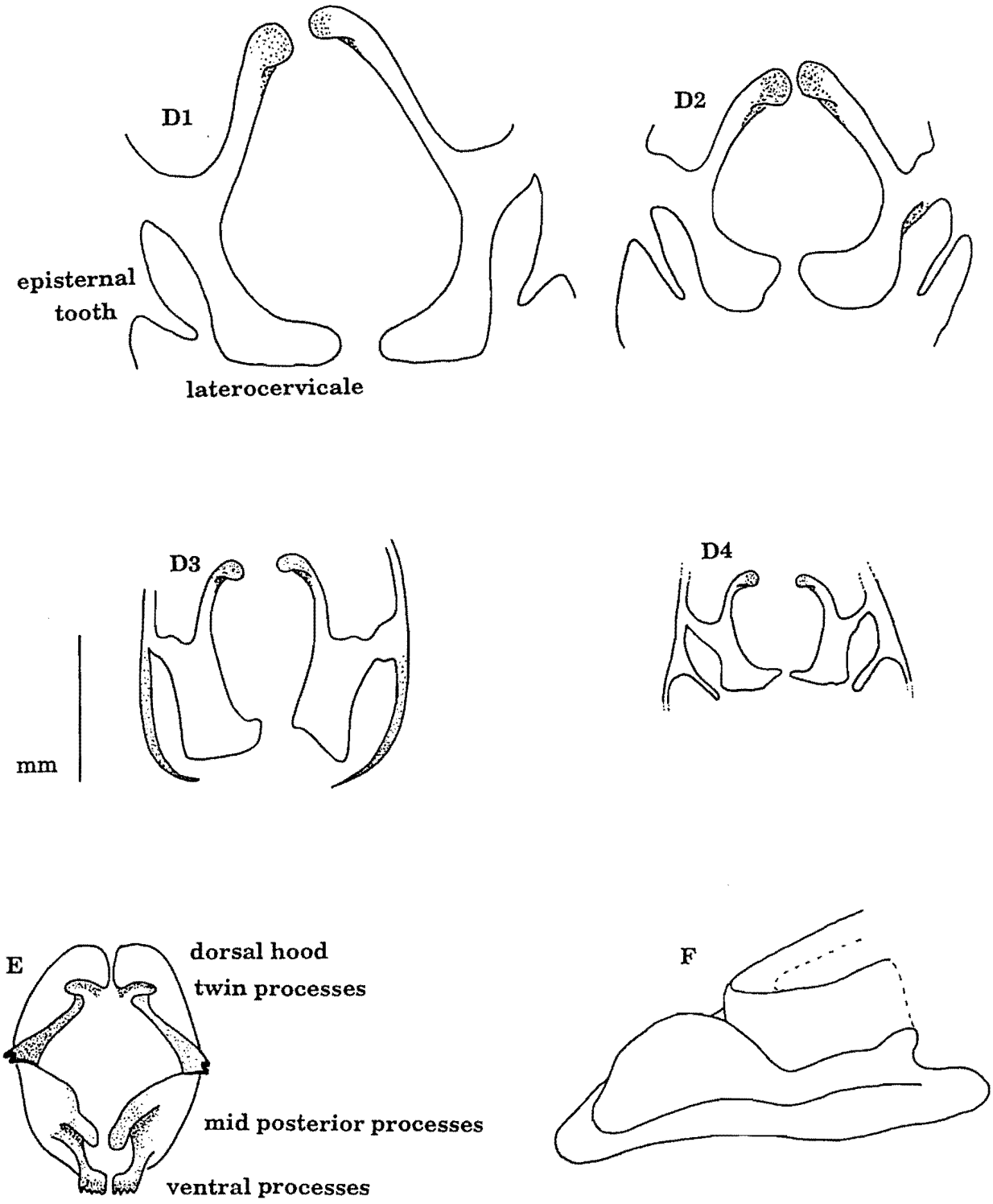

Fig. 4: Taxonomic features of New Zealand hepialids continued. (D) Episternal tooth, prothorax: D1, triangular and not reaching the ventral margin of the laterocervicale; D2, slender and not reaching the ventral margin of the laterocervicale; D3, slender and reaching the ventral margin of the laterocervicale; D4, strap-like and reaching the ventral margin of the laterocervicale. (E) Adult male pseudotegumen showing dorsal hood, twin, mid posterior and ventral processes. (F) Lateral view of the saccus, vinculum arm and flange (*Dumbletonius unimaculatus*).

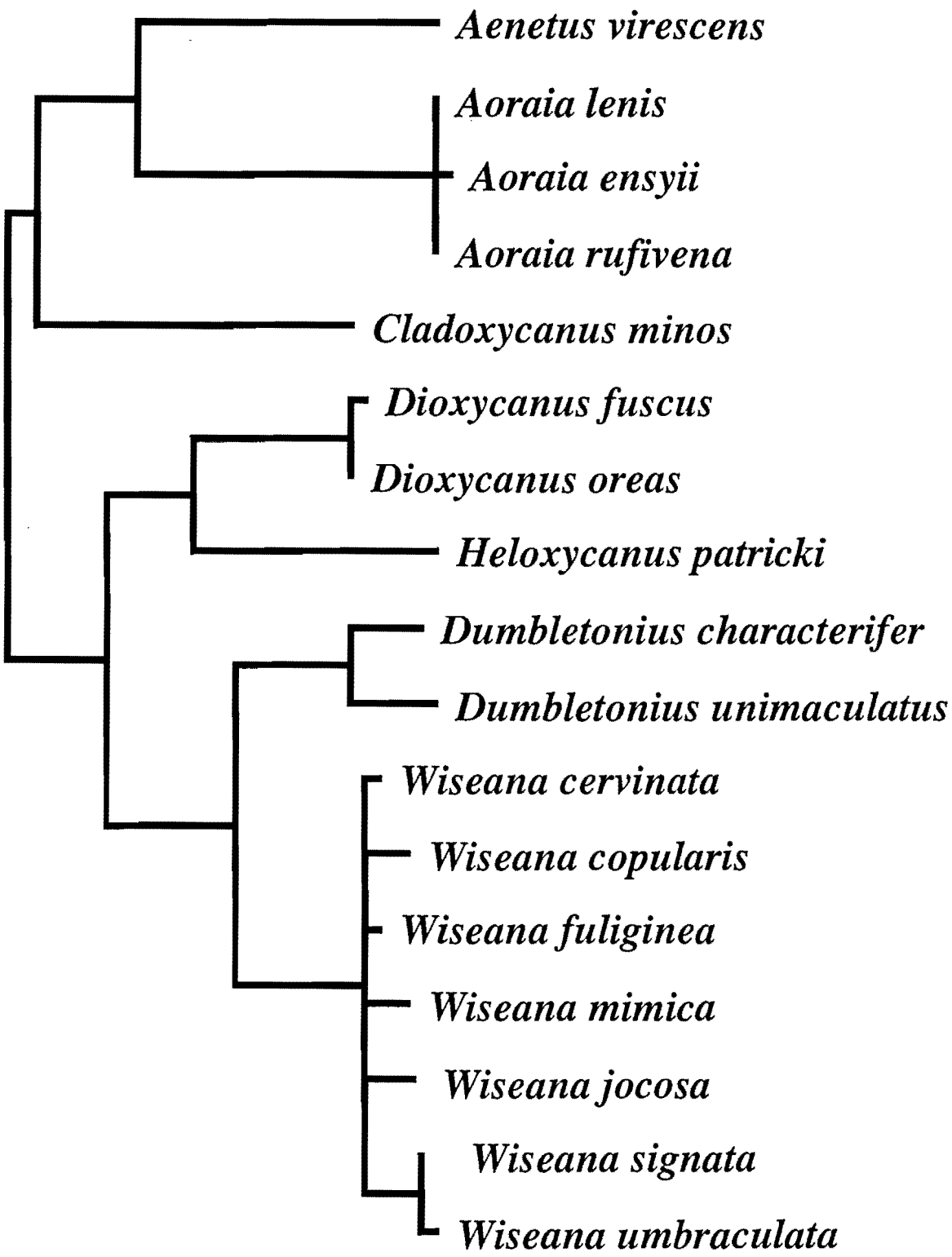

Fig. 5: Majority rule consensus phylogram of the 116 most parsimonius trees for the New Zealand Hepialidae, using mid-point rooting. Branch lengths are proportional to morphological character state change.

Wiseana - The arrangement of *Dumbletonius* species as sister taxa to *Wiseana* (Clade F) does not agree with previous hypotheses. Dugdale (1994) considered that these two taxa had little in common and considered that *Dioxycanus* and *Heloxycanus* were the sister taxa of *Wiseana*. In this analysis, Clade F (Fig. 3) is supported by two synapomorphies from adult females: cuticular processes on T8 (Character 39) and an ovipore on a strongly bilobed and erect papilla (Character 46). (However, if only male characters are analysed, *Dioxycanus* and *Heloxycanus* become the sister taxa to *Wiseana*; see below and Fig. 7).

All synapomorphies that support *Wiseana* (Clade H) come from the pseudotegumen of the male genitalia. The pseudotegumen of *Wiseana* differs from all other taxa in that the sclerites are bowed (Character 28), the pseudotegumen dorsal margin is rounded and not produced into a flange (Character 29), the mid posterior processes are apically truncate (Character 30) and the ventral processes are thick, short and apically dentate (Character 31), (Fig. 4E). However, few synapomorphies support unequivocal relationships within this clade. Although Philpott (1927) described and figured the male genitalia of six of the seven currently recognised species of *Wiseana* as *Porina*, the genus has a long history of taxonomic instability. Variability in scale pattern and shape within species (MacArthur, 1986), inability to distinguish between species on outward appearance (e.g., *W. cervinata* and *W. fuliginea* (Dugdale, 1994)), sympatric occurrence of larvae and overlapping flight periods of adults have all contributed to the uncertainty about the exact number of species. Archibald (1984) used antennal and forewing scale shape to distinguish adult males of *W. copularis, W. jocosa* and *W. mimica* which had been previously synonymised with *W. cervinata* (Dumbleton, 1966). MacArthur (1986) identified six *Wiseana* species (*W. cervinata, W. copularis, W. fuliginea, W. jocosa, W. signata and W. umbraculata)* using enzyme electrophoresis. *Wiseana mimica* was recognised as an entity but not included in his study. Herbert (1995) confirmed the identity of the seven currently recognised species, again using electrophoresis.

Only the clade comprising *Wiseana signata* and *W. umbraculata* was recovered in all 116 MP trees. This clade is supported by two synapomorphies and a high bootstrap value (91%). Both species have pallid antennae (Character 11) that are wider than the flagellomere shaft (Character 12). Antennal colour is consistently pallid, although in other *Wiseana* species, antennal colour and background scale colour appear to become darker in populations from regions with higher rainfall (e.g., *W. mimica*).

Herbert (1995), using allozyme data from *Wiseana* adults and larvae coded as locus-as-character and with *Dioxycanus oreas* as an outgroup, recovered a phylogeny with *Wiseana cervinata* and *W. jocosa* in one clade, *W. copularis* as sister taxon and *W. fuliginea* and *W. mimica* in another clade (Fig. 6, A & B). This pattern was never recovered in our analysis. However, the (*Wiseana signata, W. umbraculata*) clade recovered in our analysis, is in agreement with one of Herbert's trees (Fig. 6, A). *Wiseana signata* and *W. umbraculata* were also recovered in separate clades (Fig. 6, B). No synapomorphies supported Herbert's *W. signata* or *W. umbraculata* clades and only one synapomorphy supported each of his other clades. The presence of few or no differentiating electromorphs between *Wiseana* taxa may indicate that there has been insufficient time for the accumulation of mutational differences (Avise, 1994).

All variation in the topology of the 116 MP trees occurs within the genus *Wiseana* but is mostly not supported by unambiguous synapomorphies. This may indicate that the *Wiseana* taxa have evolved relatively recently and may be very closely related. The large number of invariant characters seen in *Wiseana* also supports this hypothesis. *Wiseana* taxa currently occupy grassland or floodplain niches. As new grassland or floodplain niches became available at some time in the past, perhaps after the retreat of glaciers approximately 850,000-14,000 years ago, they would have been occupied by the geographically-closest species most suited to those niches, which may have been ancestral *Wiseana* taxa. If this was relatively recently, then there may not have been enough time for phenotypic divergence between *Wiseana* taxa. Harvey and Pagel (1991) call this phenomenum phylogenetic niche conservation. Alternatively, some of the characters assigned homology in this data set may in fact be homoplasious, reflecting environmental or some other effect rather than relationship.

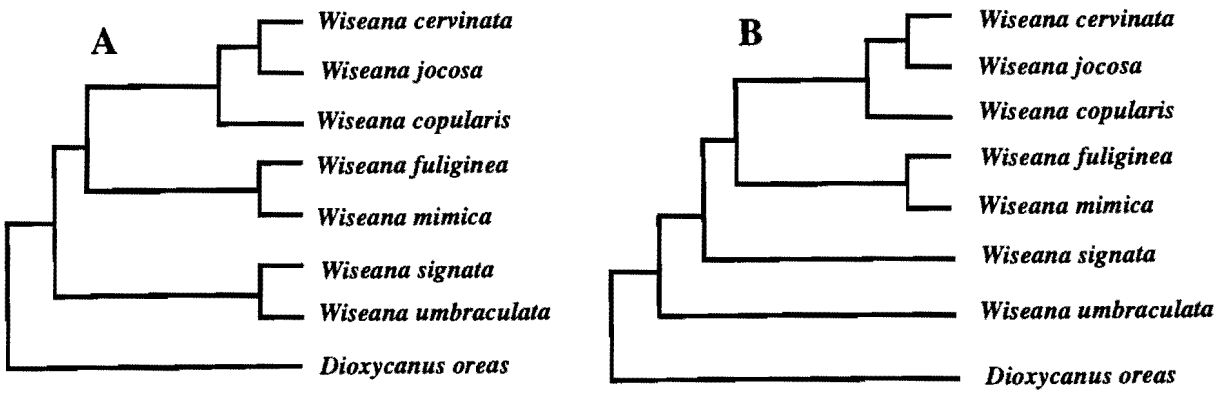

Fig. 6: (A) Tree One and (B) Tree Two, produced from the analysis of allozyme data coded as locus-as-character for *Wiseana* adults and larvae from New Zealand, using *Dioxycanus oreas* as an outgroup (from Herbert, 1995).

New Zealand - Australian hepialid relationships - The relationship of the New Zealand hepialids to other Australian hepialids has been postulated, but never subjected to cladistic analysis. Meyrick (1890) described the New Zealand fauna as "characteristically Australian". Dumbleton (1966) concluded that its closest affinities were with Australia and on the basis of wing venation characters, proposed two subfamilies Hepialinae and Oxycaninae, that included New Zealand and Australian taxa.

Grehan (1987) hypothesised evolutionary relationships within the genus *Aenetus* based on the biogeographic interpretation of larval tunnel characteristics and concluded that New Zealand *Aenetus* were more similar to *A. cohici* from New Caledonia than to *Aenetus* species from Australia, although genitalic characters indicated similarity between *A. cohici* and Australian *Aenetus*. Dugdale (1989), in his discussion of the origins and possible affinities of the New Zealand lepidopteran fauna, concluded that, overall, the New Zealand Hepialidae had greatest morphological resemblance to the Australian Hepialidae.

Characters from adult males of *Jeana timeata* Turner, *Oxycanus australis* Walker, *O. diremptus* Walker, *O. sordidus* Herrich-Schäffer, *Trictena argentata* Tindale and *T. atripalpis* Walker were added to a New Zealand male data set to investigate whether any of the Australian taxa fell within the New Zealand '*Oxycanus*' lineages.

Ten most parsimonous cladograms (length = 147, CI = 0.70, RI = 0.80 and G_1 = -0.56) were recovered. The majority rule consensus tree is shown in Fig. 7. All differences in tree topology in the 10 MP trees occurred within the genus *Wiseana*. The analysis indicated that, as expected, the Australian *Trictena* species fell outside the '*Oxycanus*' lineages along with *Aenetus* and *Aoraia* species.

The clade comprising *Cladoxycanus*, the Australian genera *Jeana* and *Oxycanus* and the New Zealand '*Oxycanus*' lineage *s. str.* was supported by three synapomorphies and a 53% bootstrap value. One synapomorphy (dorsal flange on the saccus, Character 34) supported *Jeana* as sister taxon to the (*Oxycanus*, '*Oxycanus*' *s. str.* lineage) clade and dorsal hood on the pseudotegumen (Character 26) supported the Australian genus *Oxycanus* as sister taxon to the New Zealand '*Oxycanus*' *s. str.* lineage. This result must be treated with caution, since several other Australian hepialids, e.g., *Elhamma* spp. with oxycanus-type wing venation, and *Oncopera* spp. were not included in the data set. An assessment of homology hypothesised for characters 26 and 34 should be made using an independent phylogeny, for example, derived from molecular characters.

If characters 26 and 34 are homologous they should covary with the independent phylogeny.

This result adds support for *Cladoxycanus* as a separate lineage within the New Zealand hepialid assemblage.

The New Zealand *'Oxycanus'* lineage *s. str.* is a monophyletic clade with 56% bootstrap support. There were high bootstrap values for the genera within that lineage. Relationships within the lineage were not fully resolved, with a lack of support at the node joining the *Heloxycanus-Dioxycanus* clade to *Wiseana* and within *Wiseana*.

The interspersing of extant New Zealand and Australian taxa in this analysis suggests that either the divergence of the New Zealand hepialid lineages took place before the Tasman Sea widening in the late Cretaceous or that there have been at least four dispersal events of ancestral taxa to New Zealand. If the New Zealand hepialid fauna is of Gondwanan origin, then one might expect larger radiations. These are evident only in the genera *Aoraia* and *Wiseana*, although it is possible that members of *Aenetus, Cladoxycanus, Dioxycanus, Dumbletonius* or *Heloxycanus* radiations have gone extinct. Alternatively, there may have been four dispersal events to New Zealand. One then wonders how, when and what conditions prevailed to favour dispersal and establishment? New Caledonia was the closest landmass to New Zealand between 37-25mya. However, since the Miocene (24-5 mya) Australia has been the closest (Stevens *et al.*, 1988). Westerly winds have predominated in the region since the Miocene (McGlone, 1985), which may have blown adult hepialids from Australia to the western regions of New Zealand.

Molecular techniques currently available allow these hypotheses to be tested. An estimate of time of divergence can be made using pairwise sequence divergences from DNA nucleotide sequence data and published rates of substitution for DNA, provided that the rate of change along each lineage, as measured by the relative rates test (Sarich and Wilson, 1967) is similar. If there have been no subsequent dispersal events after the Tasman Sea formation, then the estimated time since divergence between the New Zealand and Australian faunas should be large, whereas more recent dispersal events will produce a smaller estimate of time since separation.

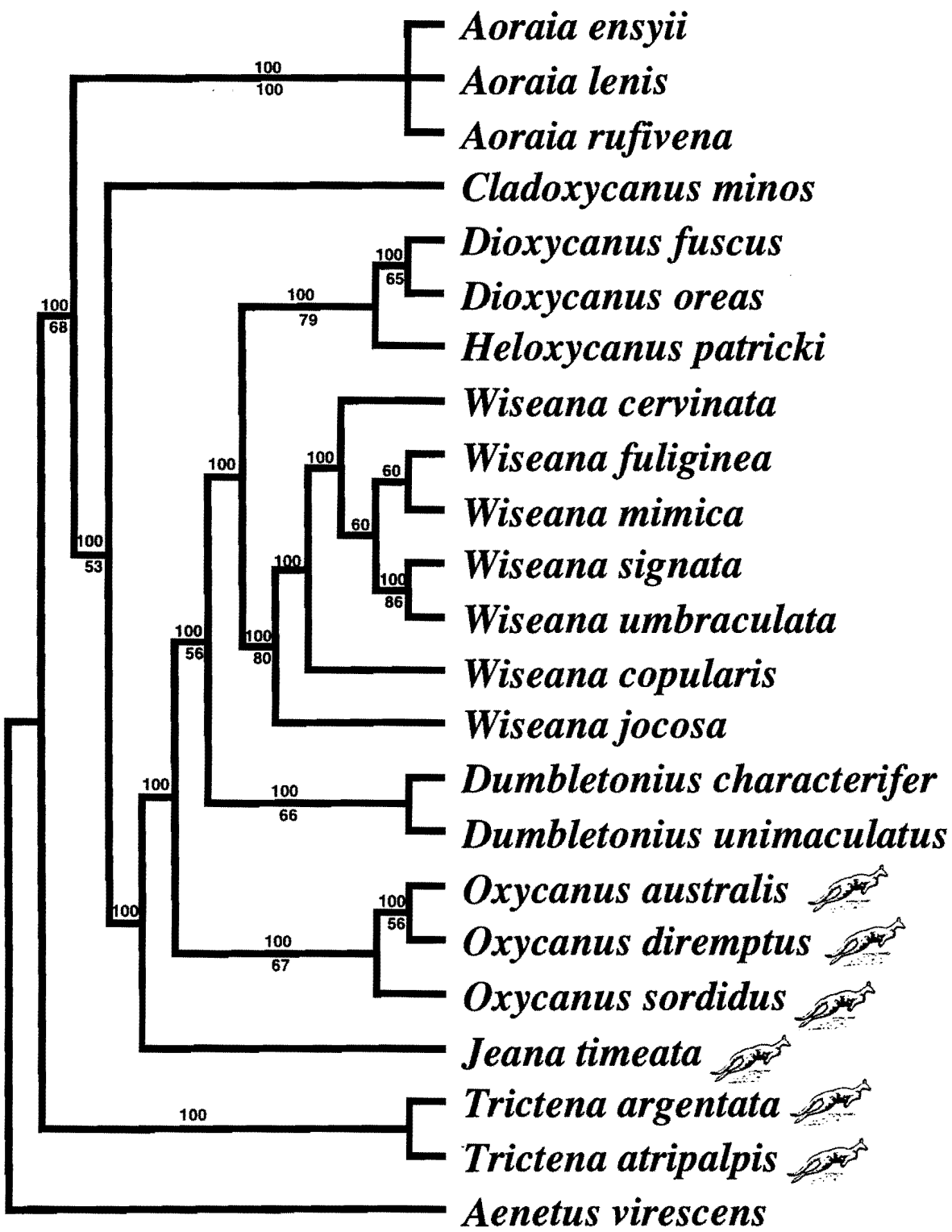

Fig. 7: Majority rule consensus tree of 10 trees from the cladistic analysis of morphological characters from New Zealand and Australian () adult male hepialids. Majority rule values are shown above the branches and bootstrap values (over 50%) below.

Conclusions

New Zealand has a distinctive hepialid fauna. The arrangement of the fauna into four lineages (Dugdale, 1994) reflects phylogenetic relationship as indicated by the results of the cladistic analysis in this study. The *Aenetus* and *Aoraia* lineages are hypothesised to have diverged before the other lineages. The *'Oxycanus'* lineage *s. str.* within New Zealand is a monophyletic group. All Australian taxa included in this study fell outside the New Zealand *'Oxycanus'* lineage *s. str.*, although some members of the Australian genera *Jeana* and *Oxycanus* may be the sister taxon to this lineage. The *'Oxycanus' Cladoxycanus* and the *'Oxycanus' s. str.* lineages share several synapomorphies but appear to be two separate lineages. *Wiseana* is strongly supported cladistically as a genus but how the species within it are related is unclear from morphological characters. The relationships between *Cladoxycanus minos* and the *'Oxycanus'* lineage *s. str.*, within the genus *Wiseana*, and between the New Zealand and Australian taxa are presently being investigated further using molecular data sets.

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Appendix 1

Character Descriptions and Character States for New Zealand hepialids.

Adult male - head

(1) Labrum/clypeus definition: (0) labrum not differentiated, (1) distinct labral sclerite present, mound-like and raised (2) distinct labral sclerite present, weakly raised.

Lack of a distinct labral sclerite, as exhibited by primitive *Fraus* species and Mnesarchaeoids, is thought to be part of the exoporian ground plan (Nielsen and Kristensen, 1989). Small labral lobes present in the *Fraus simulans* group and in the Northern Hemisphere *Hepialus humuli* are regarded as independent autapomorphic character reversals. No labral sclerite is found in *Aenetus*, *Heloxycanus*, but sclerites are present as mound-like and raised in *Aoraia* and weakly raised in *Cladoxycanus*, *Dioxycanus*, *Dumbletonius* and *Wiseana*.

(2) Mandibles: (0) Absent, (1) reduced to a plate on the epicranial wall, (2) present.

Vestigial mandibles are common in the Hepialoidea and are relatively large in some members of Hepialidae *s. str.* (Nielsen and Kristensen, 1989). Mandibles are absent from *Cladoxycanus*, *Dioxycanus fuscus*, *Dumbletonius* and *Wiseana* (except *W. jocosa*). In *Aoraia*, the mandibles are reduced to a plate on the epicranial wall. In those taxa with mandibles present, size ranges from moderate and pyriform in *Aenetus* and *Dioxycanus oreas,* to small and pyriform in *Heloxycanus* and small ovoids in *W. jocosa*.

(3) Maxillary palpi: (0) sclerotized, (1) not sclerotized.

As with all Hepialidae *s. str.*, maxillary structures including palpi are vestigial. All taxa have two segmented palpi, and differences arise in the presence or absence of sclerotization. In *Dumbletonius, Wiseana signata,* and *W. umbraculata*, the second segment is sclerotized. In those taxa with an unsclerotized apical segment, there may be a small rim or pit on the apex of the apical segment, e.g., *Wiseana copularis* and *W. fuliginea*. In some taxa the palpi are apically setose, e.g., *Wiseana cervinata* and *Aenetus virescens*.

(4) Labial palpi segmentation: (0) 3 segmented, (1) 2 segmented.

Nielsen and Kristensen (1996) regard 3-segmented labial palpi as part of the exoporian ground plan. However, reduction in segment number through loss or fusion and reduction in palpi length is common within the superfamily Hepialoidea. *Dioxycanus* and *Heloxycanus* have two segmented labial palpi and all other taxa three segmented palpi.

(5) vom Rath's organ: (0) present, (1) absent

Lack of a vom Rath's organ on the apical segment is not uncommon in the Exoporia (Nielsen and Kristensen, 1989). *Dioxycanus* and *Heloxycanus* lack vom Rath's organs.

(6) Prelabium shape: (0) obscurely bilobed, (1) strongly bilobed, (2) simple.

Ventrally, the prelabium is strongly bilobed in *Cladoxycanus minos*, although not exhibiting the degree of bilobing as seen in *Calada fuegensis* (Nielsen and Robinson, 1983: Figure 56). The prelabium is obscurely bilobed in *Aoraia* and *Dumbletonius* and simple in all other taxa.

(7) Prelabium palpal spacing: (0) contiguous, (1) separated.

There is variation in the degree of separation of the labial palpi at the point of insertion into the prelabium (distal prelabium of Kristensen and Nielsen, 1979). In *Aenetus*, the insertions are contiguous possibly because the eyes are very large and very close together. *Aenetus virescens* males can have an interocular index (Davis, 1975) of 1.6-2.2 (Dugdale, 1994). In all other taxa the palpi are well separated at point of insertion.

(8) Prelabium palpal insertion: (0) raised, (1) not raised.

In *Aenetus virescens* and *Aoraia* spp., the labial palpi are inserted into the prelabium via a raised lobe-like area (Fig. 4, A2). In all other taxa the palpi are inserted directly into the prelabium (Fig. 4, A1).

(9) Labial palpus basal segment: (0) rami absent, (1) rami present.

The basal segment of each labial palpus in *Cladoxycanus minos* has a ventrally placed, anteriorly projecting long ramus. No other taxa exhibit this feature.

(10) Clypeus shape: (0) higher than wide, (1) quadrate, (2) strip-like.

The anterioventrally facing clypeus, ventral to the lower frons, can be recognised by its two pairs of lateral anterior tentorial pits (Scoble, 1992). Clypeus shape varies from higher than wide in *Aenetus virescens*, to quadrate in *Aoraia*, *Dumbletonius* and *Wiseana*. It is reduced to a strip in *Cladoxycanus minos* and *Heloxycanus patricki*.

(11) Antennal pectination description: (0) pectinations absent, (1) tripectinate, pectinations positioned apically on flagellomere shaft, ovate apically, (2) tripectinate, pectinations positioned apically on flagellomere shaft, broad rounded apices, (3) bipectinate, posterior pectinations, ovate apically, (4) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, triangular in shape, rounded apices, (5) bipectinate, sub-pectinations apically positioned on flagellomere shaft, laterally flattened, broad, oval apices, (6) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, rectangular in shape, broad, truncate apices, (7) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, longer than deep triangles, narrow, rounded apices, (8) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, triangular, narrow rounded apices, (9) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, triangular, rounded apices, pallid.

There is much variation in antennal form in this family. There may be different numbers of rami on flagellomeres hence tripectinate and bipectinate antennae. Bipectinate antennae are found in many genera of Hepialidae *s. str.* All taxa with this feature are from the Southern Hemisphere, with the exception of *Bipectilus* Chu and Wang 1985. The only non-hepialid (*s. str.*) exoporian taxon with bipectinate antennae is *Fraus* (Nielsen and Kristensen, 1989). *Aenetus virescens* has moniliform, compressed flagellomeres and no rami. *Cladoxycanus*, and *Heloxycanus* have tripectinate antennae, while the remaining taxa are bipectinate. The position of the rami on the flagellomere varies, being posterior in *Aoraia* and apical in all other taxa. There is much variation in the general shape of the rami and the shape of the apex. *Aoraia,* and *Heloxycanus* have long, ovate rami, with an ovate apex. The remaining taxa have triangular (*Dioxycanus, W. cervinata, W. fuliginea, W. jocosa, W. mimca, W. signata* and *W. umbraculata*), rectangular (*W. copularis*) or subtriangular (*Dumbletonius*) laterally flattened rami. The shape of the apical area of the rami varies. *Dumbletonius* have rami with broad, oval apices.

Dioxycanus have narrow rounded apices. *Wiseana cervinata, W. fuliginea* and *W. jocosa* have rounded apices, while *W. copularis* has truncate apices, *W. mimica* narrow rounded apices and *W. signata* and *W. umbraculata* pallid antennal subpectinations with rounded apices. In *Wiseana* males, variation in rami shape in combination with discal cell white scale shape and genitalic characters are useful in identifying species.

(12) Antennal pectination width: (0) pectinations absent, (1) pectinations not as wide as flagellomere shaft, (2) pectinations as wide as flagellomere shaft, (3) pectinations wider than flagellomere shaft.

At mid-antennae, at the point where the ramus has cleared the flagellomere shaft, the rami in *Aoraia* are not as wide as the flagellomere shaft junction. In *Cladoxycanus*, *Dioxycanus*, *Dumbletonius, Heloxycanus* and *Wiseana*, the rami are as wide as the flagellomere shaft and in *W. signata* and *W. umbraculata* are wider than the shaft junction.

(13) Dorsum of 3-4 proximal antennal flagellomeres, (0) with linear, truncate scales, (1) linear, truncate scales absent.

Scales are not usually present on the ventral or sensory areas of lepidopteran antennae (Scoble, 1992). The dorsum of the first 3-4 proximal flagellomeres of *Aenetus virescens*, *Aoraia*, *Cladoxycanus*, *Dumbletonius* and *Heloxycanus* have sparse linear, truncate scales. *Dioxycanus* and *Wiseana* do not have these scales present.

(14) Antennae, microsensilla: (0) absent, (1) uniform, (2) proximal, on one central mound, (3) proximal, on two lateral mounds.

Microsensilla on the ventral surface of the antennal flagellomeres uniformly cover the entire surface in *Aenetus virescens*. They are present on one proximal central mound in *Cladoxycanus*, *Dumbletonius* and *Wiseana*, present proximally on two lateral mounds in *Dioxycanus* and *Heloxycanus* and absent in *Aoraia*.

(15) Sensilla chaetica on dorsal surface of flagellar segments: (0) absent, (1) present centrally.

Sensilla chaetica are present on the central dorsal region of *Aoraia*, *Cladoxycanus*, *Dioxycanus*, *Dumbletonius*, *Heloxycanus* and *Wiseana*. They are absent in *Aenetus*.

Adult Male - wings

(16) Wing venation: (0) forewing veins R_4 and R_5 arise from common stem which splits off from R_{2+3}, (1) forewing veins R_4 and R_5 arise separately from a combined R_{2+3} stem.

Oxycanine wing venation (where the forewing veins R_4 and R_5 arise separately on the combined R_{2+3} stem) is found in some Hepialidae *s. str.* taxa from New Zealand, Australia, New Guinea, Borneo, South East Asia, China, Himalayas (Dugdale, 1994) and in the South American hepialids *Dalaca guarani* Piftzner, 1914, *Aepytus catharinae* (Viette, 1951) and *Aepytus jeanneli* (Viette, 1950) (Nielsen and Robinson, 1983) (Fig. 1, B). Dumbleton (1966) divided the Hepialidae into two subfamilies, Hepialinae and Oxycaninae, based on wing venation. However, Nielsen and Robinson (1983) concluded that both subfamilies were probably paraphyletic having found oxycanine wing venation in the three South American taxa (mentioned above) not thought to form a monophyletic group. It is probable that oxycanine wing venation has arisen independently several times. *Cladoxycanus*, *Dioxycanus*, *Dumbletonius*, *Heloxycanus* and *Wiseana* have oxycanine type wing venation while *Aenetus* and *Aoraia* have the forewing veins R_4 and R_5 arising from a common stem that splits off from the R_{2+3} stem (Fig. 1, A).

(17) Hindwing veins Sc & R_1: (0) not fused apically, (1) fused apically.

In *Aenetus*, *Aoraia* and *Cladoxycanus*, Sc and R_1 are not fused apically (Fig. 4, C1) and in *Dioxycanus*, *Dumbletonius*, *Heloxycanus* and *Wiseana* are fused apically (Fig. 4, C2).

(18) Hindwing vein curvature Sc & R_1: (0) veins straight, (1) veins strongly curved.

In *Aenetus* these veins are strongly curved towards the costa. In all other taxa they are straight.

(19) Forewing scale pattern: (0) longitudinal scale pattern absent, (1) longitudinal scale pattern present.

Interpretation of wing scale patterns in Superfamily Hepialoidea is difficult due to reoccurrence of similar patterns and much intraspecific variation (Nielsen and Kristensen, 1989). These authors consider the longitudinal streak pattern part of the exoporian groundplan.

Heloxycanus patricki has a central elongate, broad pallid streak tapering basally and apically. This streak is bordered by dark scales. No other taxa exhibit this longitudinal pattern, but *Wiseana umbraculata* may have an unbroken discal streak. In North Island populations this streak can be broken thus making *W. umbraculata* indistinguisable from *W. signata*. It is then necessary to go to scale shape to distinguish these two species.

(20) Discal cell white scale shape: (0) obovate, (1) long, linear, truncate apically, (2) long, tapering evenly to a truncate apex, (3) short and broadly ovate, (4) short, pear-shaped, evenly rounded apex, (5) long, linear, broad and truncate apically, (6) very long, narrow and tapering, narrow rounded apex, (7) tapering narrow-broad truncate, (8) oval in mid-section, narrow rounded apex, (9) long and narrow with long, sharp apex, (A) very short and blunt, (B) oval in mid-section, tapers abruptly to an acute apex, (C) oval in mid-section, tapering to a rounded apex, (D) oval in mid-section, apiculate.

As with scale pattern, there is also variability in scale shape. Archibald (1984) used a combination of scale shape, antennal shape characters and adult emergence data to remove *Wiseana copularis*, *W. jocosa* and *W. mimica* from synonomy with *W. cervinata*. Herbert (1995) was able to separate *W. copularis*, *W. mimica, W. signata* and *W. umbraculata* using univariate scale measurements but could not separate *W. cervinata*, *W. fuliginea* or *W. jocosa*. Female moths could not be identified reliability using scale shape characters because of continuous overlapping characters (MacArthur, 1986). Scanning electron microscope examination of forewing white discal scales showed there was much variation between and within genera. Within the genus *Wiseana*, there was an autapomorphic character state for each species. Some *Aenetus* specimens have white discal scales and these are obovate. *Aoraia* spp. have long, linear scales with a truncate apex. *Cladoxycanus* has long, evenly broad scales tapering to a truncate apex. *Dioxycanus* spp. have short, broadly ovate scales. *Dumbletonius characterifer* has short, pear-shaped scales with an evenly rounded apex, while *D. unimaculatus* has long, linear scales with a broad, truncate apex. *Heloxycanus patricki* has very long, narrow tapering scales with a narrow rounded apex.

Wiseana cervinata has tapering broad-narrow truncate scales, with broad scales being found in northern populations and narrow ones in southern populations. This was also observed by Dugdale (1994). *Wiseana copularis* has parallel sided scales tapering to a narrow rounded apex. *Wiseana fuliginea* has long thin scales, tapering slowly to a long sharp apex. *Wiseana mimica* has scales oval in mid-section, tapering abruptly to an acute apex. *Wiseana jocosa* has very short, blunt scales. *Wiseana signata* has scales oval in mid-section, tapering to a rounded apex. *Wiseana umbraculata* scales are oval in mid-section and apiculate at the apex.

(21) Hindwing colour: (0) green, (1) brown, (2) fawn/buff, (3) yellow/orange - red/pink.

Aenetus virescens is alone in having green hindwing scales. *Aoraia, Cladoxycanus, Dioxycanus, Dumbletonius characterifer, Wiseana cervinata, W. copularis, W. fuliginea, W. jocosa, W. mimica* have brown scales. *Dumbletonius unimaculatus* scale colour ranges from yellow/orange to red/pink. *Heloxycanus patricki* and *Wiseana signata* and *W. umbraculata* have fawn/buff scales.

Adult male - prothorax

(22) Episternal tooth: (0) triangular, not reaching the ventral margin of laterocervicale, (1) slender, not reaching the ventral margin of laterocervicale, (2) slender, reaching ventral margin of laterocervicale, (3) strap-like, and reaching ventral margin of laterocervicale.

The episternal tooth is a mesal process below the broad lateral attachment of the laterocervicale. In *Aenetus*, the episternal tooth is triangular and does not reach the ventral margin of the laterocervicale (Fig. 4, D1). In *Aoraia*, the episternal tooth is slender and also does not reach the ventral margin of the laterocervicale (Fig. 4, D2). In *Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana* the episternal tooth is slender and reaches the base of the laterocervicale (Fig. 4, D3) and in *Cladoxycanus* the tooth is strap-like and reaches the laterocervicale base (Fig. 4, D4).

Adult male - legs

(23) Arolium: (0) absent, (1) present.

This character is present in all taxa except *Cladoxycanus minos*. An arolium is present in *Fraus* spp. (Nielsen and Kristensen, 1989).

Nielsen and Robinson (1983) found the arolium to be reduced or absent in five of ten southern South American hepialid genera studied.

Adult male - genitalia

(24) Sternum 8 shape: (0) apically smooth, (1) subapical teeth, (2) armed, broadly emarginate.

Segment 8 is of similar appearance to preceeding segments, although more sclerotized. This has also been observed in the South American genera *Parapielus* Viette, 1949 and *Andeabatis* Nielsen and Robinson, 1983 (Nielsen and Robinson, 1983). Sternum 8 is shorter than tergum 8 which forms a hood over the genitalia. In *Aenetus*, *Aoraia*, *Dioxycanus*, *Heloxycanus* and *Wiseana*, S8 is apically smooth. In *Dumbletonius unimaculatus* there are sub-apical teeth and in *Cladoxycanus minos*, the apex is broadly emarginate.

(25) T9: (0) strongly sclerotized region, (1) present as a faint sclerite, (2) absent.

In *Aenetus*, T9 is present as a strongly sclerotized region, and in *Cladoyxcanus* and *Heloxycanus* as a faint sclerite. T9 is apparently absent from *Aoraia, Dioxycanus, Dumbletonius* and *Wiseana*.

(26) Extension of pseudotegumen into a dorsal hood: (0) absent, (1) present, below height of twin processes, (2) present, reaching above height of twin processes.

The pseudotegumen may be produced into a dorsal hood *sensu* Dugdale (1994) which extends dorsally as far as the twin processes (see Character 27) in *Dioxycanus*, *Dumbletonius characterifer* and *Heloxycanus patricki*. In *Dumbletonius unimaculatus* and *Wiseana*, the dorsal hood is extended above the twin processes. The dorsal hood is absent from *Aenetus*, *Aoraia* and *Cladoxycanus* (Fig. 4, E).

(27) Twin processes: (0) absent, (1) present, small, (2) present, large, (3) present, very large.

Twin processes *sensu* Dugdale (1994): These sclerotized processes are part of the pseudotegumen, anterolateral to the anus. They support the membrane associated with the anus.

Twin processes are absent from *Aenetus, Aoraia,* and *Cladoxycanus*. They are present in all other taxa, being small in *Wiseana fuliginea* (mean = 0.07 mm, SD = 0.02, n = 7), *W. jocosa* (mean = 0.18 mm, SD = 0.06, n = 11), *W. mimica* (mean = 0.12 mm, SD = 0.05, n = 11), and *W. signata* (mean = 0.15 mm, SD = 0.03, n = 11), large in *Dioxycanus* (mean = 0.41 mm, SD = 0.03, n = 3), *Heloxycanus* (mean = 0.39 mm, SD = 0.05, n = 10), *Wiseana cervinata* (mean = 0.40 mm, SD = 0.04, n = 14), *W. copularis* (mean = 0.33 mm, SD = 0.04, n = 16), and *W. umbraculata* (mean = 0.44 mm, SD = 0.04, n = 16) and very large in *Dumbletonius* (mean = 0.74 mm, SD = 0.0, n = 4). Size of twin processes is used to separate the outwardly indistinguishable *Wiseana cervinata* and *W. fuliginea* and *Wiseana signata* and *W. umbraculata* (Fig. 4, E).

(28) Pseudotegumen dorsal margin shape: (0) straight, parallel sided, (1) straight, decreasing separation dorsal to ventral, (2) splayed dorsally, parallel ventrally, (3) parallel dorsally, decreasing separation ventrally, (4) curved, not straight.

Pseudotegumen *sensu* Nielsen and Kristensen (1989) sclerites surround the anus and phallocrypt. There is variation in the way the sclerites are positioned and shaped dorsally. In *Aenetus* the sclerites are straight dorsally to ventrally and parallel to each other. In *Dioxycanus, Heloxycanus* and *Dumbletonius characterifer* the sclerites are straight but the distance between them decreases from dorsal to ventral parts. In *Aoraia lenis* and *A. rufivena* and *Cladoxycanus* the sclerites are dorsally splayed but ventrally parallel. The opposite occurs in *Dumbletonius unimaculatus* and *Aoraia ensyii* where the sclerites are straight and parallel dorsally but decrease in distance apart ventrally. All *Wiseana* species have curved/bowed sclerites.

(29) Pseudotegumen dorsal margin: (0) knife-edged, bare, smooth flange, (1) knife-edged, small irregular teeth on flange, (2) knife-edged, bare, smooth, flange, lateral sharp teeth-like processes, (3) rounded, thickened margin, no flange.

In *Aenetus*, *Cladoxycanus*, *Heloxycanus* and *Dioxycanus*, the pseudotegumen dorsal margin is a knife-edged, bare and smooth flange. In *Aoraia* and *Dumbletonius characterifer*, the margin is knife-edged but with small irregular teeth. The margin of *Dumbletonius unimaculatus* is bare and smooth, but lateral of the margin is a row of small sharp pointed teeth-like processes. In *Wiseana* the dorsal margin is not knifed-edged but thickened, rounded and not produced into a flange.

(30) Mid posterior process shape: (0) absent, (1) present, apically acute, (2) present, apically truncate.

The mid posterior processes *sensu* Dugdale (1994) are paired, sclerotized processes on the posterior dorsal margin of pseudotegumen. These processes are absent from *Aenetus*, *Cladoxycanus*, *Dumbletonius unimaculatus* and *Heloxycanus*. In *Aoraia*, *Dioxycanus* and *Dumbletonius characterifer*, the processes are apically acuminate. In *Wiseana*, the processes are apically truncate. In *Wiseana umbraculata*, they are upcurved (Fig. 4, E).

(31) Ventral processes: (0) absent, (1) present, moderately thick, short, apically acute, (2) present, moderately thick, short, apically acute, anterior margin extended to fuse with trulleum, (3) long, slender, subspinose-acuminate apically, (4) moderately thick, short, apically truncate.

In *Aenetus* and *Cladoxycanus* the ventral processes are absent. In *Dioxycanus*, *Dumbletonius unimaculatus* and *Heloxycanus* these processes are moderately thick, short and apically acuminate. In *Dumbletonius characterifer* they are moderately thick and short and the anterior margin is extended and fused with the dorsal margin of the trulleum. In *Aoraia*, these processes are relatively long, slender and subspinose-acuminate apically. In *Wiseana* the processes are moderately thick and short with apically dentate inner processes. In some cases the ventral processes in *Wiseana* are bridged by a sclerotized bar or are narrowly membranous in midline (Fig. 4, E).

(32) Lateral processes on pseudotegumen: (0) absent, (1) present, weak, (2) present, strong.

Lateral processes are absent in *Aenetus, Aoraia, Cladoxycanus, Dumbletonius, Wiseana cervinata, W. fuliginea, W. mimica, W. signata* and *W. umbraculata*. A line of weak processes extends laterally on the pseudotegumen from the vertical dorsal margin of the pseudotegumen to the ventral processes, in *Dioxycanus, Wiseana copularis* and *W. jocosa*. In *Heloxycanus*, explanate toothed processes are found laterally on the pseudotegumen.

(33) Supraphallic papilla: (0) absent, (1) present, reduced, (2) present, short, thumb-like, (3) present, long, finger-like.

Found on the membranous area between the pseudotegumen, the supraphallic papilla is ventral to the anus and dorsal to the phallus opening. The papilla covers the opening at rest. This papilla is absent in *Aenetus* and *Cladoxycanus*, present but reduced in *Heloxycanus* and *Wiseana*, short and thumb-like in *Aoraia* and long and finger-like in *Dioxycanus* and *Dumbletonius*.

(34) Extension of posterior margin of saccus: (0) absent, (1) present, small flange, (2) present, moderately large flange, heavy sclerotization, concavity.

A sclerotized flange may be present on the posterior margin of the vinculum/saccus complex. A small flange is present in *Dioxycanus*, *Dumbletonius characterifer*, *Heloxycanus* and *Wiseana*. A moderate sized flange with heavy sclerotization and a medial concavity is present in *Dumbletonius unimaculatus*. The flange is absent in *Aenetus, Aoraia* and *Cladoxycanus*.

(35) Extension of lateral and distal margins of saccus: (0) absent, (1) present.

The lateral and distal margins of the saccus may be extended beyond the cavity to form a saccus 'skirt'. In *Aenetus, Aoraia, Dioxycanus* and *Heloxycanus* there is no saccus skirt. In *Cladoxycanus minos, Dumbletonius* and *Wiseana* a saccus skirt is present. The angulate saccus skirt margin differentiates *Wiseana fuliginea* from outwardly identical *W. cervinata*.

(36) Trulleum shape and sclerotization: (0) no strongly defined region, (1) broadly v-shaped, lightly sclerotized, mesal prominence, (2) broadly rectangular, strongly sclerotized, centrally widened or deeply concave.

Nielsen and Kristensen (1989) regard the free, unfused and unsclerotized trulleum as seen in *Fraus* spp. as the plesiomorphic condition. In *Aenetus*, no strongly defined sclerite exists. In *Aoraia*, the sclerite is broadly v-shaped and lightly sclerotized with a mesal, conical process. Strongly sclerotized, broadly rectangular, centrally widened or deeply concave sclerites are found in *Cladoxycanus, Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana.*

(37) Valvae: (0) acuminate process absent, (1) acuminate process present.

In *Aenetus* and *Aoraia* there are large, decurved, acuminate processes on the proximal half of the valvae (Fig. 4, B2). All other taxa are unarmed, setose and lobate (Fig. 4, B1).

Adult female - genitalia

(38) T7 cuticular processes: (0) absent, (1) present.

Cuticular processes are present laterally on the distal margin of T7 in *Dioxycanus* and *Dumbletonius* and absent from all other taxa.

(39) T8 cuticular processes: (0) absent, (1) present.

Cuticular processes are absent on *Aenetus*, *Aoraia* and *Heloxycanus* and present in all other taxa.

(40) S7 & 8: (0) fused, (1) widely separated, (2) narrowly separated.

Sterna 7 & 8 are fused in *Aenetus, Cladoxycanus, Dioxycanus* and *Dumbletonius*. In *Aoraia*, S7 & S8 are widely separated by transversely folded integument. In *Heloxycanus* and *Wiseana* the sterna are narrowly separated.

(41) Position of spiracle 8 on pleural area: (0) below anterolateral corner, (1) anteroventral to anterolateral corner, (2) anterior to extended posterolateral corner.

In *Aenetus* and *Heloxycanus* spiracle 8 is located below the anterolateral corner of T8. In *Aoraia*, the spiracle is anterolateral to the anterolateral corner and in *Cladoxycanus, Dioxycanus, Dumbletonius* and *Wiseana* is anterior to the extended posterolateral corner of T8.

(42) S9: (0) reduced, (1) triangular, (2) large, long, broad.

Sternum 9 is modified posteriorly into a median piece supporting the ventral floor of the antrum. In *Aenetus, Dumbletonius* and *Wiseana*, S9 only exists as side-pieces and a median-piece. S9 is triangular in *Aoraia* and in *Cladoxycanus, Dioxycanus* and *Heloxycanus* is long and broad.

(43) S9 side and median pieces: (0) junctions not discernible, (1) side and median pieces separated by clefts, (2) side and median pieces separated by weakly sclerotized zone.

The side and median pieces of S9 are separated by varying degrees. In *Aenetus*, *Dumbletonius* and *Wiseana* no junctions are obvious. In *Aoraia, Dioxycanus* and *Heloxycanus*, side and median pieces are separated by clefts and in *Cladoxycanus* by a weakly sclerotized zone.

(44) Sclerotization of dorsal plate midline: (0) absent, (1) weak sclerotization, (2) strong sclerotization.

The dorsal plate is the bilobed terminal sclerite comprised of T9 and T10 (Nielsen and Kristensen, 1989). There is no sclerotization of the dorsal plate midline in *Aenetus, Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana*. There is weak sclerotization in *Aoraia* and strong sclerotization in *Cladoxycanus*.

(45) Paranal groups of setae on diaphrama: (0) absent, (1) present.

Paranal groups of seta are present on the diaphrama of *Heloxycanus, Dumbletonius* and *Wiseana* and are absent from all other taxa.

(46) Position of ovipore: (0) ovipore simple, (1) ovipore on a weakly bilobed, erect papilla, (2) ovipore on a strongly bilobed, erect papilla.

In *Dumbletonius* and *Wiseana* the ovipore is on a papilla which is strongly bilobed and erect. In *Cladoxycanus*, *Dioxycanus* and *Heloxycanus*, the papilla is weakly bilobed and erect, and in *Aenetus* and *Aoraia* the ovipore is simple.

(47) Intergenital lobes: (0) open, (1) firmly apposed, (2) fused for all or part of length.

Below the ovipore are a pair of transverse folds, the intergenital lobes, the medial ends of which may be free, appressed or fused. The cleft/sinus between the lobes is where the sperm is presumed to travel from the bursa complex to the oviduct. The open cleft is believed to be the plesiomorphic condition (Nielsen and Kristensen, 1989). The intergenital lobes in *Aenetus*, *Aoraia* and *Dioxycanus* are open, in *Heloxycanus* are firmly apposed and in *Cladoxycanus, Dumbletonius* and *Wiseana* are fused for all or part of the length.

(48) Antrum floor: (0) membranous, (1) sclerotized, (2) thick and folded, (3) strongly sclerotized.

Ventral to the intergenital lobes is the vestibule or antrum of the copulatory pore. The antrum floor in *Aenetus* and *Aoraia* is membranous. It is sclerotized in *Cladoxycanus*, *Dioxycanus* and *Wiseana*, thick and folded in *Heloxycanus* and very strongly sclerotized in *Dumbletonius*.

(49) Ductus bursae: (0) spines present, (1) spines absent.

Anterior to the antrum, is the ductus bursae of the bursa copulatrix. The ductus bursae is generally slender and widens into the ovoid/globose corpus bursae. Spines are found in the ductus bursae of *Aenetus* and *Dioxycanus* and are absent from all other taxa. Spines are also reported from *Fraus* spp. (Nielsen and Kristensen, 1989).

(50) T8 apical tuft: (0) absent, (1) present.

On the caudal margin of T8, there is a broad tuft of long hair-like scales. These are present in *Cladoxycanus, Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana* and absent in *Aenetus* and *Aoraia*.

Larvae

(51) Arrangement of stemmata on head capsule: (0) in two parallel rows, (1) in one straight and one curved row, (2) in two parallel arcs.

Arrangement of stemmata: In *Aenetus*, the six stemmata are arranged in two parallel rows. In *Aoraia*, the dorsal three stemmata form a straight line while the ventral three form an arc around the antennal base. In *Cladoxycanus, Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana* the six stemmata form two parallel arcs around the antennal base.

(52) Head capsule setae SO_3, G_2, G_1: (0) in a strong curve, (1) in a straight line.

In *Aenetus*, *Cladoxycanus*, *Dioxycanus*, *Dumbletonius* and *Wiseana*, these setae form a strong curve and in *Aoraia* and *Heloxycanus* form a straight line.

(53) Mesothorax prosternum: (0) absent, (1) present.

A small prosternum is present on the mesothorax of all taxa except *Heloxycanus*.

(54) Metathorax prosternum: (0) absent, (1) present.

This sclerite is present in *Aenetus, Aoraia, Dioxycanus* and *Dumbletonius* and absent from *Cladoxycanus, Heloxycanus* and *Wiseana*.

(55) Abdominal segments A3-6 prosternum: (0) fused with V_1 pinaculum, (1) small, separate, (2) large, separate.

The prosternum is fused with the V_1 pinaculum in *Aoraia*, small and separate in *Cladoxycanus, Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana*, and large and separate in *Aenetus*.

(56) Metathoracic seta L_3: (0) on rhomboidal SD_1, SD_2 pinaculum, (1) not on rhomboidal SD_1, SD_2 pinaculum.

In *Aenetus* and *Aoraia*, L_3 is on the rhomboidal SD_1 or SD_2 pinaculum. In all other taxa L_3 is not on SD_1 or SD_2.

Pupae

(57) Antennal pedicel and scape: (0) antennae smooth, (1) broad, stout, outwardly decurved thorn-like process dorsally, (2) crenulate carina.

In *Aenetus*, the scape and pedicel and part of the antennal sheath form a crenulate carina or blade. In *Cladoxycanus, Dumbletonius* and *Wiseana*, there is a broad, stout, outwardly decurved thorn-like process dorsally. In *Aoraia, Dioxycanus* and *Heloxycanus* the antennae are smooth.

(58) Vertex: (0) sunken with mesal furrow, (1) produced into a cone, (2) produced into 2 divergent, strongly sclerotized cones.

The vertex in *Aenetus, Dioxycanus* and *Dumbletonius* is sunken with a mesal furrow. In *Cladoxycanus*, the vertex is produced into a cone and in *Aoraia, Heloxycanus* and *Wiseana* is produced into two divergent, strongly sclerotized cones.

(59) Frons: (0) plane, (1) convex, (2) convex with conical process, (3) convex with decurved bifurcate process.

The frons in *Aenetus* is plane, convex in *Aoraia, Dumbletonius* and *Heloxycanus*, convex with a conical process in *Dioxycanus* and convex with a decurved, bifurcate process in *Cladoxycanus* and *Wiseana*.

(60) Gena: (0) planoconvex, (1) on a prominent mound.

The gena is planoconvex in *Aenetus, Aoraia, Dioxycanus* and *Heloxycanus* and on a prominent mound in *Cladoxycanus, Dumbletonius* and *Wiseana*.

(61) Length of labial and maxillary plates: (0) of similar length, (1) labial plate extends further than maxillary plate.

In *Cladoxycanus* and *Heloxycanus*, the labial plate extends further than the maxillary plate, and in all other taxa does not end conspicuously further than the maxillary plate.

(62) Mesonotum: (0) low prominence absent, (1) low prominence present.

A low prominence is present on the forewing base in *Aoraia, Dumbletonius* and *Wiseana* and absent from *Aenetus, Cladoxycanus, Dioxycanus* and *Heloxycanus*.

(63) Chaetotaxy of abdominal segment A1: (0) seta D_1 & D_2 present, (1) seta D_1 present.

Setae D_1 and D_2 are present on abdominal segment A_1 in *Aenetus, Aoraia* and *Cladoxycanus* and D_2 is absent from *Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana*.

(64) Carina sublaterally on A4-6, anterolateral of the spiracle: (0) absent, (1) present.

A short carina is present sublaterally on A4-6 anteroventral of the spiracle in *Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana* and absent from *Aenetus, Aoraia* and *Cladoxycanus*.

Appendix 2: Data matrix of the morphological data for the seventeen species of New Zealand hepialids used in the cladistic analysis. (For description of character states see Appendix 1).

	1 1234567890	1111111112 1234567890	2222222223 1234567890	3333333334 1234567890	4444444445 1234567890	5555555556 1234567890	6666 1234
Aenetus virescens	0210020000	0001000100	0010000000	0000001000	0000000000	0011202000	0000
Aoraia enysii	1110001001	3100100001	1110200311	3020011001	1111000010	1111000210	0100
Aoraia lenis	1110001001	3100100001	1110200211	3020011001	1111000010	1111000210	0100
Aoraia rufivena	1110001001	3100100001	1110200211	3020011001	1111000010	1111000210	0100
Cladoxycanus minos	2010011112	2202110002	1302100200	0000120010	2222012111	2010111131	1000
Dioxycanus fuscus	2011121101	8213111003	1210212101	1131020110	2210010101	2011110020	0011
Dioxycanus oreas	2211121101	8213111003	1210212101	1131020110	2210010101	2011110020	0011
Dumbletonius characterifer	2000001101	5202111004	1210213111	2031120110	2000122311	2011111011	0111
Dumbletonius unimaculatus	2000001101	5202111005	3211223320	1032120110	2000122311	2011111011	0111
Heloxycanus patricki	0211121102	1203111016	2210112100	1211020002	0210111211	2100110210	1011
Wiseana cervinata	2010021101	4212111007	1210222432	4011120012	2000122111	2010111231	0111
Wiseana copularis	2010021101	6212111008	1210222432	4111120012	2000122111	2010111231	0111
Wiseana fuliginea	2010021101	4212111009	1210221432	4011120012	2000122111	2010111231	0111
Wiseana jocosa	2210021101	421211100A	1210221432	4111120012	2000122111	2010111231	0111
Wiseana mimica	2010021101	721211100B	1210221432	4011120012	2000122111	2010111231	0111
Wiseana signata	2000021101	931211100C	2210221432	4011120012	2000122111	2010111231	0111
Wiseana umbraculata	2000021101	931211100D	2210222432	4011120012	2000122111	2010111231	0111

Chapter 3

Phylogeny and biogeography of *'Oxycanus'* lineages of Hepialid moths from New Zealand inferred from sequence variation in the mtDNA COI and II gene regions.

B.Brown, R.M. Emberson and A.M. Paterson

Abstract

The phylogeny of the New Zealand hepialid moths was estimated from a 527 base pair nucleotide sequence from the mitochondrial DNA cytochrome oxidase subunit I and II gene regions. New haplotypes were identified for *Wiseana cervinata, W. copularis* and *W. signata*. Phylogenetic reconstructions using maximum parsimony and maximum likelihood methods indicated that the four hepialid lineages *Aenetus, Aoraia, 'Oxycanus' Cladoxycanus* and *'Oxycanus' s. str.*, hypothesised by Dugdale (1994) based on a morphological taxonomic revision, were monophyletic. Addition of exemplars from the Australian genera *Fraus, Jeana, Oxycanus* and *Trictena* to the data set supported the monophyly of the New Zealand *'Oxycanus'* lineages. Times of divergence for the various lineages were estimated using rates of 2-2.3% pairwise sequence divergence per one million years. Estimated times of divergence for *Wiseana* taxa fitted well with known geological events and suggest the genus may have diverged 1-1.5 mya.

Key words - hepialid, *'Oxycanus' Cladoxycanus, 'Oxycanus' s. str.*, phylogeny, mtDNA, COI & II, biogeography.

Status - Prepared for submission to Insect Molecular Biology

Introduction

Family Hepialidae in New Zealand has been divided into four informal lineages to reflect the four distinctive groupings found in a morphological taxonomic revision (Dugdale, 1994). The two *'Oxycanus'* lineages, so named because of possible affinities to *'Oxycanus'* lineages in Australia, New Guinea and Asia (Dugdale, 1994) were the focus of this study. The *'Oxycanus'* lineage *sensu stricto* comprised four genera: *Dioxycanus* with two species found in the sub-alpine zones of the South Island ranges; *Dumbletonius,* with two species found in forests of North Island and upper South Island; *Heloxycanus,* a monotypic genus found in areas of *Sphagnum* moss bogs in southern South Island; and *Wiseana,* with seven species inhabiting flood plains, grasslands and now, improved pastureland. The *'Oxycanus'* lineage *Cladoxycanus,* has one member, *Cladoxycanus minos*, which shares some morphological characteristics with the *'Oxycanus'* lineage *s. str.*, but is distinctive because of reduced features in the pseudotegumen region of the male genitalia (Dugdale, 1994). Two other lineages were hypothesised: the *Aenetus* lineage comprising *Aenetus virescens* (New Zealand's largest moth species) found only on North Island; and the *Aoraia* lineage comprising 13 species, many with distributions restricted to the sub-alpine zone in South Island's Central Otago region.

Previous research interest in this family in New Zealand has focused on *Aenetus virescens* (Grehan, 1979, 1981, 1987a & b) and the genus *Wiseana*. Taxonomic research to establish the number and distribution of *Wiseana* species has been hampered by intra and interspecific variability in scale shape, colour and pattern of adults and lack of characteristics to distinguish between the larvae of the different species (see Dumbleton, 1966; French, 1973). It was not until the allozyme work of MacArthur (1986) and Herbert (1995) that seven *Wiseana* species were confirmed. All species are described in Dugdale (1994) under a morphological species concept. Other research has focused on the control and management of *Wiseana* species identified as pests (Barlow and Carpenter, 1981; Latch, 1983; Barratt *et al.*, 1990; van Toor *et al.*, 1993; Herbert, 1995). The larvae of *Wiseana cervinata*, *W. copularis*, *W. fuliginea* and *W. mimica* defoliate improved pasture, reduce feed availability for stock and can cause significant economic loss to farmers. Herbert (1995) observed *W. cervinata* and *W. copularis* in damaged pastures throughout New Zealand as well as *W. fuliginea* in South Island pastures. Dugdale (1994) lists *W. mimica* as a pest species.

A phylogeny for the *'Oxycanus'* lineages (Dugdale, 1994) has been reconstructed from the analysis of morphological data (Chapter 2). That study indicated that the lineages of Dugdale (1994) reflect phylogenetic relationships. There was strong support for the monophyly of the *'Oxycanus'* lineages through synapomorphies and high bootstrap values. Cladistic analysis of the morphological data indicated that the Australian genera *Jeana* and *Oxycanus* may be a sister group to the New Zealand *'Oxycanus'* lineage *s. str.*, although the majority of *Oxycanus* taxa were not examined and nor were taxa from the Australian hepialid genera *Elhamma* and *Oncopera*. The relationship of *Cladoxycanus minos* to the *'Oxycanus'* lineage *s. str.* and relationships within the genus *Wiseana* were unable to be resolved using morphological data. In the case of *Wiseana*, there was a lack of synapomorphies to support relationships (except between *Wiseana signata* and *W. umbraculata*). When morphological data lack synapomorphies, contain conflicting data or fail to produce a robust phylogeny, or when an independent phylogeny is needed for investigations into the evolution of morphological characters, molecular data sets may be useful (e.g., Brower, 1994a; Brown *et al.*, 1994b).

Mitochondrial DNA (mtDNA) is commonly used as its high copy number per cell allows easy recovery. The molecule is maternally inherited, non-recombining and has genes that evolve at different rates (Moritz *et al.*, 1987; Rand, 1994; Simon *et al.*, 1994; but see Zhang and Hewitt (1996) for discussion of pseudogenes confounding phylogenetic inference from mtDNA). Mitochondrial DNA is a popular choice for reconstructing phylogenetic relationships in animal species (Harrison, 1989; Avise, 1991; DeSalle, 1992; Sperling and Harrison, 1994; Funk *et al.*, 1995; Howland and Hewitt, 1995), as well as for identifying species' origins, analysis of population structure, phylogeography and molecular evolution (Bogdanowisz *et al.*, 1993; Vogler and DeSalle, 1993; Rand, 1994). The cytochrome oxidase I and II genes (COI & II) are often used in insect studies (see Table 5 in Simon *et al.*, 1994). Partial COI & II sequences have previously been used to resolve relationships in Lepidoptera. For example, Brown *et al.* (1994a) were able to resolve relationships within the genus *Greya* (Lepidoptera: Prodoxidae) and found the molecular and morphological phylogenies mostly congruent. Sperling and Hickey (1994) assessed population structure and species limits within the spruce budworm (*Choristoneura* spp.) complex.

We undertook a molecular study of New Zealand hepialids using a region of the mitochondrial cytochrome oxidase I and II genes to: (i) resolve the phylogeny and establish whether the *'Oxycanus'* lineages are monophyletic, (ii) answer biogeographical questions regarding the origins of the New Zealand hepialid fauna and (iii) produce an independent estimate of phylogeny to enable the study of morphological character evolution.

Material and Methods

Collections - Specimens of New Zealand hepialids were collected by light trapping, placed directly into 96% ethanol and stored at 4°C before DNA extraction. Herbert (1995) reported some clinal variation with latitude within populations of *Wiseana cervinata*. Consequently, where possible for each species, specimens were collected from populations at a range of locations (Figs. 1 & 2) (Appendix 1).

Additional specimens were provided by Australian colleagues to test the monophyly of the New Zealand *'Oxycanus'* lineages. Included in this study are the Australian taxa *Fraus simulans*, *Jeana timeata*, *Oxycanus australis*, *O. diremptus*, *O. sordidus*, *O. sphragidias*, *Trictena argentata* and *T. atripalpis*. Collection locations of the Australian taxa are listed in Appendix 2.

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

DNA extraction, PCR and Nucleotide sequencing - Muscular tissue from the thorax of specimens was homogenised and total DNA extracted using a proteinase-K digestion and high salt precipitation (White *et al.*, 1990). A region spanning the 3´end of the cytochrome oxidase subunit I (COI) gene, the leucine tRNA gene, and the 5´end of the cytochrome oxidase II subunit (COII) gene were amplified via the polymerase chain reaction (PCR) (Saiki *et al.*, 1988) using the primers A3389 (5´TCATAAGTTCA(A/G)TATCATTG) and S2792 (5´ATACCTCGACGTTATTCAGA) (Brown *et al.*, 1994a). These primer names correspond to the position of their 3´ end based on the *Drosophila yakuba* sequence (Clary and Wolstenholme, 1985). 25 µl reactions comprised 2.5 µl of 10x *Taq* buffer (Boehringer Mannheim), 2.5 µl 10x dNTPs, 0.625 µl 20 mM magnesium, 1.25 µl 10 µM A3389 and S2792 primers and 0.2 µl 5 u/µl *Taq* DNA polymerase (Boehringer Mannheim).

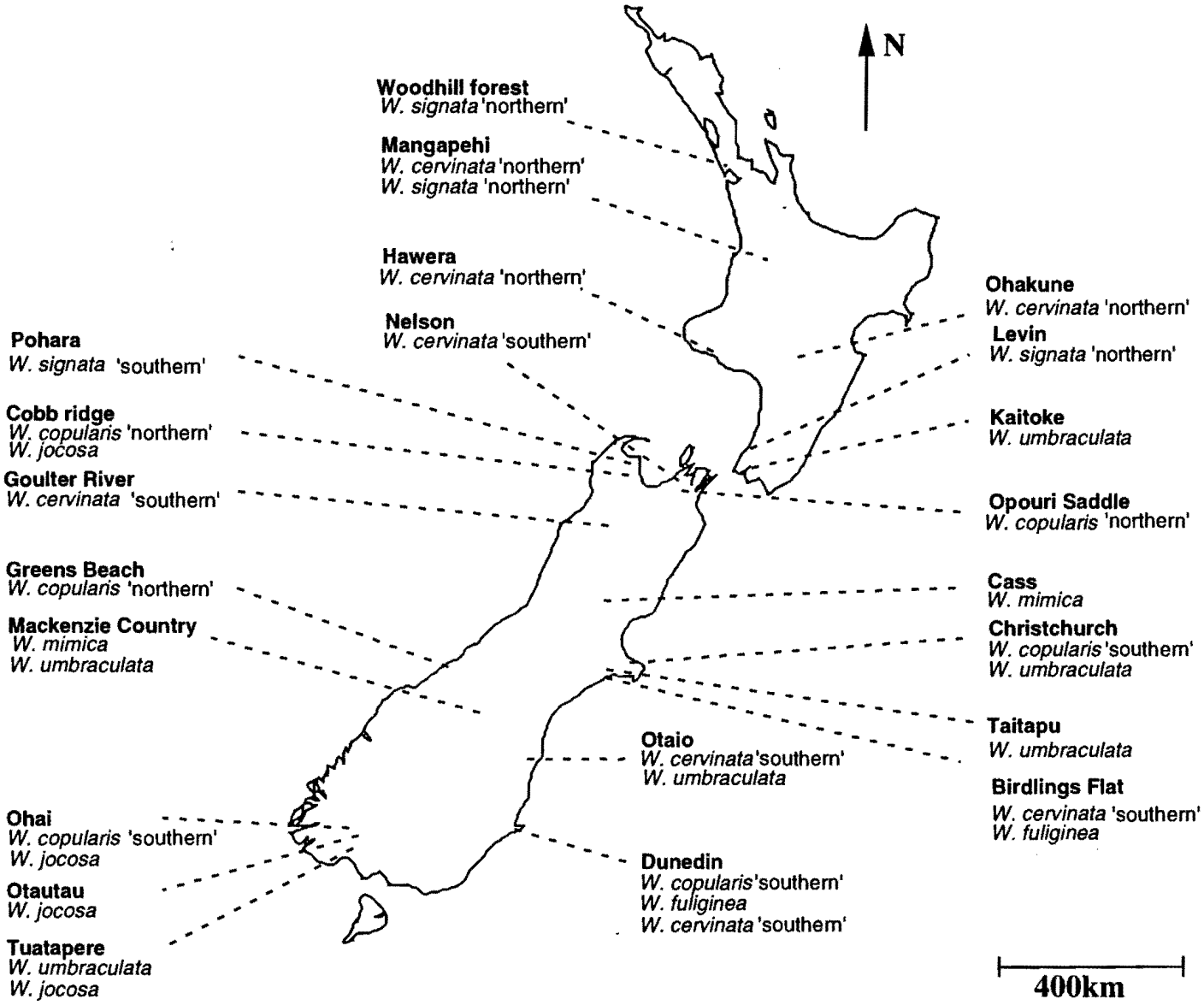

Fig. 1: Map of New Zealand showing the location of collection sites for *Wiseana* taxa.

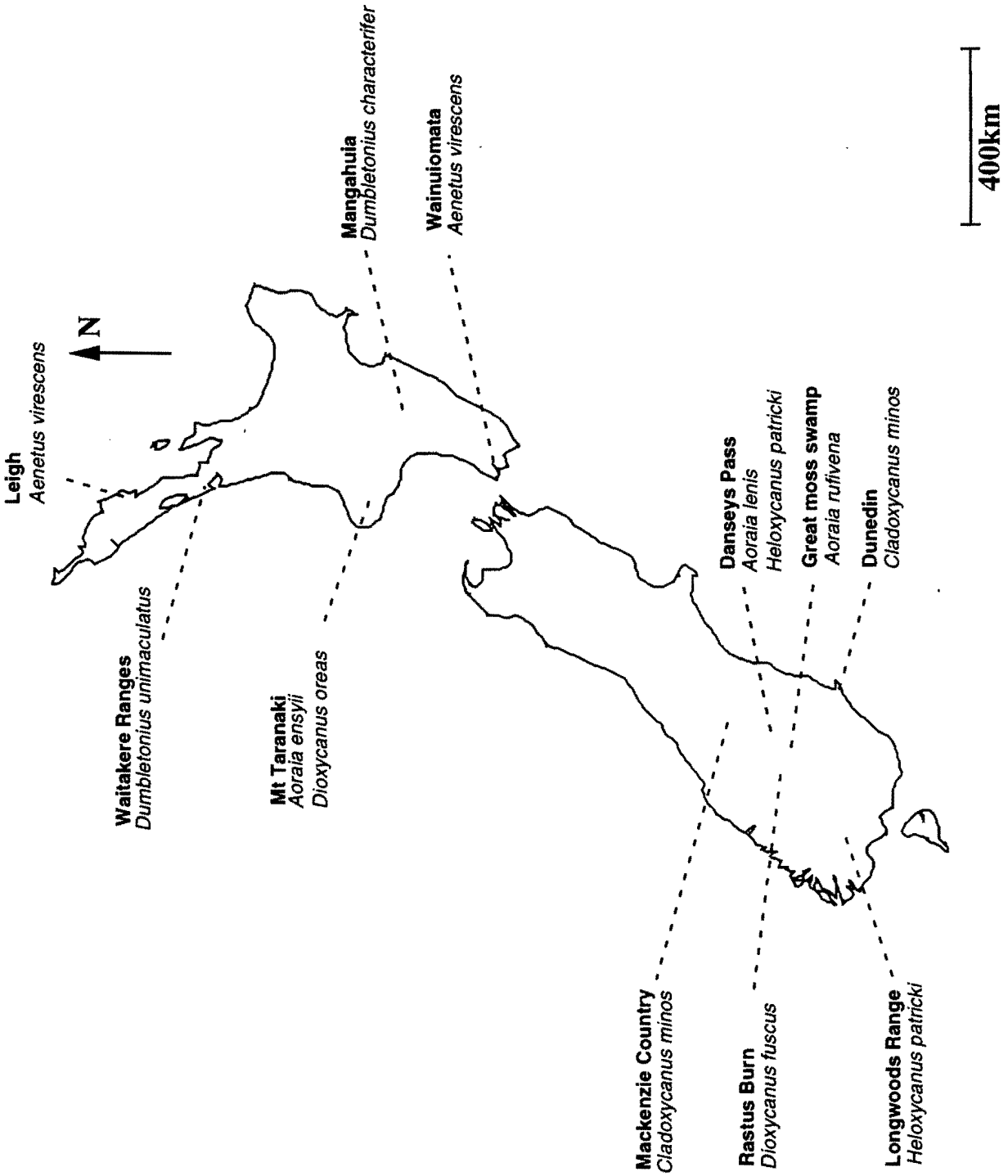

Fig. 2: Map of New Zealand showing the location of collection sites for *Aenetus virescens*, *Aoraia* spp., *Cladoxycanus minos*, *Dioxycanus* spp. and *Dumbletonius* spp.

A Perkin- Elmer 2400, thermal cycler was used with a cycling profile of 94°C for 2 minutes pre-PCR followed by 93°C for 20 sec, 50°C for 40 sec and 72°C for 1 min for 33 cycles with a 5 minute extension at 72°C after the final cycle. Excess primers and salts were removed from the double stranded PCR product by precipitation with 3M ammonium acetate and isopropanol. The product was rinsed in 70% alcohol and air dried before resuspension in 5-10 µl of deionized water.

Direct sequencing of the PCR product was carried out using the Prism™ Ready Reaction Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer) following the manufacturer's recommendations. The annealling temperature in this reaction was 50°C for the A3389 primer and 45°C for S2792. Sequencing was carried out on an ABI 377 Automatic Sequencer. For each specimen, the sequence from both sense and antisense strands was obtained. At least two specimens were sequenced from every population sampled for each species.

Sequence Alignment and Translation - For each sequence, a 28 base pair (bp) section before the A3389 primer and a 38 bp section after the S2792 primer were not able to be reliably identified due to excessive dye incorporation or loss of signal and were eliminated from the alignment process. The remaining 527 bp region, corresponding to the region between positions 2830 and 3360 in the *Drosophila yakuba* sequence (Clary and Wolsenholme, 1985), was aligned using the default parameters of the alignment programme Clustal W (Thompson *et al.*, 1994). The sequence was also translated to amino acids using the programme DNAMAN (Lynnon Biosoft, 1994-95).

Sequence Analysis - We searched for maximum parsimony (MP) trees in the unordered, unweighted nucleotide data with PAUP 3.1 (Swofford, 1993), using the heuristic option and 10 stepwise addition replicates. In protein coding genes such as COI & II, that are structurally and functionally conservative, not all sites are equally free to vary (Nei, 1991; Simon *et al.*, 1994; Lunt *et al.*, 1996) e.g. first and third codon positions may change without altering the amino acid, but those positions may be subject to several substitutions (multiple hits) that obscure the phylogenetic signal and cause an underestimation of genetic distance (Fitch, 1986). Three different weighting schemes were employed. Third positions were weighted zero and other positions one, or third positions were weighted one and other positions two or five. In mitochondrial DNA there tend to be more transitions at lower levels of divergence (Liu and Beckenbach, 1992; Moritz and Hillis, 1996) while at higher divergences, transitions are replaced by transversions (DeSalle *et al.*, 1987).

Consequently, transitions were weighted one while transversions were weighted zero, two, five or ten.

Maximum likelihood (ML) trees were recovered using Phylip 3.4 (Felsenstein, 1991), under the Kimura two-parameter model (Kimaru, 1980), which compensates for rate heterogeneity across sites. Amino acids were analysed using Protdist in Phylip 3.4. Bootstrap proportions (Felsenstein, 1985), measuring the frequency of a branch's occurrence in the resampling of pseudoreplicates from the data set, were calculated.

Results

The Nucleotide Sequence - The aligned nucleotide sequence for a 527 bp region of the mitochondrial DNA (mtDNA) COI & II genes for 20 haplotypes of New Zealand's hepialid moths is shown in Appendix 3. As with other insect mtDNA (Beckenbach *et al.*, 1993; Funk *et al.*, 1995) few indels were observed. There was one insertion after position 3075 (immediately before the stop codon at the end of COI) in *Aenetus* and *Cladoxycanus* and one deletion at position 3078 (immediately after the stop codon at the end of the tRNA) in *Heloxycanus*.

Of the 527 nucleotides, 61 were parsimony informative (11.6%). Twenty four of the parsimony informative sites occurred in COI region, four in the tRNA and 33 in the COII region. Thirty eight of the parsimony informative sites (62%) occurred at third positions, 17 at 1st positions (28%) and two at 2nd positions (3%).

Sixty three percent of sites had adenines (A) or thymines (T) present for all haplotypes. This value is lower than others reported for the COI region: 76-90% in *Apis* (Crozier, 1990), 80-85% in Coleoptera (Howland and Hewitt, 1995) and 69% in the meadow grasshopper (Lunt *et al.*, 1996) and lower than the 73-75% reported for COII in *Drosophila* (Beckenbach *et al.*, 1993), although we sequenced only part of the COI & II gene regions. A's and T's were distributed relatively evenly between COI, tRNA and COII, with values of 58%, 66% and 65% respectively. Frequency of occurrence of A's and T's was also evenly spread between codon positions for each region, with each having approximately 35% (range 25-37%). These values are lower than those reported by Sperling and Hickey (1994) who found 61-63% A's and T's in 1st and 2nd positions in COI, 65-70% for the same positions in COII and 92-93% for 3rd positions in *Choristoneura* species.

The transition: transversion ratio was 3:1. Thirty seven of the 61 informative characters (61%) were transitions, with 18 in 3rd positions, two in 2nd and 17 in 1st positions. The majority of transitions (57%) were T -> C. Only two C -> T transitions occurred. Twenty four of the 61 informative characters (39%) were transversions. Four transversions occurred in the tRNA region while the remaining 21 transversions were in 3rd positions of COI or COII. All were A -> T transversions or multistate transition/transversions.

As has been reported in other insect studies (DeSalle *et al.*, 1987; Beckenbach *et al.*, 1993; Brown *et al.*, 1994b), there were more transitions at low levels of divergence and more transversions at deeper levels of divergence.

Distance estimates - Corrected divergences using the Kimura two-parameter model (Kimura, 1980) were calculated for all pairwise combinations of haplotypes (Table 1). Distances between the ingroup and outgroup haplotypes ranged from 9.5% between *Aenetus* and *Cladoxycanus* to 8.1% between *Aoraia lenis* and *Cladoxycanus*. The distance between *Cladoxycanus* and the *'Oxycanus' s. str.* was 7.2%. Differences within the genus *Wiseana* ranged from 2.7% between the *Wiseana signata* 'southern' haplotype and *Wiseana mimica*, to 0.2% between *W. mimica* and *W. fuliginea*.

The Search - A heuristic search of the unordered and unweighted nucleotide sequence resulted in six maximum parsimony (MP) trees: tree length (TL) 182, consistency index (CI) 0.55, retention index (RI) 0.65, G_1 statistic -0.79. The majority rule consensus tree with bootstrap values is shown in Figure 3.

Inspection of the six MP trees showed three differing arrangements. *Cladoxycanus* occurred either as a long, unresolved branch basal to *D. characterifer* or occurred in a clade with *Dumbletonius characterifer* or *D. unimaculatus*. On trees where *Cladoxycanus* occurred alone or in a clade with *D. characterifer*, the order of the remaining clades was ((*Heloxycanus, Dioxycanus*), (*Dumbletonius unimaculatus, Wiseana*)). On trees where *Cladoxycanus* was in a clade with *D. unimaculatus*, the order of the clades was (*D. characterifer,* ((*Heloxycanus, Dioxycanus*), ((*Cladoxycanus, D. unimaculatus*), *Wiseana*))). Within the genus *Wiseana,* the *W. signata* haplotypes either grouped together (Trees 4, 5, 6) or the *W. signata* 'southern' haplotype grouped with *W. umbraculata* (Trees, 1, 2, 3).

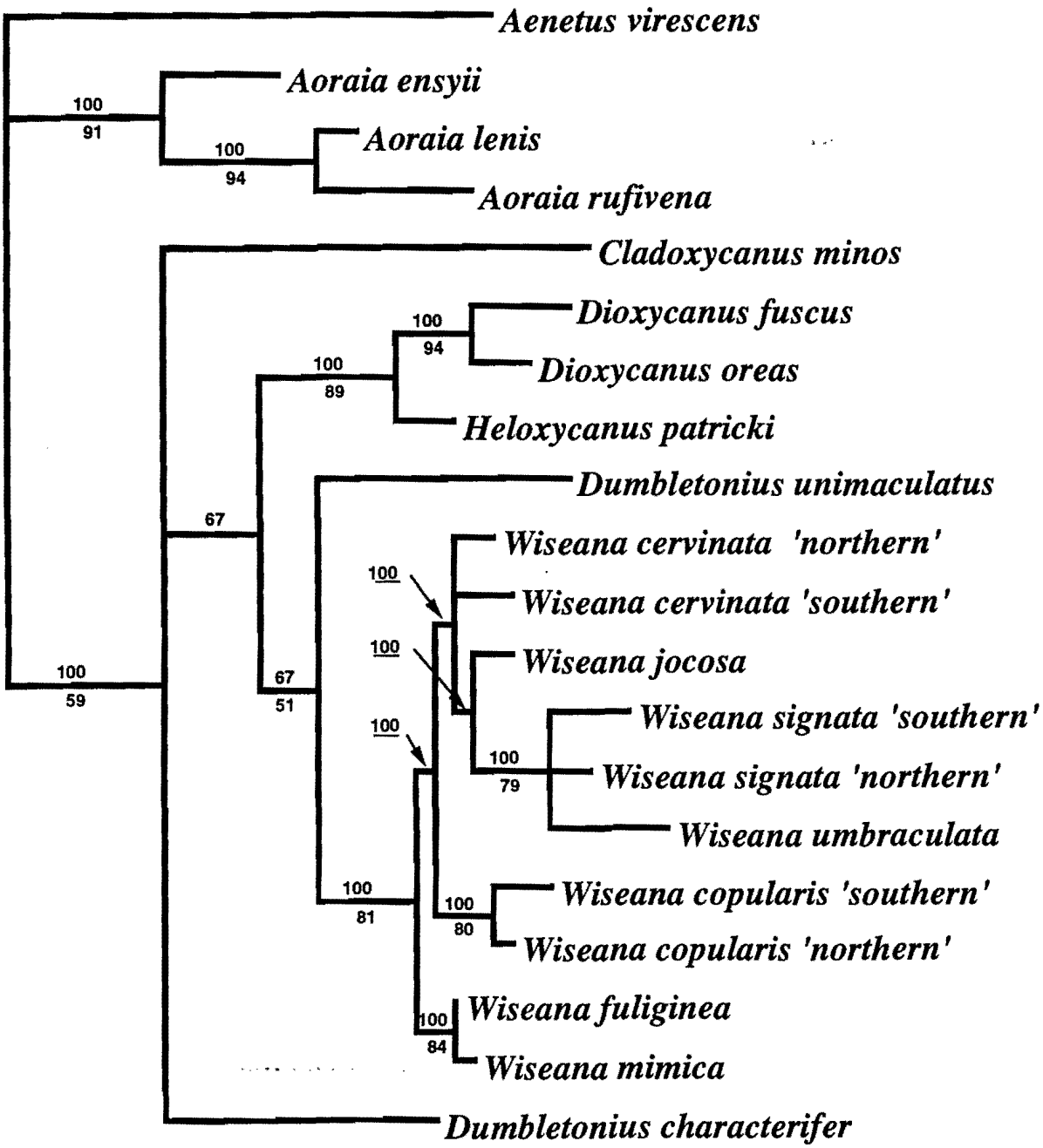

Figure 3: Majority rule consensus phylogram of the six most parsimonious trees from the analysis of mtDNA COI & II sequence data from New Zealand hepialids. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule values are shown above the branches and bootstrap proportions (>50%) below.

Table 1: Percentage sequence divergences among mtDNA COI & II haplotypes of New Zealand hepialid moths, corrected for multiple hits using the Kimura (1980) two-parameter model. Percentage sequence divergences are above the diagonal and standard errors are below.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *Aenetus virescens*	-	0.072	0.064	0.078	0.095	0.082	0.076	0.08	0.091	0.067	0.08	0.082	0.074	0.076	0.076	0.078	0.078	0.089	0.08	0.078
2. *Aoraia ensyii*	0.012	-	0.029	0.043	0.081	0.078	0.071	0.053	0.066	0.055	0.059	0.057	0.062	0.062	0.055	0.055	0.057	0.068	0.062	0.057
3. *Aoraia lenis*	0.011	0.008	-	0.019	0.081	0.069	0.063	0.057	0.071	0.047	0.057	0.059	0.055	0.059	0.053	0.053	0.055	0.059	0.055	0.055
4. *Aoraia rufivena*	0.013	0.009	0.006	-	0.089	0.089	0.082	0.072	0.091	0.066	0.076	0.074	0.074	0.078	0.071	0.071	0.074	0.078	0.074	0.074
5. *Cladoxycanus minos*	0.014	0.013	0.013	0.014	-	0.066	0.072	0.072	0.068	0.064	0.07	0.068	0.072	0.072	0.066	0.072	0.068	0.072	0.072	0.064
6. *Dioxycanus fuscus*	0.013	0.013	0.012	0.014	0.012	-	0.015	0.059	0.064	0.023	0.043	0.045	0.043	0.047	0.045	0.041	0.047	0.049	0.043	0.045
7. *Dioxycanus oreas*	0.012	0.012	0.012	0.013	0.013	0.006	-	0.053	0.053	0.017	0.039	0.041	0.041	0.045	0.039	0.037	0.041	0.049	0.039	0.045
8. *Dumbletonius characterifer*	0.013	0.011	0.011	0.013	0.013	0.011	0.011	-	0.057	0.043	0.049	0.047	0.049	0.051	0.045	0.051	0.047	0.064	0.051	0.053
9. *Dumbletonius unimaculatus*	0.014	0.012	0.012	0.014	0.012	0.012	0.011	0.011	-	0.051	0.044	0.046	0.041	0.037	0.039	0.041	0.041	0.049	0.046	0.049
10. *Heloxycanus patricki*	0.012	0.011	0.011	0.012	0.012	0.007	0.006	0.009	0.01	-	0.037	0.039	0.035	0.039	0.033	0.035	0.035	0.047	0.037	0.043
11. *Wiseana cervinata* 'northern'	0.013	0.011	0.011	0.013	0.012	0.009	0.009	0.01	0.01	0.009	-	0.011	0.017	0.013	0.011	0.011	0.013	0.021	0.013	0.021
12. *Wiseana cervinata* 'southern'	0.013	0.011	0.011	0.013	0.012	0.01	0.009	0.01	0.01	0.009	0.004	-	0.019	0.015	0.013	0.011	0.015	0.023	0.015	0.023
13. *Wiseana copularis* 'southern'	0.013	0.011	0.011	0.013	0.012	0.009	0.009	0.01	0.009	0.008	0.006	0.006	-	0.007	0.017	0.019	0.019	0.027	0.023	0.027
14. *Wiseana copularis* 'northern'	0.013	0.011	0.011	0.013	0.012	0.01	0.01	0.01	0.009	0.009	0.005	0.006	0.004	-	0.013	0.015	0.015	0.023	0.019	0.027
15. *Wiseana fulginea*	0.013	0.011	0.011	0.012	0.012	0.01	0.009	0.01	0.009	0.008	0.005	0.005	0.006	0.005	-	0.011	0.002	0.025	0.017	0.021
16. *Wiseana jocosa*	0.013	0.011	0.011	0.012	0.013	0.009	0.009	0.01	0.009	0.008	0.004	0.005	0.006	0.006	0.004	-	0.012	0.019	0.012	0.019
17. *Wiseana mimica*	0.013	0.011	0.011	0.013	0.012	0.01	0.009	0.01	0.009	0.008	0.005	0.006	0.006	0.006	0.002	0.005	-	0.027	0.019	0.023
18. *Wiseana signata* 'southern'	0.014	0.012	0.011	0.013	0.013	0.01	0.01	0.01	0.01	0.01	0.007	0.006	0.008	0.007	0.007	0.006	0.008	-	0.012	0.019
19. *Wiseana signata* 'northern'	0.013	0.011	0.011	0.013	0.013	0.009	0.009	0.01	0.01	0.009	0.005	0.006	0.007	0.006	0.006	0.005	0.006	0.005	-	0.015
20. *Wiseana umbraculata*	0.013	0.011	0.011	0.013	0.012	0.01	0.01	0.01	0.01	0.009	0.006	0.007	0.008	0.008	0.007	0.006	0.007	0.006	0.006	-

Weighting - The objective of *a priori* weighting is to make best use of the collected data given our current understanding of the mechanisms of and constraints on DNA sequence evolution (Simon *et al.*, 1994; but see Brower and DeSalle, 1994). Weighting does not always produce the results that theory predicts. For example, even though third positions in protein coding regions are known to experience multiple hits that may contribute to increased homoplasy (Simon *et al.*, 1994) removal of these positions may not be useful (Weller *et al.*, 1994).

Elimination of third positions in this analysis removed 38 of the 61 parsimony informative sites (18 transitions and 20 transversions), with a corresponding loss of resolution throughout the tree. Increasing the weighting at first and second positions in effect gave heavier weight to transitions because all nucleotide substitutions in these positions were transitions. This resulted in a tree identical to the unweighted MP tree apart from arrangements within the genus *Wiseana*.

Theoretically, transversions should recover divergences at deeper levels in a phylogeny (De Salle *et al.*, 1987; Cracraft and Helm Bychowski, 1991), although transversion weighting may be affected by base composition and secondary structure (Brower and DeSalle, 1994). In this analysis, transversion parsimony also resulted in a loss of resolution throughout the tree. Weighting transversions 2: transitions as 1, recovered a tree identical to the unweighted MP tree, indicating that the combination of tranversions and transitions contributed information at all levels in this phylogeny and should be retained.

Maximum Likelihood - Maximum likelihood analysis produced three trees under the Kimura two-parameter model (Kimura, 1980). The unrooted phylogram is shown in Figure 4. The only difference in the arrangement of the three ML trees was in the positioning of the *Wiseana cervinata* 'southern' haplotype. The arrangement of the other clades was the same as that found in one third of the MP trees: (*Cladoxycanus*, (*Dumbletonius characterifer*, ((*Heloxycanus*, *Dioxycanus*), (*Dumbletonius unimaculatus*, *Wiseana*)))).

Amino Acids - The inferred CO I amino acid sequence (Appendix 4) was part of the more variable M12 and COOH terminal regions identified by Lunt *et al.* (1996). The 5′ end of the COII region had few variable sites with reference to the *Drosophila yakuba* model (Clary and Wolsenholme, 1985). A single T stop codon was found at the end of the COI and tRNA as has been found in other insects (Crozier and Crozier, 1993; Mitchell *et al.*, 1993). Twenty eight of the 174 codons coded for leucine.

The frequency of leucine codons that began with T was 89% which would have contributed to the A,T bias observed in this region. Distance analysis of the amino acid data set produced 50 trees, the consensus of which supported *Cladoxycanus* as basal taxon and *Dumbletonius unimaculatus* as sister taxon to the genus *Wiseana*, but was not informative regarding the remainder of the relationships.

Figure 4: Unrooted majority rule consensus phylogram for the three trees produced by maximum likelihood analysis of mtCOI & II data from New Zealand hepialids. Branch lengths are proportional to the number of unambiguous nucleotide changes.

Addition of Australian taxa - *Fraus simulans*, recognised as a primitive hepialid (Nielsen and Kristensen, 1989) was used to root the cladogram when several Australian taxa were added to the analysis (Appendix 5). Two MP trees were produced (TL 296, CI 0.44, RI 0.59, G_1 -0.37). The majority rule consensus tree with bootstrap values is shown in Figure 5. The two trees differed only in their placement of taxa in the (*Wiseana signata, W. umbraculata*) clade. Either the two *W. signata* haplotypes grouped together or the *W. signata* 'southern' haplotype grouped with *W. umbraculata*. New Zealand *Aenetus* and *Aoraia* taxa and all Australian taxa fell outside the New Zealand *'Oxycanus'* lineages. The Australian genus *Oxycanus* appeared to be paraphyletic, with *Oxycanus sphragidias* in the same clade as *T. argentata* and *T. atripalpis*. The clade comprising the Australian taxa *Jeana timeata, Oxycanus australis, O. diremptus* and *O. sordidus* was the sister taxa to the New Zealand *'Oxycanus'* lineages. As in one third of the MP trees from the analysis of New Zealand taxa alone, *Dumbletonius characterifer* was recovered in a clade with *Cladoxycanus*. However there was poor bootstrap support at this node and at other deeper nodes. The order of the remaining taxa was ((*Heloxycanus, Dioxycanus*), (*Dumbletonius unimaculatus, Wiseana*)). Maximum likelihood analysis produced three trees, which differed only in their placement of the *Wiseana cervinata* haplotypes. The arrangement of the remaining taxa was the same as that recovered for the MP analysis described above.

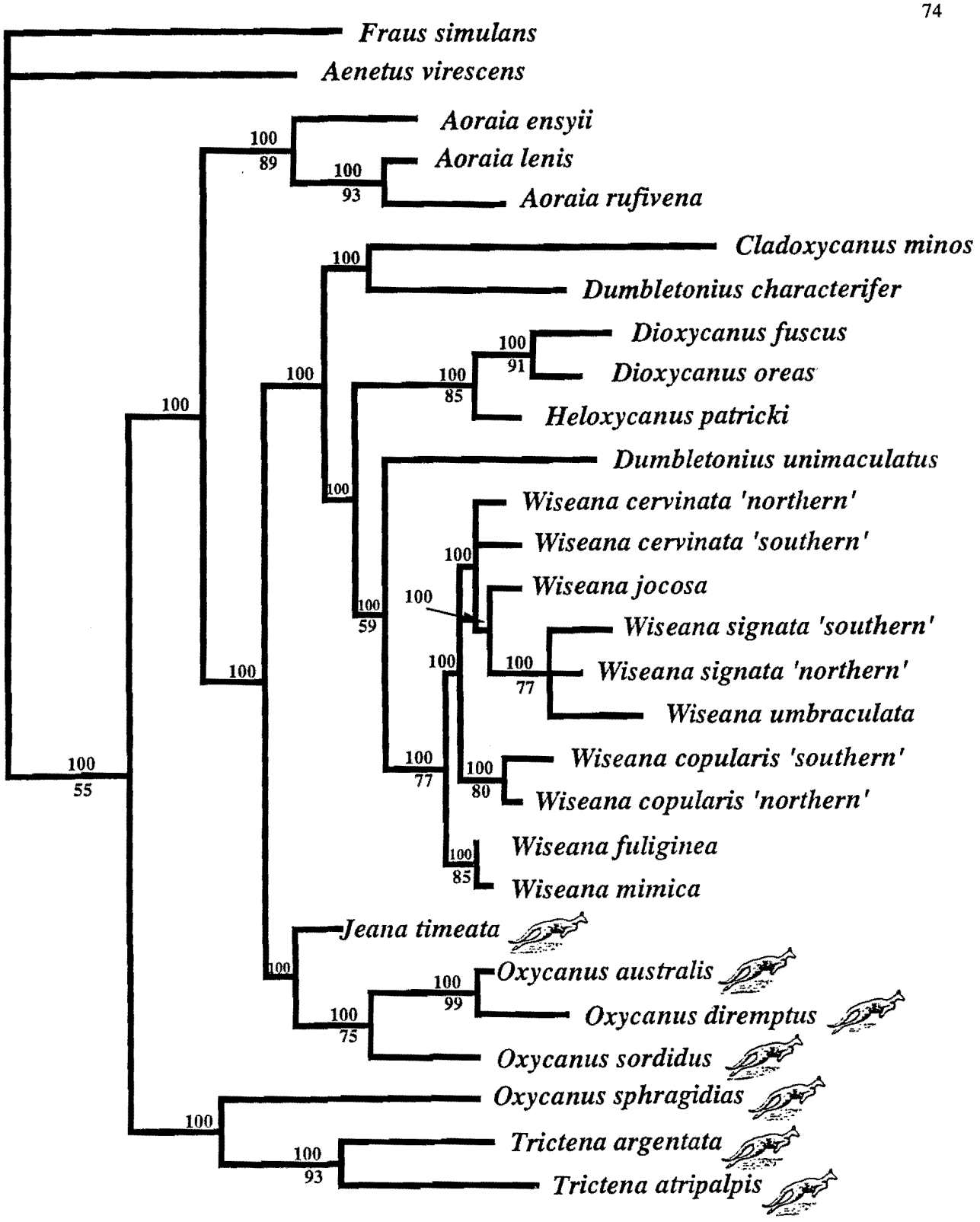

Figure 5: Majority rule consensus phylogram of the two most parsimonious trees from the analysis of mtDNA COI & II sequence data from New Zealand and Australian hepialids. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule values are shown above the branches and bootstrap proportions (>50%) below.

Discussion

Analysis of sequences from the COI & II regions of mtDNA produced four groupings that correspond to the four lineages: *Aenetus*, *Aoraia*, *'Oxycanus' Cladoxycanus* and *'Oxycanus' s. str.*, hypothesised by Dugdale (1994). This analysis supports the monophyly of the *'Oxycanus'* lineages. Recovery of the same branching order under maximum parsimony and maximum likelihood and stability of the branch topology under various weighting schemes gives confidence in the phylogenetic signal from the data used (Miyamoto and Cracraft, 1991; Miyamoto and Fitch, 1995) and the accuracy of the methods (Kim, 1993).

Aenetus* and *Aoraia - *Aenetus* and *Aoraia* occurred as separate branches, basal to the *'Oxycanus'* lineages, in all analyses. Addition of the Australian taxa to the data set showed *Aenetus* and *Aoraia*, along with the Australian *Trictena* spp. and *Oxycanus sphragidias* to fall outside the New Zealand *'Oxycanus'* lineages and their sister group which included some members of the Australian genera *Oxycanus* and *Jeana timeata*. This ingroup corresponds to part of the subfamily Oxycaninae (Dumbleton, 1966), which all have 'oxycanus' type wing venation. However, *Oxycanus sphragidias* also has the 'oxycanus' wing venation pattern. *Aenetus*, *Aoraia* and *Trictena* were all included in Dumbleton's subfamily Hepialinae. Nielsen and Robinson (1983) concluded that both subfamilies were probably paraphyletic, but testing this hypothesis was beyond the scope of our study.

Cladoxycanus - One third of the MP trees and all the ML trees supported *Cladoxycanus* as the basal taxon of the *'Oxycanus'* lineages. A further third of the MP trees placed *Cladoxycanus* in a basal clade with *Dumbletonius characterifer*. The fact that *Cladoxycanus* is so morphologically different to the remaining taxa, yet also shares some morphological synapomorphies with them, leads us to believe that it is the basal taxon in the New Zealand *'Oxycanus'* lineages and worthy of the separate lineage status given by Dugdale (1994). Invoking long branch attraction as an explanation of the *Cladoxycanus-Dumbletonius characterifer* clade is justified by the recovery of two separate branches under maximum likelihood. This method is not as sensitive to the long branch attraction problem because multiple substitutions that occur on long branches are corrected for (Huelsenbeck, 1997).

'Oxycanus' lineage s. str. - Analyses supported the branching order within this lineage to be: (*Dumbletonius characterifer,* ((*Heloxycanus, Dioxycanus*), (*Dumbletonius unimaculatus, Wiseana*))). This differs from our morphological analysis (Chapter 2) where *Dumbletonius characterifer* and *D. unimaculatus* were recovered in a clade together. In the molecular analysis, a combination of few synapomorphies and long branches with many autapomorphies may have caused instability in the branching order of *Dumbletonius characterifer* and *D. unimaculatus*. However, *Dumbletonius characterifer* mostly occurred as a monotypic taxon, and *D. unimaculatus* occurred as sister taxon to the genus *Wiseana* in all the ML trees, two thirds of the MP trees and all the weighted scenarios.

This result suggests that morphological characters such as twin processes, antennal rami shape and antrum floor sclerotization interpreted as homologies for *Dumbletonius characterifer* and *D. unimaculatus* in Chapter 2 may be homoplasious or perhaps plesiomorphic. Both *D. characterifer* and *D. unimaculatus* have autapomorphic morphological features. *Dumbletonius characterifer* has an apically acute forewing with an oblique termen, ocellate scale pattern and bicoloured scales while *Dumbletonius unimaculatus* has apically rounded forewings with a convex termen and no bicoloured scales. Differences also occur in the pseudotegumen of the male genitalia with *D. unimaculatus* having a large invaginated sclerite behind the subanal papilla. Only one population of each taxon was sampled in this study because of difficulties locating accessible populations and difficulties in coordinating trapping events with moth flight events. Identification of additional haplotypes within either of these species would help break up long branches and possibly stabilise the placement of these taxa in the phylogeny.

Dioxycanus - The *Dioxycanus* spp. always occurred in a clade with *Heloxycanus* and this relationship was supported by five synapomorphies and a 89% bootstrap value. In two thirds of the MP trees and all the ML trees the (*Heloxycanus, Dioxycanus*) clade occurred as sister group to the (*D. unimaculatus, Wiseana*) clade. A similar topology was recovered from the cladistic analysis of morphological characters (Chapter 2), with the (*Heloxycanus, Dioxycanus*) clade as sister group to the (*Dumbletonius,* Wiseana) clade.

Wiseana - In this analysis, new haplotypes for *Wiseana cervinata*, *W. copularis* and *W. signata* were identified. The *Wiseana cervinata* 'northern' haplotype was found in specimens from Mangapehi, Hawera and Ohakune all on North Island (Appendix 1) and the 'southern' haplotype from specimens from Nelson, Goulter River, Birdlings Flat, Otaio and Dunedin, all on South Island. These two groupings correspond to geographical groupings north and south of a latitudinal line approximately 40°S, that have previously been identified based on adult emergence times and differences in discal cell white scale shape (Dugdale, 1994; B. Brown, unpublished data) and allozymes (Herbert, 1995).

The *Wiseana copularis* 'northern' haplotype was found by chance (and subsequently sequenced) when populations were being surveyed with restriction enzymes for the development of a diagnostic test for *Wiseana* species. Specimens from three populations had already been sequenced for *W. copularis,* but they represented the same 'southern' haplotype even though they were from populations at Ohai, Dunedin and Christchurch. The 'northern' haplotype with a northern and western South Island distribution was found in specimens from Opouri Saddle, Pohara, Cobb Ridge and Greens Beach. Restriction fragment length polymorphism (RFLP) patterns (Chapter 7) revealed that the *W. copularis* 'southern' haplotype, had a southern and eastern South Island distribution, being found in specimens from Kaka Point, Mackenzie Country and Queenstown. Both 'southern' and 'northern' haplotypes were found in equal numbers in specimens collected at Queenstown on a single night, at a single location. Subsequent to the identification of two *W. copularis* haplotypes, it was confirmed (John Dugdale, personal communication) that some variation in genitalic characters had been noted between specimens from southern South Island (Gore) and those from northern South Island (Opouri Saddle), in the preparation of Dugdale (1994). *Wiseana copularis* is also found on North Island. No specimens were available, although attempts were made to collect there.

Two haplotypes for *W. signata* were identified. Specimens from the South Island location of Pohara differed from North Island specimens from Woodhill forest, Mangapehi and Levin.

Although the biological significance of these haplotypes and the extent of their geographical boundaries needs to be investigated further, these findings are significant as they may indicate the extent to which vicariance events have produced many cryptic taxa within the New Zealand lepidopteran fauna. The occurrence of two *Wiseana cervinata* haplotypes may also explain the different results reported from field trials investigating control measures for the larvae of *Wiseana cervinata* in Hamilton (Wrenn *et al.*, 1985) and South Otago (Stewart and Ferguson, 1989), using the moulting inhibitor diflubenzuron (Dimilin). Herbert (1995) identified 'northern' form *Wiseana cervinata* larvae in the Hamilton area and a *W. cervinata* 'southern' form in the South Otago area using allozymes. These regions correspond with the location of the two haplotypes found in this study.

Some relationships within the genus *Wiseana* were able to be resolved. Several clades were identified but the relationships between these were not strongly supported. Lack of synapomorphies may indicate rapid evolution with insufficient time for nucleotide substitutions to accumulate on short internodes (Martin and Pashley, 1992). Similar sequence divergence percentages between *W. jocosa* and *W. cervinata* 'southern' and 'northern'; *W. mimica* and *W. copularis* 'southern'; *W. mimica* and *W. cervinata* 'southern'; and *W. umbraculata* and *W. signata* 'southern' also suggest that taxa diverged within a very short time. All nucleotide substitutions within the genus *Wiseana* were transitions indicating that this group has probably evolved very recently (Swofford *et al.*, 1996). Morphology may provide significant evidence to support branch order at these points (see Brown *et al.*, 1994b) but in the case of *Wiseana*, no morphological synapomorphies could be identified.

Wiseana fuliginea and *W. mimica* always occurred in a clade together. This relationship was also recovered by Herbert (1995) from an allozyme data set. Corrected divergences for the *Wiseana fuliginea* and *W. mimica* haplotypes only differed by 0.19%, whereas *Wiseana signata*, *W. copularis* and *W. cervinata* haplotypes differed by 1.1%, 0.9% and 0.7% respectively. *Wiseana fuliginea* was synonomised with *W. cervinata*, until MacArthur (1986) defined it enzymatically. Superficially from colour and scale pattern, *W. fuliginea* males are indistinguishable from *W. mimica* and *W. cervinata* males. However, *Wiseana fuliginea* males can be distinguished from *W. cervinata* by their pointed scales, and several genitalic characters: short twin processes, angulate saccus margin, strong lateral apophysis on the vinculum base and bowed outer margin of the pseudotegumen (Dugdale, 1994). *Wiseana fuliginea* and *W. mimica* are distinguished by antenna rami shape, with *W. fuliginea* having short triangular rami and *W. mimica* long triangular rami.

The topotype of *W. mimica* was not included in this study and neither were specimens from populations found at altitude extremes (Kyeburn, 1300m) which emerge in December or from populations found at latitude extremes (Invercargill, 46°30'S) which emerge as early as September (John Dugdale, personal communication).

Only one nucleotide difference was found between the *W. fuliginea* and *W. mimica* haplotypes for the region sequenced, which suggests that they may have diverged very recently. Biologically, *Wiseana fuliginea* falls within the distribution range of *W. mimica*, apart from populations in Canterbury at Lincoln, Birdlings Flat and Kaiapoi. Adult emergence times for *W. fuliginea* (October - December) fall within those for *W. mimica* (September - February) and larvae of both are implicated in pasture defoliation (Herbert, 1995; Dugdale, 1994). Herbert (1995) reported habitat preferences for both *W. fuliginea* and *W. mimica* larvae as being summer dry soils, moist soils, lowland to low alpine, newly reclaimed pasture near tussock grassland. Herbert (1995) found few electrophoretic differences between the adults or the larvae of *W. fuliginea* and *W. mimica*. Adults only differed electrophoretically at one of the 30 loci investigated. At the Gp-2 locus, *W. fuliginea* had the 'b' allele and *W. mimica* the 'a' allele. The *W. fuliginea* and *W. mimica* caterpillars could not be reliably separated using fixed differences at loci, although there were frequency differences at the Gpi-1 locus.

The two *W. copularis* haplotypes always occurred together in a clade that was supported by three synapomorphies. *Wiseana jocosa* always grouped with the *W. signata* haplotypes and *W. umbraculata*. Cladistic analysis of the morphological data recovered *W. signata* and *W. umbraculata* in a clade that was a sister group to the remainder of the *Wiseana* species. This pattern was not recovered from the molecular data, although *W. signata* and *W. umbraculata* always occurred together. The relationships between the two *W. cervinata* haplotypes was unable to be resolved, but they are never recovered in a clade together in any of the analyses, suggesting they are not close sister taxa.

Biogeographical implications - The origins and age of the New Zealand hepialid fauna are unknown, although historically Australia has been regarded as the source of the fauna (Meyrick, 1890; Dumbleton, 1966) due to morphological similarities. Hepialids may have been part of the original Gondwanan fauna with ancestors of the present day New Zealand hepialid fauna isolated by the widening of the Tasman Sea, approximately 60 mya. However, a Gondwanan origin is questionable given the evidence presented by Pole (1994) that most of New Zealand's extant flora may date from only the late Miocene or Pliocene.

Alternatively, the ancestors of each lineage may have arrived in New Zealand as the result of one or several dispersal events from close landmasses. New Caledonia was the closest landmass to New Zealand between 37-25 mya, however, since the Miocene (24-5 mya) Australia has been the closest (Stevens *et al.*, 1988). Westerly winds have predominated in the region since the Miocene (McGlone, 1985), which may have assisted wind-blown adult hepialids from Australia to the western regions of New Zealand. Survival after such a journey is favoured by several physical factors; adults are large, robust and non-feeding and females are highly fecund, producing up to 1000 eggs each (Barratt *et al.*, 1990; pers obs). Based on a cladistic analysis of morphological characters (Chapter 2), it was hypothesised that there may have been at least four hepialid dispersal events from Australia to New Zealand.

If there have been no subsequent dispersal events after the Tasman Sea formation, then the estimated time since divergence between the New Zealand and Australian faunas should be large, whereas more recent dispersal events will produce a smaller estimate of time since separation.

We hypothesise, based on the COI & II sequence data presented, that the New Zealand '*Oxycanus*' lineages have radiated in situ after splitting from an ancestor shared with the Australian taxa *Jeana* and *Oxycanus*. An estimate of time of divergence can be made using pairwise sequence divergences and published rates of substitution for mtDNA, provided that the rate of change along each lineage, as measured by the relative rates test (Sarich and Wilson, 1967), is similar. Rates of 2% and 2.3% pairwise sequence divergence per one million years have been calculated by DeSalle *et al.* (1987) and Brower (1994b) respectively. De Salle's value was calculated from NADH and rRNA sequences of Hawaiian *Drosophila* and Brower's from mtDNA regions of recently diverged arthropod taxa. These values are similar to that estimated by Brown *et al.* (1979) for mtDNA divergence between primate lineages.

Estimation of divergence times based on 2-2.3% of pairwise sequence divergence per one million years supports the New Zealand hepialid fauna being of recent rather than Gondwanan origin. Using this estimate of divergence, the *'Oxycanus'* lineage *Cladoxycanus* would have shared a common ancestor with its Australian sister group approximately 3-4 mya, the *Aoraia* lineage with the *Cladoxycanus* and *'Oxycanus' s. str.* lineages 3-5 mya and *Aenetus* with the *Cladoxycanus* and *'Oxycanus' s. str.* lineages 4-5 mya. Radiation within the genus *Wiseana* may have occurred more recently around 1-1.5 mya and would correspond to the end of the Pliocene/ beginning of Pleistocene era, when there was uplifting of the Southern Alps and other ranges such as the Rock and Pillars (Stevens *et al.*, 1988), resulting in the creation of new subalpine and tussock grassland niches. Approximately 850,000 ya marks the end of twelve major glacial periods which began 2.4 mya (Stevens *et al.*, 1988). The Central North Island was glaciated, as were the Tararua Ranges. The estimated time of separation of the *Wiseana signata* haplotypes is close to this period at 800,000-1 mya. Separation of the *W. copularis* and *W. cervinata* haplotypes is estimated at 4-500,000 and 3-350,000 ya respectively. The separation of ancestral populations of these taxa may have occurred during the eight further glacial periods between 850,000 ya and 14,000 ya. Suitable tussock grasslands niches predominated the landscape during stadials and new niches became available as the glaciers retreated during the interstadials (McGlone, 1985).

The accuracy of the estimations of mtDNA evolution rate is unknown, but confidence limits are thought to be very large (Frank Prüser, personal communication). The 2-2.3% sequence divergence per one million years gave times of separation, especially within the genus *Wiseana,* that fit well with known geological events. The rate was also found to give an estimate of separation time for ground weta lineages from the Rock and Pillar Range, Central Otago, that corresponded to mountain range expansion during the Kaikoura Orogeny (Tania King, personal communication).

Estimated times of divergence for the basal lineages *Aenetus* and *Aoraia* are much more recent than had been assumed based on morphological character assessment (John Dugdale, personal communication). Larval biology, and larval and pupal structure support New Zealand *Aenetus* being part of a group of genera from the Pacific and South East Asia regions. Included in this group are *Endoclita*, *Sahyadrassus*, *Phassus*, *Trichophassus* and *Zelotypia* (John Dugdale, personal communication). *Aenetus* taxa are also found on Australia, New Caledonia, New Guinea and the islands of the Banda Arc.

The origin of New Zealand *Aenetus* is uncertain and even if the estimated time of divergence is a 10-fold underestimate, this still does not support a Gondwanan origin. No *Aenetus* specimens other than from New Zealand were available for this study, so it is not known how closely related New Zealand *Aenetus* is to other taxa, although there are known morphological differences between the New Zealand *Aenetus* and Australian and New Caledonian taxa (Dugdale, 1994).

If the current distribution of *Aenetus* taxa is due to dispersal over stretches of water, then it is surprising that *Aenetus* has not dispersed from New Zealand's North Island across the 40-100 kilometre wide Cook Strait to South Island, where suitable host trees such as *Carpodetus serratus* are common. Possibly the distribution of *Aenetus* throughout North Island is very recent.

Underestimation of the times of divergence for the basal taxa *Aenetus* and *Aoraia* may be due to an underestimation of the amount of change that has occurred along these branches due to multiple hits (De Salle *et al.*, 1987). This hypothesis is supported by an increased number of transversion substitutions with increased sequence divergence. Sperling *et al.* (1997) found Brower's estimate of 2% sequence divergence/my to give up to a 10-fold underestimate of divergence times for *Limnoporus* water striders compared with an estimated time of divergence based on fossil evidence and Prüser and Mossakowski (1997) reported sequence divergence rates of <1%/my for Mediterranean carabid beetles.

Comparison of molecular and morphological data sets - The molecular data set presented here and the morphological data set presented in Chapter 2, agree on the order of branching within the *'Oxycanus'* lineages apart from the placement of *Dumbletonius characterifer* and relationships within the genus *Wiseana*. Molecular data place *Dumbletonius characterifer* as the most basal of the *'Oxycanus' s. str.* taxa either alone or in a clade with *Cladoxycanus minos,* while morphological data place it in a clade with *D. unimaculatus*. Molecular data place *Wiseana jocosa* in a clade with *Wiseana signata* and *W. umbraculata,* while the morphological data place it in a clade with *W. cervinata. W. copularis, W. fuliginea* and *W. mimica.* Placement of the (*W. fuliginea, W. mimica*) clade differs between the molecular and morphological analyses, with the clade being recovered basally in all molecular analyses, but not in the morphological analysis. Comparison of the 166 morphological and six molecular trees using the Kishino Hasegawa (1989) test showed that the differences between the two data sets were highly significant ($p<0.0001$). A lack of phylogenetic signal combined with noise in both data sets may have contributed to this result.

The COI & II region may be evolving too slowly to be informative for very recent divergences and/or the factor(s) contributing to the changes in the morphology may be correlated with environmental adaption rather than reflecting phylogenetic relationship.

Conclusions

Mitochondrial DNA data recover four clades within the New Zealand hepialid taxa corresponding to the four lineages hypothesised by Dugdale (1994). The *Aenetus* and *Aoraia* lineages fall outside the *'Oxycanus'* lineages. The New Zealand *'Oxycanus'* lineages form a monophyletic group, with their nearest relatives most likely being Australian. Relationships within the genus *Wiseana* were not totally resolved. All nucleotide substitutions within this genus were transitions suggesting recent radiation, while lack of resolution deeper within the clade suggests rapid radiation. Estimated times of divergence and the number of autapomorphic characters for each *Wiseana* species support this hypothesis.

An independent estimation of relationships within this family in New Zealand is being investigated with a faster evolving, nuclear gene region.

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Appendix 1: Collection locations for New Zealand hepialids sequenced in this study.

Species	Location	Code*	Grid Ref.*	Collection date	Collector
Aenetus virescens	Leigh	AK	R09 725-443	14.x.1995	J. Duckworth
	Wainuiomata	WN	R27 767-914	18.x.1995	O. Spearpoint
Aoraia ensyii	Mt Taranaki	TK	P20 040-128	22.iii.1995	P. Peckham
Aoraia lenis	Danseys Pass	CO	I41 027-806	6.iv.1995	B. Brown
Aoraia rufivena	Great Moss Swamp	CO	H43 711-169	7.iv.1995	B. Brown
Cladoxycanus minos	Mackenzie Country	MK	H39 760 465	23.iv.1996	E.G. White
	Dunedin	DN	I44 133-783	21.iv.1995	B.H. Patrick
Dioxycanus fuscus	Rastus Burn	CO	F41 805-643	22.i.1995	B. Brown
Dioxycanus oreas	Mt Taranaki	TK	P20 040-128	4.xii.1995	P. Peckham
	Island Saddle	MB	N30 930-926	28.xii.1994	B. Brown
Dumbletonius characterifer	Mangahuia	TO	S19 234-217	11.xii.1995	B. Brown
Dumbletonius unimaculatus	Waitakeres	AK	Q11 492-796	15.xi.1995	P.J. Wigley
Heloxycanus patricki	Danseys Pass	CO	I41 027-806	6.iv.1995	B. Brown
	Longwoods Range	SL	D46 110-363	31.iv.1997	B. Brown
Wiseana cervinata	Mangapehi	WO	S17 116-960	19.i.1995	L. Burgess
	Hawera	TK	Q21 290-790	10.xii.1995	B. Brown
	Ohakune	RI	S20 162-958	19.xii.1994	R.M. Emberson
	Nelson	NN	N27 182-844	23.x.1995	W.P.Thomas
	Goulter River	MB	N28 288-581	23.x.1995	B. Brown
	Birdlings Flat	MC	M37 860-090	22.xi.1994	B. Brown
	Otaio	SC	J39 621-248	10.xi.1995	M. Bowie
	Dunedin	DN	I44 133-783	20.xi.1995	B.H. Patrick
Wiseana copularis	Christchurch	MC	M35 792-433	28.xi.1994	B. Brown
	Ohai	SL	D45 187-627	8.xii.1994	B. Brown
	Opouri Saddle	MB	P27 713-073	25.i.1995	B. Brown
	Cobb Ridge	NN	M26 843-113	28.i.1995	B. Brown
	Greens Beach	WD	I34 188-974	10.ii.1995	B. Brown
Wiseana fuliginea	Birdlings Flat	MC	M37 860-090	22.xi.1994	B. Brown
	Dunedin	DN	I44 133-783	27.x.1995	B. Brown
Wiseana jocosa	Cobb Ridge	NN	M26 843-113	16.xi.1995	B. Brown
	Ohai	SL	D45 187-627	8.xii.1994	B. Brown
	Tuatapere	SL	D46 004-387	30.x.1996	B. Brown
	Otautau	SL	D45 187-627	9.xii.94	B. Brown
Wiseana mimica	Cass	NC	K34 963-090	29.x.1995	B. Brown
	Mackenzie Country	MK	H38 802-615	17.x.1995	E.G. White

Appendix 1 continued.

Species	Location	Code[a]	Grid Ref.[b]	Collection date	Collector
Wiseana signata	Woodhill Forest	AK	Q10 367-934	15.i.1995	L. Burgess
	Mangapehi	WO	S17 116-960	19.1.1995	L. Burgess
	Levin	WN	S25 977-496	20.iii.1997	J.I. Townsend
	Pohara	NN	N25 012-421	30.i.1995	B. Brown
Wiseana umbraculata	Kaitoke	WN	S26 918-113	7.xii.1995	B. Brown
	Christchurch	MC	M35 776-423	27.xi.1994	B. Brown
	Taitapu	MC	M36 718-267	24.xi.1994	B. Brown
	Mackenzie Country	MK	H38 801 622	10.xii.1995	E.G. White
	Otaio	SC	J39 621-248	19.xi.1995	M. Bowie
	Tuatapere	SL	D46 004-387	9.xii.1995	B. Brown

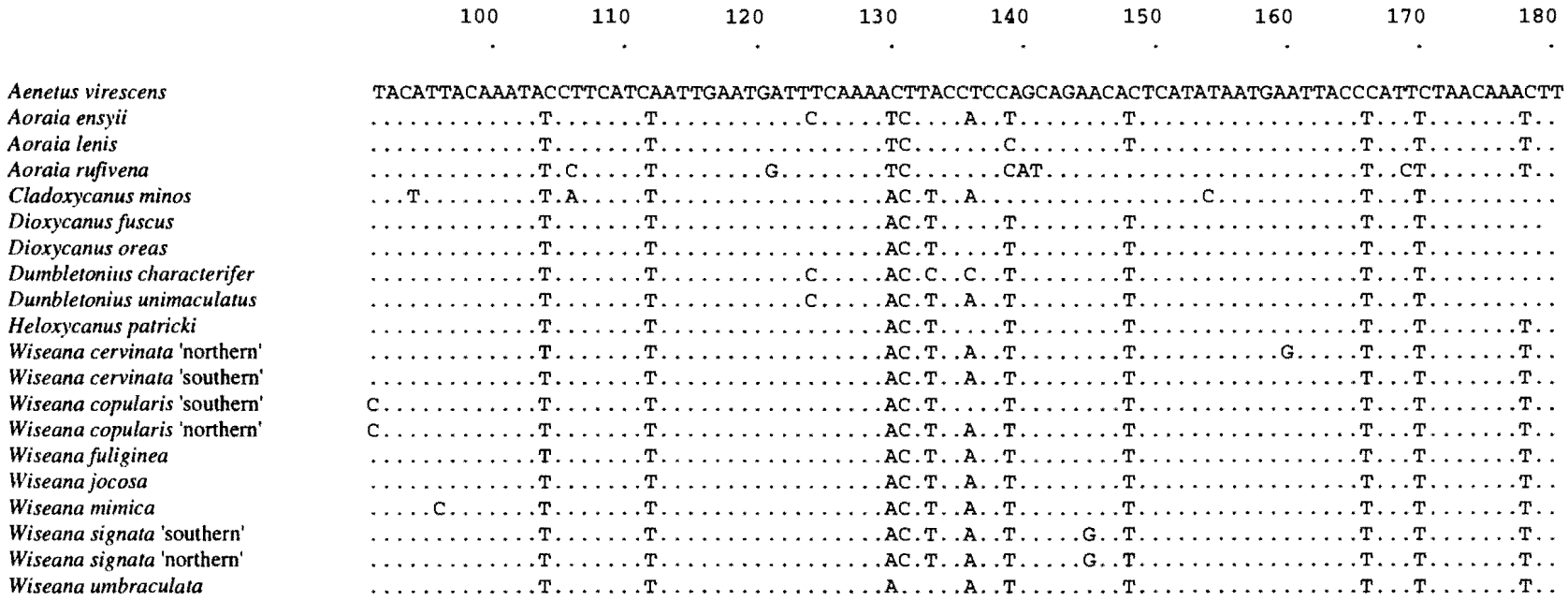
(a) System of areas and codes for recording specimen localities in New Zealand (Crosby *et al.*, 1976).
(b) Grid references from NZMS 260 series maps.

Appendix 2: Collection locations for Australian hepialids sequenced in this study.

Species	Location	State	Collection date	Collector
Fraus simulans	Mt. Field NP	Tasmania	26.iii.1997	R.M. Emberson
Jeana timeata	Collinsvale	Tasmania	18.iv.1996	M.A. Williams
Oxycanus australis	Mole Creek	Tasmania	13.iv.1996	B.Brown
O. diremptus	MacQuarie	ACT	2.v.1995	E.D. Edwards
O. sphragidias	Edgar Dam	Tasmania	10.iv.1997	R.M. Emberson
O. sordidus	Friendly Beach	Tasmania	19.iv.1996	B.Brown
Trictena argentata	Orford	Tasmania	8.iv.1996	B.Brown
T. atripalpis	O'Connor	ACT	2.v.1995	I.D. Naumann

Appendix 3: Aligned nucleotide sequence from mtDNA COI & II gene regions for 20 New Zealand hepialid taxa. Dots indicate nucleotides matching top line in each block.

```
                                10        20        30        40        50        60        70        80        90
                                .         .         .         .         .         .         .         .         .
Aenetus virescens               TTCTTTAGGATCTAATATTTCAATAATTGGTGTTATAATAATATTATTAATTATTTGAGAATCAATAATTAATAAACGAATAAATTTATT
Aoraia ensyii                   ...A.....T.....................A.....................................T.GA.....
Aoraia lenis                    .........T.....................G..A.....................................T.GA.....
Aoraia rufivena                 .........T.....................A..A.........A.....................................T.GA.....
Cladoxycanus minos              ....C....T........C.....................C.................T.......GA........T........
Dioxycanus fuscus               ...AC.......A.....................................................T.......GA........T........
Dioxycanus oreas                ...A........A.....................................................GA........T........
Dumbletonius characterifer      .........T........C.............A.....................................GA........T........
Dumbletonius unimaculatus       ...A.....T.....................................................G....GA........C........
Heloxycanus patricki            ...A.....T..A.....................................................GA........T........
Wiseana cervinata 'northern'    ...A.....................................................GA........T........
Wiseana cervinata 'southern'    ...A.....................C.....................GA..G.....T........
Wiseana copularis 'southern'    ...A.....................................GA........T........
Wiseana copularis 'northern'    ...A.....................................GA........C........
Wiseana fuliginea               ...A.....................................GA......G.T........
Wiseana jocosa                  ...A.....................................GA......G.T..C.....
Wiseana mimica                  ...A.....................................GA......G.T........
Wiseana signata 'southern'      ...A.....................C.....................GG........T........
Wiseana signata 'northern'      ...A.....................................GA........T........
Wiseana umbraculata             ...A.....................................GA........T........
```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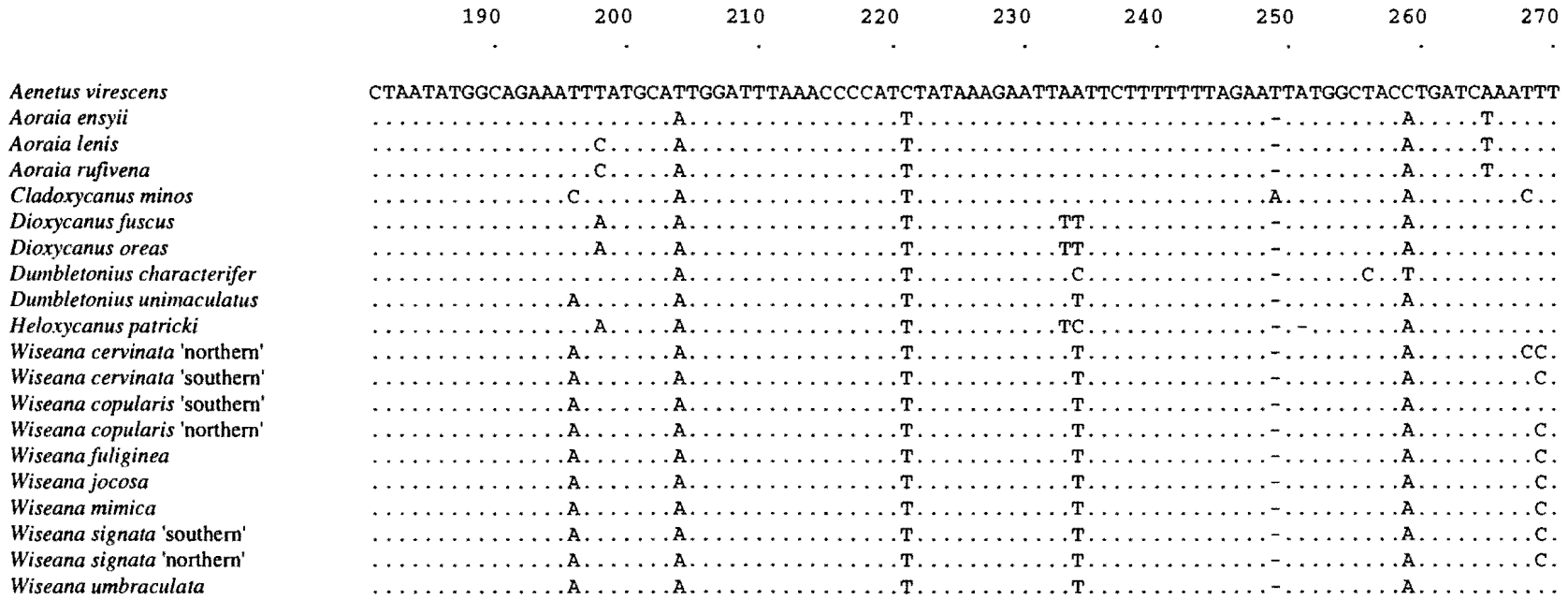
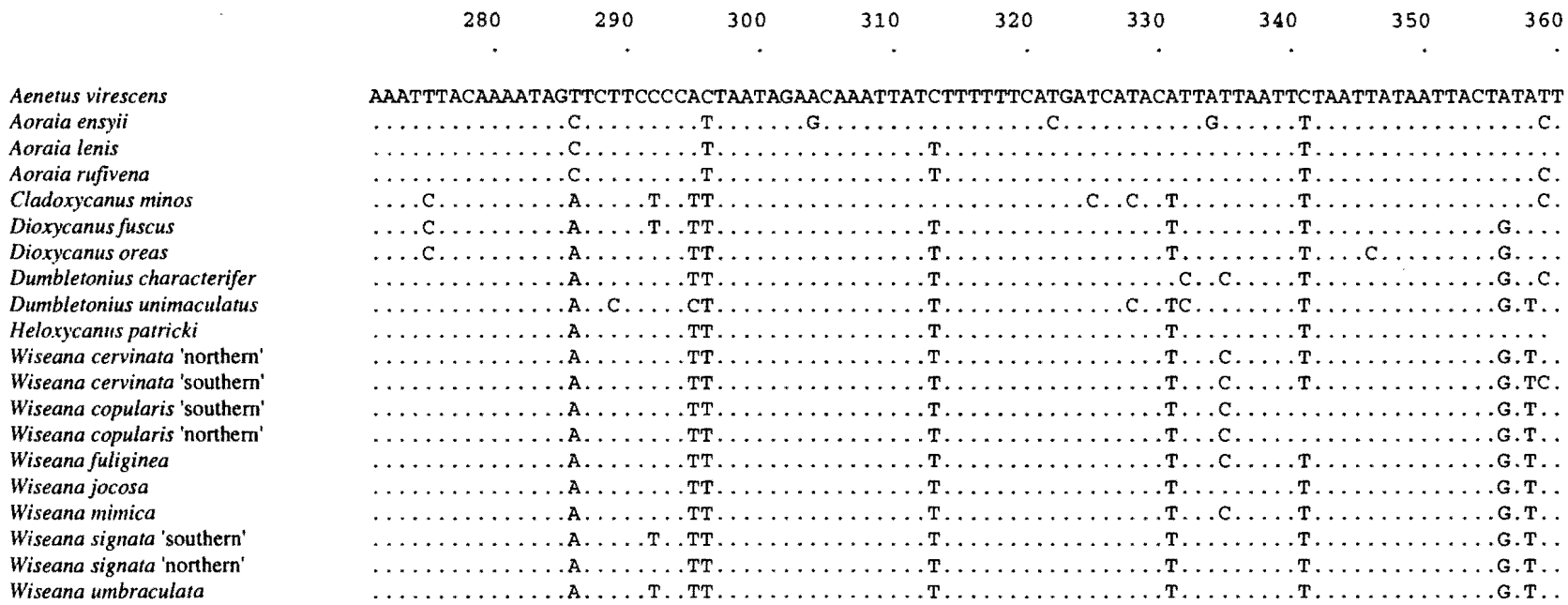
Aenetus virescens TACATTACAAATACCTTCATCAATTGAATGATTTCAAAACTTACCTCCAGCAGAACACTCATATAATGAATTACCCATTCTAACAAACTT
Aoraia ensyii
Aoraia lenis
Aoraia rufivena
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
Dumbletonius unimaculatus
Heloxycanus patricki
Wiseana cervinata 'northern'
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata


190 200 210 220 230 240 250 260 270
Aenetus virescens CTAATATGGCAGAAATTTATGCATTGGATTTAAACCCCATCTATAAAGAATTAATTCTTTTTTTAGAATTATGGCTACCTGATCAAATTT
Aoraia ensyii
Aoraia lenis
Aoraia rufivena
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
Dumbletonius unimaculatus
Heloxycanus patricki
Wiseana cervinata 'northern'
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata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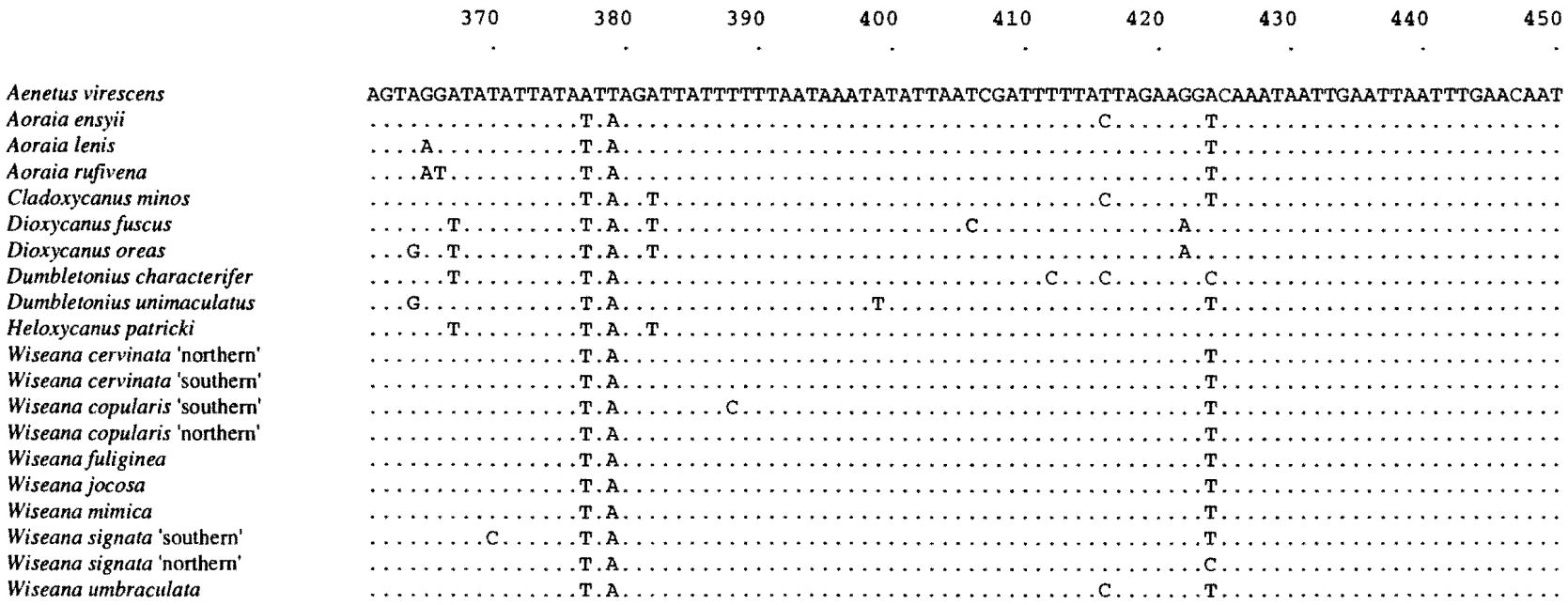
Aenetus virescens AAATTTACAAAATAGTTCTTCCCCACTAATAGAACAAATTATCTTTTTTCATGATCATACATTATTAATTCTAATTATAATTACTATATT
Aoraia ensyii .....C.....T.....G.....C.....G.....T.....C.
Aoraia lenis .....C.....T.....T.....T.....
Aoraia rufivena .....C.....T.....T.....T.....C.
Cladoxycanus minos ....C.....A.....T..TT.....C..C..T.....T.....C.
Dioxycanus fuscus ....C.....A.....T..TT.....T.....T.....T.....G....
Dioxycanus oreas ....C.....A.....TT.....T.....T.....T....C.....G....
Dumbletonius characterifer .....A.....TT.....T.....C..C.....T.....G..C.
Dumbletonius unimaculatus .....A..C.....CT.....T.....C..TC.....T.....G.T..
Heloxycanus patricki .....A.....TT.....T.....T.....T.....
Wiseana cervinata 'northern' .....A.....TT.....T.....T...C.....T.....G.T..
Wiseana cervinata 'southern' .....A.....TT.....T.....T...C.....T.....G.TC.
Wiseana copularis 'southern' .....A.....TT.....T.....T...C.....G.T..
Wiseana copularis 'northern' .....A.....TT.....T.....T...C.....G.T..
Wiseana fuliginea .....A.....TT.....T.....T...C.....T.....G.T..
Wiseana jocosa .....A.....TT.....T.....T.....T.....G.T..
Wiseana mimica .....A.....TT.....T.....T...C.....T.....G.T..
Wiseana signata 'southern' .....A.....T..TT.....T.....T.....T.....G.T..
Wiseana signata 'northern' .....A.....TT.....T.....T.....T.....G.T..
Wiseana umbraculata .....A.....T..TT.....T.....T.....T.....G.T..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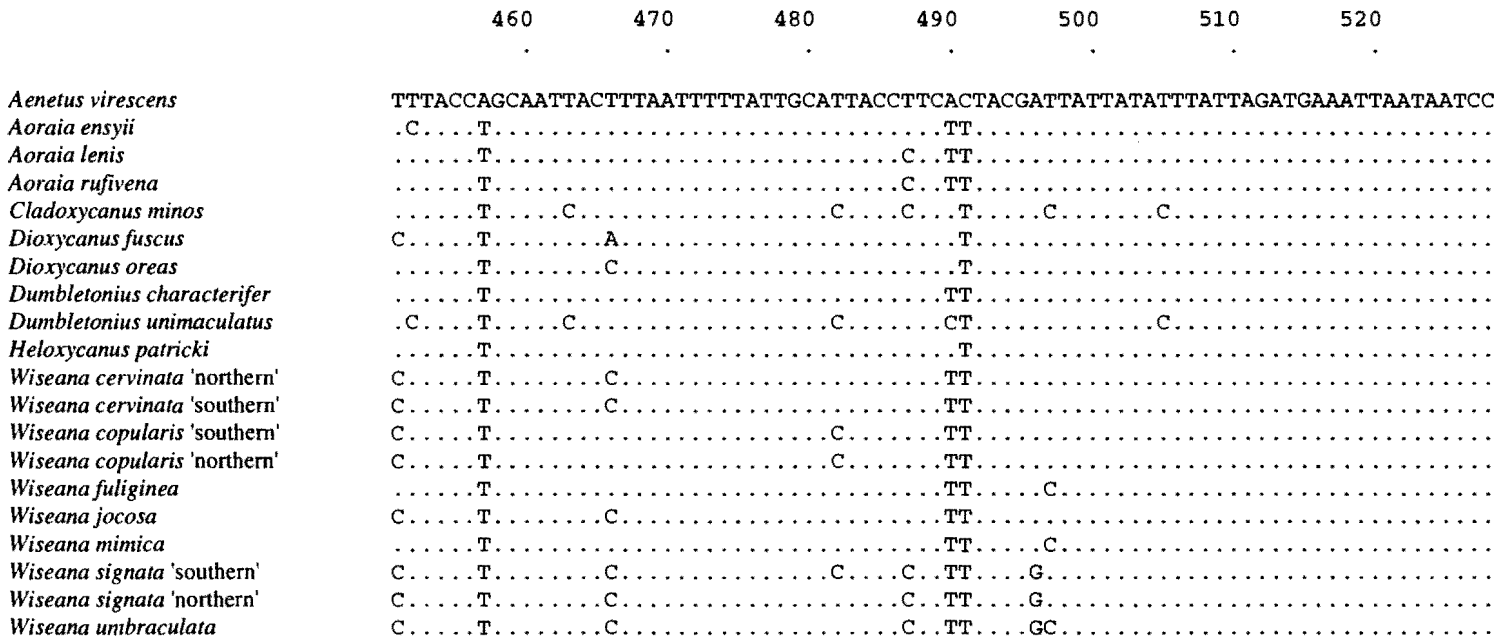
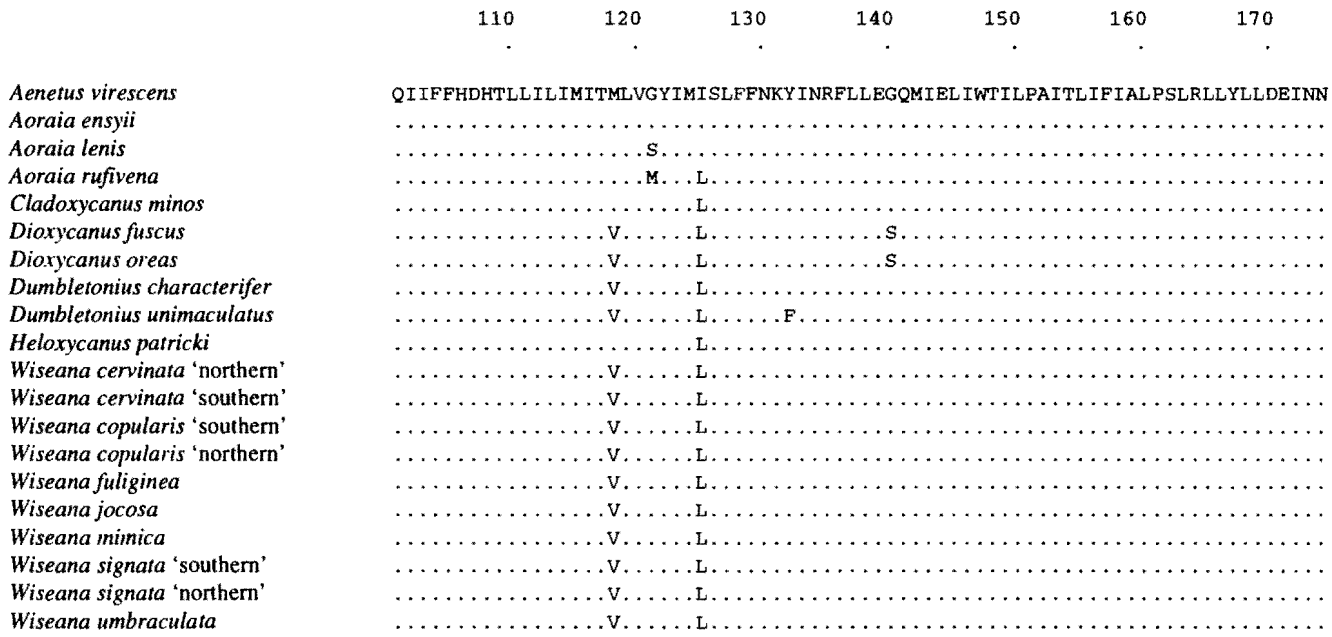
Aenetus virescens AGTAGGATATATTATAATTAGATTATTTTTTAATAAATATATTAATCGATTTTTATTAGAAGGACAAATAATTGAATTAATTTGAACAAT
Aoraia ensyii ..............T.A.....C.....T.....
Aoraia lenis ....A.....T.A.....T.....
Aoraia rufivena ....AT.....T.A.....T.....
Cladoxycanus minos ..............T.A..T.....C.....T.....
Dioxycanus fuscus ......T.....T.A..T.....C.....A.....
Dioxycanus oreas ...G..T.....T.A..T.....A.....
Dumbletonius characterifer ......T.....T.A.....C...C.....C.....
Dumbletonius unimaculatus ...G.....T.A.....T.....T.....
Heloxycanus patricki ......T.....T.A..T.....
Wiseana cervinata 'northern' ..............T.A.....T.....
Wiseana cervinata 'southern' ..............T.A.....T.....
Wiseana copularis 'southern' ..............T.A.....C.....T.....
Wiseana copularis 'northern' ..............T.A.....T.....
Wiseana fuliginea ..............T.A.....T.....
Wiseana jocosa ..............T.A.....T.....
Wiseana mimica ..............T.A.....T.....
Wiseana signata 'southern' .....C.....T.A.....T.....
Wiseana signata 'northern' ..............T.A.....C.....
Wiseana umbraculata ..............T.A.....C.....T.....


460 470 480 490 500 510 520
Aenetus virescens TTTACCAGCAATTACTTTAATTTTTATTGCATTACCTTCACTACGATTATTATATTTATTAGATGAAATTAATAATCC
Aoraia ensyii .C....T.....TT.....
Aoraia lenis ......T.....C..TT.....
Aoraia rufivena ......T.....C..TT.....
Cladoxycanus minos ......T.....C.....C....C...T.....C.....C.....
Dioxycanus fuscus C.....T.....A.....T.....
Dioxycanus oreas ......T.....C.....T.....
Dumbletonius characterifer ......T.....TT.....
Dumbletonius unimaculatus .C....T.....C.....C.....CT.....C.....
Heloxycanus patricki ......T.....T.....
Wiseana cervinata 'northern' C.....T.....C.....TT.....
Wiseana cervinata 'southern' C.....T.....C.....TT.....
Wiseana copularis 'southern' C.....T.....C.....TT.....
Wiseana copularis 'northern' C.....T.....C.....TT.....
Wiseana fuliginea ......T.....TT.....C.....
Wiseana jocosa C.....T.....C.....TT.....
Wiseana mimica ......T.....TT.....C.....
Wiseana signata 'southern' C.....T.....C.....C....C..TT....G.....
Wiseana signata 'northern' C.....T.....C.....C..TT....G.....
Wiseana umbraculata C.....T.....C.....C..TT....GC.....

Appendix 4: Aligned amino acid sequence from mtDNA COI & II gene regions for 20 New Zealand hepialid taxa. Dots indicate nucleotides matching top line in each block.

```
                                10        20        30        40        50        60        70        80        90        100
                                .         .         .         .         .         .         .         .         .         .
Aenetus virescens               SLGSNISMIGVMMMLLIIWESMINKRMNLFTLQMPSSIEWFQNLPPAEHSYNELPILTNSNMAEIYALDLNPIYKELILFLEMATWSNLNLQNSSSPLME
Aoraia ensyii                   ..........................IS.....S.............................M.............................
Aoraia lenis                    ..........................IS.....S.............................H.M.....I.....................
Aoraia rufivena                 ............M..........IS.....S..........M..................H.M.............................
Cladoxycanus minos              ......................S..I.....S.....K.....................T..M.............................
Dioxycanus fuscus               ......................S..I.....S.....K.....................N.M.....FF.....................
Dioxycanus oreas                ......................S..I.....S.....K.....................N.M.....FF.....................
Dumbletonius characterifer      ......................S..I.....S.....K.....................M.....L.......................
Dumbletonius unimaculatus       ......................S..I.....S.....K.....................N..M.....F.....................
Heloxycanus patricki            ......................S..I.....S.....K.....................N.M.....FL.....................
Wiseana cervinata 'northern     ..........................I.....S.....K.....................N..M.....F.....................
Wiseana cervinata 'southern'    ..........................I.....S.....K.....................N..M.....F.....................
Wiseana copularis 'southern'    ..........................I.....S.....K.....................N..M.....F.....................
Wiseana copularis 'northern'    ..........................I.....S.....K.....................N..M.....F.....................
Wiseana fuliginea               ..........................V.....S.....K.....................N..M.....F.....................
Wiseana jocosa                  ..........................V.....S.....K.....................N..M.....F.....................
Wiseana mimica                  ..........................V....S..S.....K.....................N..M.....F.....................
Wiseana signata 'southern'      ..........................I.....S.....K.....................N..M.....F.....................
Wiseana signata 'northern'      ..........................I.....S.....K.....................N..M.....F.....................
Wiseana umbraculata             ..........................I.....S.....K.....................N..M.....F.....................
```


110 120 130 140 150 160 170
Aenetus virescens QIIFFHDHTLLILIMITMLVGYIMISLFFNKYINRFLLEGQMIELIWTILPAITLIFIALPSLRLLYLLDEINN
Aoraia ensyii
Aoraia lenis S
Aoraia rufivena M L
Cladoxycanus minos L
Dioxycanus fuscus V L S
Dioxycanus oreas V L S
Dumbletonius characterifer V L
Dumbletonius unimaculatus V L P
Heloxycanus patricki L
Wiseana cervinata 'northern' V L
Wiseana cervinata 'southern' V L
Wiseana copularis 'southern' V L
Wiseana copularis 'northern' V L
Wiseana fuliginea V L
Wiseana jocosa V L
Wiseana mimica V L
Wiseana signata 'southern' V L
Wiseana signata 'northern' V L
Wiseana umbraculata V L

Appendix 5: Aligned nucleotide sequence from mtDNA COI & II gene regions for 20 New Zealand and eight Australian hepialid taxa. Dots indicate nucleotides matching top line in each block.

```
                          10        20        30        40        50        60        70        80        90
                          .         .         .         .         .         .         .         .         .
Fraus simulans            TCATTAGGATCAAATATTTCAATAATTGGAGTAATAATAATACTGTTAATTATTTGAGAATCAATAATTAATAAACGAGTAAATTTATTTACACTACAAA
Aenetus virescens         ..T........T.................T..T.........T.A.................................A..............T.......
Aoraia ensyii             ........T..T.................T............T.A.................................A.T.GA.........T.......
Aoraia lenis              ..T.....T..T.................G............T.A.................................A.T.GA.........T.......
Aoraia rufivena           ..T.....T..T.................................A.A.................................A.T.GA.........T.......
Cladoxycanus minos        ..TC....T..T.....C...........T..T.........T.AC.................T.......GA......A.T...........TT.......
Dioxycanus fuscus         ...C.....................................T..T.........T.A.................T.......GA......A.T...........T.......
Dioxycanus oreas          .....................................T..T.........T.A.....................GA......A.T...........T.......
Dumbletonius characterifer..T.....T..T.....C...........T.....................T.A.....................GA......A.T...........T.......
Dumbletonius unimaculatus .........T..T.................T..T.........T.A.....................G....GA......A.C...........T.......
Heloxycanus patricki      .........T.....................T..T.........T.A.....................GA......A.T...........T.......
Jeana timeata             ..T.....T..T.................T..T.........T.A.....................GA......A.T...........T.......
Oxycanus australis        ..T.....T..T..C..............T..T.........T.A.................T.......GA......A.T...........T.......
Oxycanus diremptus        ..T.....C..T.................T..T.........T.A.................T.....C.GA......A.T...........T.......
Oxycanus sordidus         ..T.....T..T.................T..T.........T.A.................T.......GA......A.T..C.........T.......
Oxycanus sphragidias      ..T.........T.................T..T.........T.A.....................GA......A.T..C.........T.......
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Trictena atripalpis       ..........................................T.A.................T.............A.T...............................
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Wiseana cervinata 'northern' ..........T.................T..T.........T.AC...................GA..G...A.T...........T.......
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Wiseana copularis 'northern' ..........T.................T..T.........T.A.....................GA......A.C........C...T.......
Wiseana fuliginea         ..........T.................T..T.........T.A.....................GA........T...........T.......
Wiseana jocosa            ..........T.................T..T.........T.A.....................GA........T..C.........T.......
Wiseana mimica            ..........T.................T..T.........T.A.....................GA........T...........TC......
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Wiseana signata 'northern' ..........T.................T..T.........T.A.....................GA......A.T...........T.......
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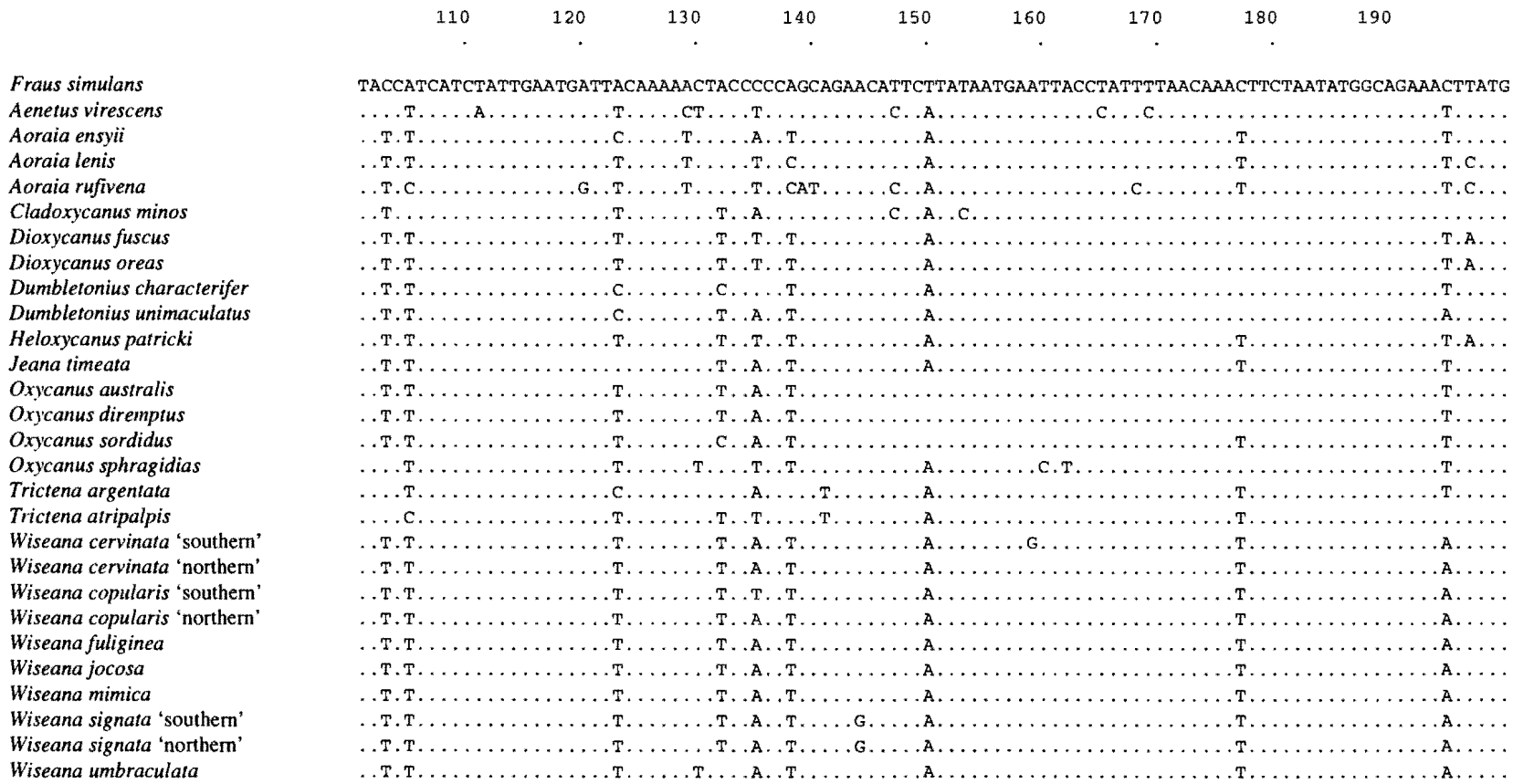
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Dioxycanus fuscus ..T.T.................T........T..T..T..........A.....................................T.A...
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Dumbletonius characterifer ..T.T.................C........C.....T..........A.....................................T.....
Dumbletonius unimaculatus ..T.T.................C........T..A..T..........A.....................................A.....
Heloxycanus patricki ..T.T.................T........T..T..T..........A.....................T.................T.A...
Jeana timeata ..T.T.........................T..A..T..........A.....................T.................T.....
Oxycanus australis ..T.T.................T........T..A..T...T.....
Oxycanus diremptus ..T.T.................T........T..A..T...T.....
Oxycanus sordidus ..T.T.................T........C..A..T.....................T.................T.....
Oxycanus sphragidias ....T.................T......T....T..T..........A.........C.T.....................T.....
Trictena argentata ....T.................C...........A.....T........A.....................T.................T.....
Trictena atripalpis ....C.................T........T..T.....T........A.....................T.....................
Wiseana cervinata 'southern' ..T.T.................T........T..A..T..........A........G.................T.................A.....
Wiseana cervinata 'northern' ..T.T.................T........T..A..T..........A.....................T.................A.....
Wiseana copularis 'southern' ..T.T.................T........T..T..T..........A.....................T.................A.....
Wiseana copularis 'northern' ..T.T.................T........T..A..T..........A.....................T.................A.....
Wiseana fuliginea ..T.T.................T........T..A..T..........A.....................T.................A.....
Wiseana jocosa ..T.T.................T........T..A..T..........A.....................T.................A.....
Wiseana mimica ..T.T.................T........T..A..T..........A.....................T.................A.....
Wiseana signata 'southern' ..T.T.................T........T..A..T.....G.....A.....................T.................A.....
Wiseana signata 'northern' ..T.T.................T........T..A..T.....G.....A.....................T.................A.....
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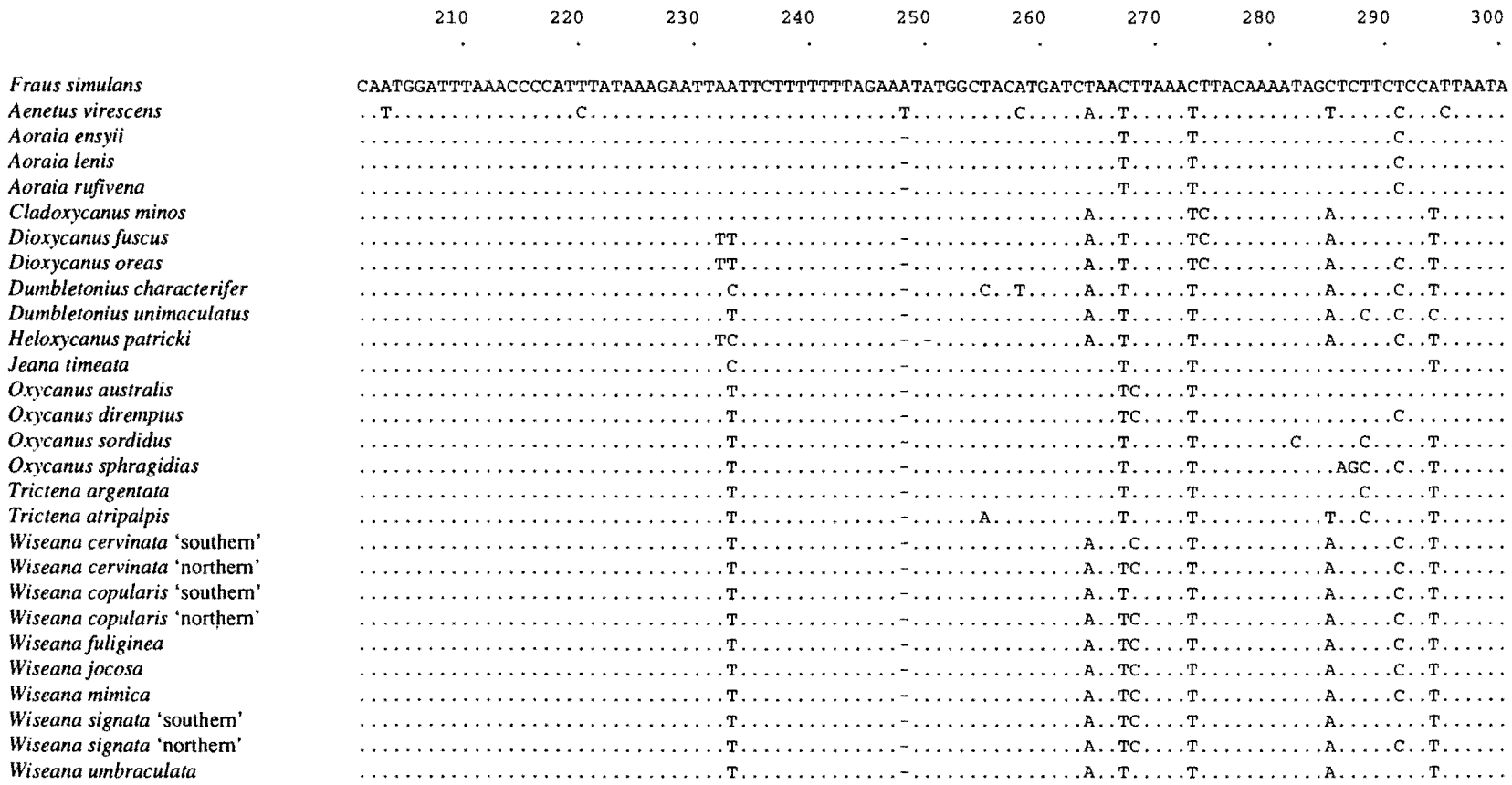
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Aenetus virescens ..T.....C.....T.....C.....A..T.....T.....T.....C...C.....
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Aoraia lenis .....-.....T.....T.....C.....
Aoraia rufivena .....-.....T.....T.....C.....
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Dioxycanus fuscus .....TT.....-.....A..T.....TC.....A.....T.....
Dioxycanus oreas .....TT.....-.....A..T.....TC.....A.....C..T.....
Dumbletonius characterifer .....C.....-.....C..T.....A..T.....T.....A.....C..T.....
Dumbletonius unimaculatus .....T.....-.....A..T.....T.....A..C..C..C.....
Heloxycanus patricki .....TC.....-.-.....A..T.....T.....A.....C..T.....
Jeana timeata .....C.....-.....T.....T.....T.....
Oxycanus australis .....T.....-.....TC....T.....
Oxycanus diremptus .....T.....-.....TC....T.....C.....
Oxycanus sordidus .....T.....-.....T.....T.....C.....C.....T.....
Oxycanus sphragidias .....T.....-.....T.....T.....AGC..C..T.....
Trictena argentata .....T.....-.....T.....T.....C.....T.....
Trictena atripalpis .....T.....-.....A.....T.....T.....T..C.....T.....
Wiseana cervinata 'southern' .....T.....-.....A...C....T.....A.....C..T.....
Wiseana cervinata 'northern' .....T.....-.....A..TC....T.....A.....C..T.....
Wiseana copularis 'southern' .....T.....-.....A..T.....T.....A.....C..T.....
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Wiseana fuliginea .....T.....-.....A..TC....T.....A.....C..T.....
Wiseana jocosa .....T.....-.....A..TC....T.....A.....C..T.....
Wiseana mimica .....T.....-.....A..TC....T.....A.....C..T.....
Wiseana signata 'southern' .....T.....-.....A..TC....T.....A.....T.....
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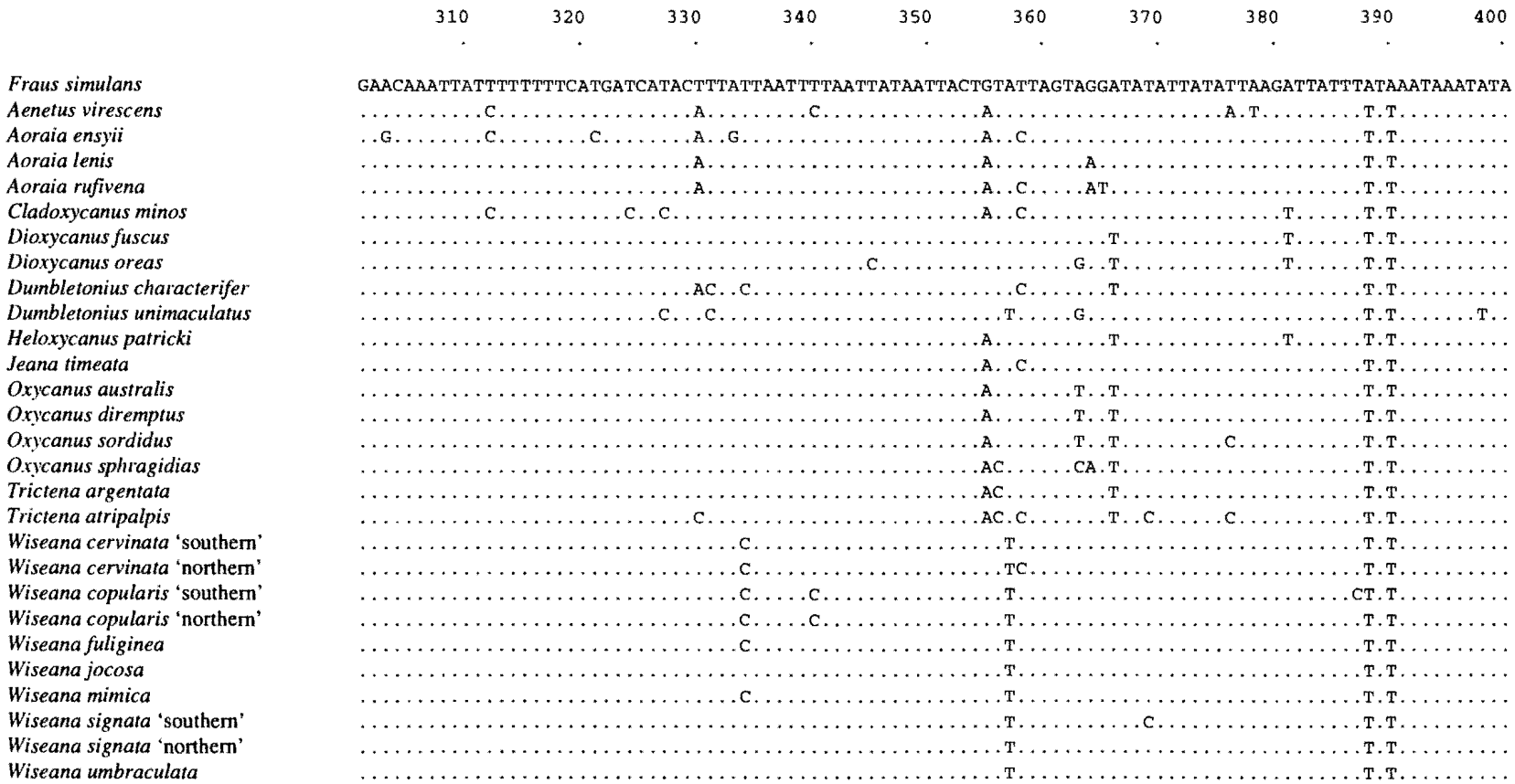
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Fraus simulans GAACAAATTATTTTTTTTCATGATCATACTTTATTAATTTTAATTATAATTACTGTATTAGTAGGATATATTATATTAAGATTATTTATAAATAAATATA
Aenetus virescens
Aoraia ensyii
Aoraia lenis
Aoraia rufivena
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
Dumbletonius unimaculatus
Heloxycanus patricki
Jeana timeata
Oxycanus australis
Oxycanus diremptus
Oxycanus sordidus
Oxycanus sphragidias
Trictena argentata
Trictena atripalpis
Wiseana cervinata 'southern'
Wiseana cervinata 'northern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
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Wiseana mimica
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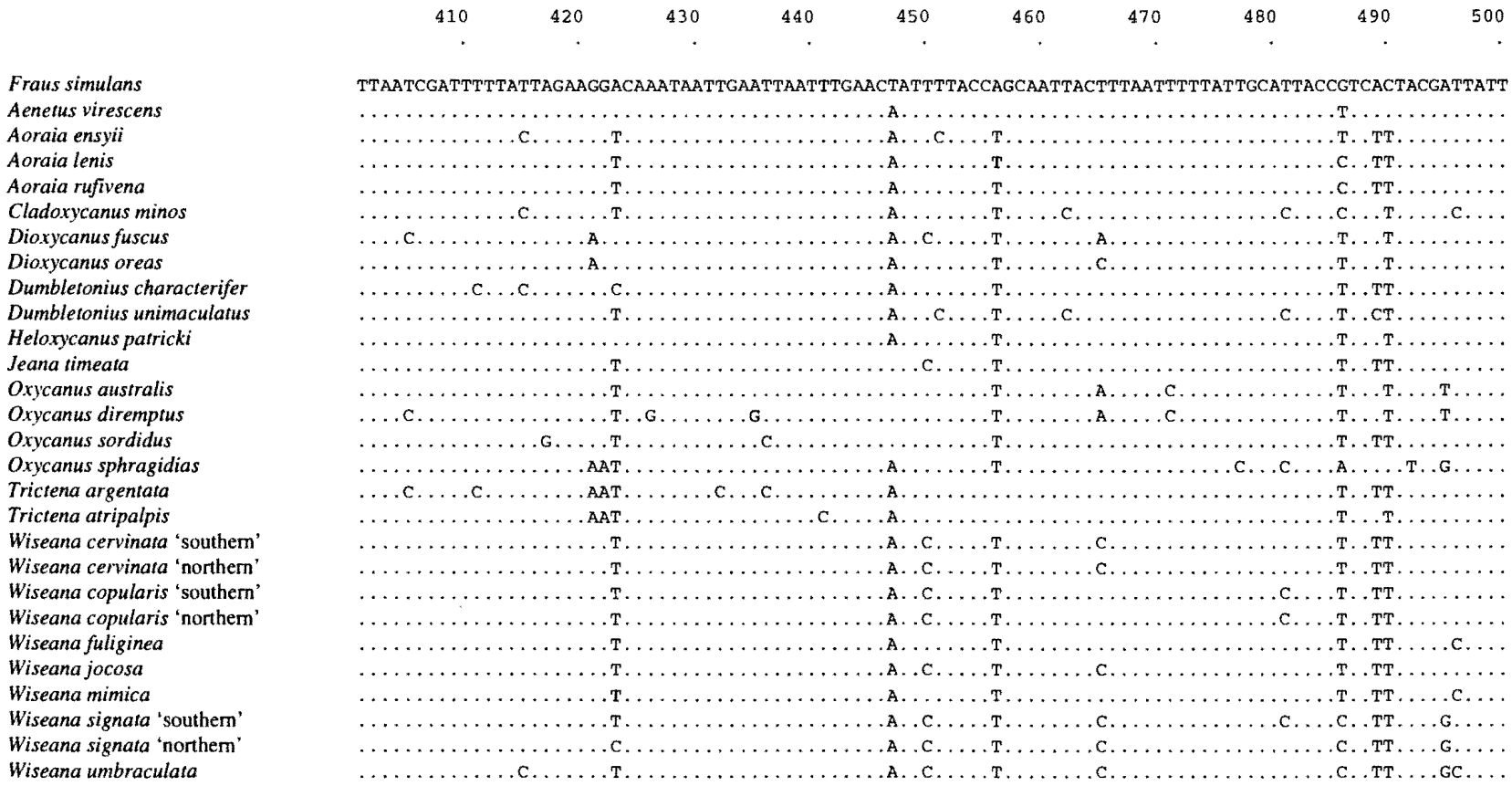
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Fraus simulans TTAATCGATTTTTATTAGAAGGACAAATAATTGAATTAATTTGAACTATTTTACCAGCAATTACTTTAATTTTTATTGCATTACCGTCACTACGATTATT
Aenetus virescens
Aoraia ensyii
Aoraia lenis
Aoraia rufivena
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
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Jeana timeata
Oxycanus australis
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Oxycanus sordidus
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Trictena argentata
Trictena atripalpis
Wiseana cervinata 'southern'
Wiseana cervinata 'northern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata

	510 520
Fraus simulans	ATATCTTTAGATGAAATTAATAATCC
Aenetus virescens	....T.A.....
Aoraia ensyii	....T.A.....
Aoraia lenis	....T.A.....
Aoraia rufivena	....T.A.....
Cladoxycanus minos	...CT.A.....
Dioxycanus fuscus	....T.A.....
Dioxycanus oreas	....T.A.....
Dumbletonius characterifer	....T.A.....
Dumbletonius unimaculatus	...CT.A.....
Heloxycanus patricki	....T.A.....
Jeana timeata	....T.A.....
Oxycanus australis	....T.A.....
Oxycanus diremptus	....T.A.....
Oxycanus sordidus	....T.A.....
Oxycanus sphragidias	....T.A.....
Trictena argentata	....T.A.....
Trictena atripalpis	...CT.A.....
Wiseana cervinata 'southern'	....T.A.....
Wiseana cervinata 'northern'	....T.A.....
Wiseana copularis 'southern'	....T.A.....
Wiseana copularis 'northern'	....T.A.....
Wiseana fuliginea	....T.A.....
Wiseana jocosa	....T.A.....
Wiseana mimica	....T.A.....
Wiseana signata 'southern'	....T.A.....
Wiseana signata 'northern'	....T.A.....
Wiseana umbraculata	....T.A.....

Chapter 4

Phylogeny of the New Zealand *'Oxycanus'* lineages of hepialid moths (Lepidoptera: Hepialidae) inferred from nrDNA ITS2 region

B. Brown, R.M. Emberson and A.M. Paterson

Abstract

The phylogeny of the New Zealand hepialid moths was estimated using nucleotide sequence data from the ITS2 region of the nuclear, ribosomal DNA. Relationships between *Wiseana signata* 'southern' and *W. signata* 'northern' and *W. umbraculata* were resolved, but other relationships within the genus *Wiseana* were not. The phylogeny recovered for the New Zealand *'Oxycanus'* lineages was congruent with those from a mitochondrial DNA COI & II data set, although the relationship between *Cladoxycanus minos* and the members of the *'Oxycanus'* lineage *s. str.* was not resolved. Addition of more divergent taxa from New Zealand's *Aenetus* and *Aoraia* lineages and from the Australian hepialid genera *Jeana, Oxycanus* and *Trictena* produced topologies not recovered previously from mtDNA or morphological data sets.

Key words - New Zealand, hepialids, *'Oxycanus' Cladoxycanus, 'Oxycanus' s. str.*, phylogeny, nrDNA ITS2.

Status - Thesis only

Introduction

All hepialid moths in New Zealand are included in the subfamily Hepialidae *s. str.* (Nielsen and Scoble, 1986), the largest subfamily in the superfamily Hepialoidea. The subfamily, comprising 500 species in 80 genera (Common, 1990), is cosmopolitan in distribution, although many taxa are found in South America, South Africa and Australia (Dugdale, 1994). The New Zealand Hepialidae comprises seven genera: *Aenetus, Aoraia, Cladoxycanus, Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana*. The 27 currently recognised and described species, all of which are endemic to New Zealand, were divided into four separate lineages after a morphological, taxonomic revision (Dugdale, 1994). No explicit analysis was undertaken to reconstruct their phylogenetic relationships. Consequently, this research was undertaken to reconstruct the phylogenetic relationships within the New Zealand *'Oxycanus'* lineages of hepialid moths.

Two New Zealand lineages have been hypothesised based on differences in male and female genitalia; the *'Oxycanus'* lineage *s. str.* and the *'Oxycanus'* lineage *Cladoxycanus*. The *'Oxycanus'* lineage *s. str.* comprises 12 taxa in the four genera: *Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana*. Taxa in this lineage have antennal flagellomeres with basal and apical prominences, with the dorsal surfaces of the proximal antennal flagellomeres unscaled or sparsely scaled. Males have a well developed pseudotegumen with twin processes supporting the anal tube. Females have an anogenital field that is higher than wide and an ovipore at or above mid-field height. The ovoid corpus bursae may have a large or small appendix and the caudal margin of tergum 8 has a long, broad tuft of hair-like scales (Dugdale, 1994).

The *'Oxycanus'* lineage *Cladoxycanus* is represented in New Zealand by *Cladoxycanus minos*. Compared with the taxa in the restricted *'Oxycanus'* lineage, *Cladoxycanus* has no arolium on the adult pretarsus and no sclerotised bridge between the apices of the pseudotegumen. The labial palpi have rami on the basal segments and the forewing discal cell apex occurs before half wing length. The female dorsal plate has sclerotised setose lobes fused in the dorsal midline to form a mesal tubercle. The larvae are without metathoracic or abdominal sclerites (Dugdale, 1994).

A cladistic analysis of a morphological data set (Chapter 2) indicated that the lineages of Dugdale (1994) do reflect phylogenetic relationships.

However, morphological data could not resolve whether *Cladoxycanus* was a basal taxon of the *'Oxycanus'* lineage *s. str.* or a separate lineage as hypothesised by Dugdale (1994).

Relationships within the genus *Wiseana* were also not able to be resolved, apart from identifying a close relationship between *Wiseana signata* and *W. umbraculata*. Morphological data indicated that the *'Oxycanus'* lineage *s. str.* in New Zealand was monophyletic and that its sister group included taxa from the Australian genera *Oxycanus* and *Jeana*.

Analysis of a molecular data set from the mitochondrial DNA (mtDNA) cytochrome oxidase subunit 1 and 2 (COI & II) gene regions (Chapter 3) gave support for the lineages of Dugdale, the monophyly of the New Zealand *'Oxycanus'* lineages and placed the Australian genera *Oxycanus* and *Jeana* as sister group to the New Zealand *'Oxycanus'* lineages. New haplotypes were identified within the genus *Wiseana* and some relationships within *Wiseana* were resolved. *Wiseana fuliginea* and *W. mimica* formed a clade together, as did the *W. copularis* 'northern' and 'southern' haplotypes. The *Wiseana signata* haplotypes were always recovered in a clade with *W. umbraculata*. Within the genus *Wiseana*, lack of synapomorphies to support clades and the presence of autapomorphic characters in both the morphological and molecular data sets resulted in poor resolution of phylogenetic relationships and suggested recent and/or rapid radiation.

In an attempt to resolve relationships within the genus *Wiseana* and to provide an independent estimate of relationships within the New Zealand hepialids, we have sequenced the internal transcribed spacer-2 (ITS2) region of the nuclear ribosomal DNA (nrDNA). In arthropods, the nrDNA array comprises many tandem repeats of the 18S, 5.8S and 28S ribosomal RNA (rRNA) genes and the intervening ITS1 and 2 spacers, with each repeat being separated by the non-transcribed spacer region (NTS) (Beckingham, 1982), now known as the intergenic spacer region (IGS). The ITS2 region separates the 5.8S and 28S rRNA genes while the ITS1 spacer separates the 18S and 5.8 genes. Functional analysis of the ITS regions (van der Sande *et al.*, 1992) indicated that ITS2 region is involved in the processing of the 3′ end of the 5.8S molecule and the 5′ end of the 28S molecule, with the secondary structure of the ITS2 being important in the processing reactions. The ITS regions are processed out of the final rRNA product.

DNA sequence from the nuclear ribosomal DNA (nrDNA) has been utilised to recover phylogenies from distantly related through to very closely related taxa (for reviews see Hillis and Dixon, 1991; Brower and DeSalle, 1994). The ITS2 region has been used successfully to recover phylogenetic relationships at both the species and genus level in animals and plants (Torres *et al.*, 1990; Odorico and Miller, 1997). The region has also been widely used to resolve relationships between very closely related taxa, for example between six *Aedes* mosquito species (Wesson *et al.*, 1992), *Anopheles gambiae* species complex (Paskewitz *et al.*, 1993), *Drosophila* species within the *D. melanogaster* subgroup (Schlötterer *et al.*, 1994), sibling species of the *Culex pipiens* mosquito complex (Severini *et al.*, 1996) and between three species of wasp in the genus *Nasonia* (Campbell *et al.*, 1993). Finally, the ITS2 region has been used to develop species diagnostic tests based on the polymerase chain reaction (PCR) (Saiki *et al.*, 1988) with mosquitoes (Porter and Collins, 1991; Crabtree *et al.*, 1995; Cornel, Porter and Collins, 1996).

The region is also suitable for determining hybridization events and reticulate evolution in animals (Odorico and Miller, 1997) and plants (Sang *et al.*, 1995). The ITS2 region evolves faster than the 5.8S and 28S coding regions that flank it (Brown *et al.*, 1972; Tautz *et al.*, 1987; Severini *et al.*, 1996) and so potentially may have more phylogenetically informative nucleotide substitutions for closely related species (Stewart *et al.*, 1983; Porter and Collins, 1991). Conserved gene regions on either side of the ITS2 region can be a source of primers for the PCR reaction. The inferred secondary structure of the region may provide information on species relationships (Wesson *et al.*, 1992) and improve the accuracy of the sequence alignment by identifying correct placement of gaps (Kjer, 1995).

Our aims in this study were to: (i) describe and compare sequence variation within the ITS2 region of hepialid moths from New Zealand, (ii) use this variability to determine phylogenetic relationships between the *'Oxycanus'* lineages, (iii) determine phylogenetic relationships within the genus *Wiseana* and (iv) compare the estimate of phylogenetic relationships from the ITS2 region with previous estimates from morphological and molecular data sets.

Material and Methods

Collections - Specimens of all New Zealand hepialids were collected by light trapping, placed directly into 96% ethanol and stored at 4°C prior to DNA extraction. Intra-individual and intra-species differences have been reported from studies utilising ITS regions (Vogler and DeSalle, 1994; Paskewitz *et al.*, 1993). Consequently, where possible for each species, specimens were collected from populations at a range of locations (see Chapter 3, Appendix 1). Additional specimens were provided by Australian colleagues to test the monophyly of the New Zealand *'Oxycanus'* lineages. Included in this analysis are the Australian taxa *Jeana timeata*, *Oxycanus australis*, *O. diremptus*, *O. sordidus*, *Trictena argentata* and *T. atripalpis*. Collection locations of the Australian taxa are listed in Chapter 3, Appendix 2.

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

DNA extraction, PCR and Nucleotide sequencing - Internal muscular tissue from the thorax of specimens was homogenised and total DNA extracted using a proteinase-K digestion and high salt precipitation (White *et al.*, 1990). The ITS2 region was amplified via the polymerase chain reaction (PCR) (Saiki *et al.*, 1988) using the primers FFA (5´TGTGAACTGCAGGACACAT) (Karen Armstrong, *pers comm*) and ITS4 (5´TCCTCCGCTTATTGATATGC) (White *et al.*, 1990). An additional primer pair from within the ITS2 region, was designed and used with species that could not be amplified using the FFA and ITS4 primers. The two additional primers were HA (5´ ACTCCTGTCTGAGGGCCGGCTGT) and HB (5´ GGATATCGCGTCTGCCTCGATC) (Figure 1). Species amplified with each primer pair are listed in Appendix 1.

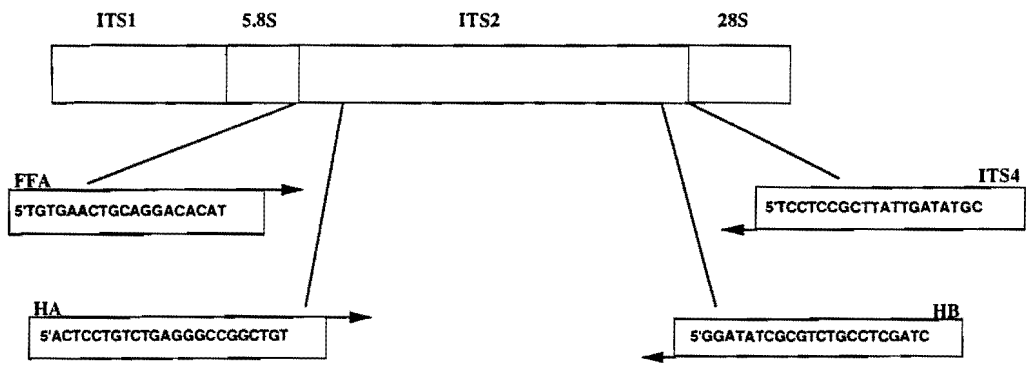

Figure 1: Diagram showing the location of the internal transcribed spacer-2 (ITS2) region of the nuclear ribosomal DNA (nrDNA), amplified in this study. Location of primers used in the polymerase chain reaction (PCR) are shown.

Twenty five µl reactions comprised 2.5 µl of 10x buffer, 3.5 µl 1 mM dNTPs, 0.625 µl 20 mM magnesium (no extra magnesium was added when the HA/HB primers were used), 5% dimethyl sulphoxide (DMSO), 1.5 µl 2 µM FFA/HA and ITS4/HB primers and 0.2 5 U/µl *Taq* DNA polymerase (Boehringer Mannheim). 0.9 µl of 20 ng/µl DNA was used in each reaction. A Perkin- Elmer 2400, thermal cycler was used with a cycling profile for the FFA and ITS4 primers of 95°C for 2 minutes and 97°C for 2 seconds pre-PCR followed by 56°C for 1 minute, 72°C for 45 seconds and 95°C for 1 minute for 38 cycles with a 5 minute extension at 72°C after the final cycle. The cycling profile for the HA and HB primers varied only in that the annealing temperature was 64°C and 40 cycles were used. The resulting double stranded PCR product was precipitated with 3M ammonium acetate and isopropanol, rinsed in 70% ethanol and air dried before resuspension in 2-10 µl of de-ionized water.

A single product band was produced for most species. Where two or more bands were produced, the PCR product was run on a 2% low electroendosmosis (LE) agarose gel (Boehringer Mannheim) with a 100 base pair (bp) ladder (Gibco BRL) for a minimum of two hours following which the target band was cut from the gel and the DNA extracted by a freeze and squeeze method (Hengen, 1994). Direct sequencing of the PCR product was carried out using the Prism™ Ready Reaction Dye Deoxy Terminator Cycle Sequencing kit (Perkin Elmer) following the manufacturer's recommendations. 1.25-4.5 µl of 10-30 ng/µl PCR product was used in each sequencing reaction. The annealing temperature in this reaction was 56°C for the FFA/ITS4 primers and 64°C for the HA/HB primers. Sequencing was carried out on an ABI 377 Automatic Sequencer. For each specimen, sequence from both the sense and antisense strands was obtained. At least two specimens were sequenced from every population sampled for each species.

Data Analysis - Sequences were aligned using the default parameters of the alignment programme Clustal W (Thompson *et al.*, 1994). As approximately half the species were amplified using the HA/HB primer pair which amplifies only part of the ITS2 region, 74 nucleotides (nt) were trimmed off the 5′ end of species amplified using the FFA/ITS4 primers and 87 nt off the 3′ end. A data set comprising only *Wiseana* species was analysed initially. Additional taxa from the New Zealand *'Oxycanus'* lineages and the more divergent New Zealand and Australian taxa were added to subsequent data sets for analysis. Removal of all gaps introduced during the alignment process, resulted in a less resolved phylogeny. Therefore, gaps were included in the analysis of each data set.

We searched for maximum parsimony (MP) trees in the unordered and unweighted data with PAUP 3.1 (Swofford, 1993), using the heuristic option and ten stepwise addition replicates. Maximum likelihood (ML) trees were recovered using Phylip 3.4 (Felsenstein, 1991), under the Kimura two-parameter model (Kimura, 1980). Bootstrap proportions (Felsenstein, 1985) measuring the frequency of a branch's occurance in the resampling of pseudoreplicates from the data set, were calculated.

Secondary Structure - The RNA secondary structure was predicted based on minimum free energy, using RNAdraw (Matzura and Wennborg, 1996). This programme incorporates the dynamic programming algorithm of Zuker and Stiegler (1981) and follows the folding rules and energy computations and parameters of Turner *et al.* (1988), Freier *et al.* (1986) and Jaeger *et al.* (1989).

Results

Amplification - The ITS2 region for New Zealand's hepialid moths varied in length from approximately 700 nt in *Aenetus virescens* to 450 in *Wiseana* spp. and falls within the range of lengths (200-1000 nt) reported for eukaryotes (Paskewitz *et al.*, 1993). Amplification of a single target band proved difficult for *Aenetus virescens* and *Aoraia* species regardless of primer combinations tried, addition of 5% DMSO, or variation of concentration or volume of PCR reagents or conditions. This may have been caused by poor primer fit or the presence of internal priming sites.

Cloning the ITS regions is common practice (Wesson *et al.*, 1992; Schlötterer *et al.*, 1994; Cornel *et al.*, 1996) so that intra-individual polymorphisms can be detected. However, lack of shadow bands occurring with the expected band, when PCR product is electrophoresed on a gel, may also indicate lack of intra-individual polymorphisms (Fritz *et al.*, 1994). A pilot study of the region with representatives of each genus from the Family Hepialidae in New Zealand found the primers FFA/ITS4 amplified only a single band and absence of shadow bands indicated no intra-individual polymorphisms (B.Brown, unpublished data). Later, it was observed that an additional band approximately 20-75 nt different in length was amplified in some individuals regardless of primer combinations used. Individuals sequenced from a *Dumbletonius unimaculatus* population from the Waitakere Ranges, Auckland (AK) produced either one or two bands approximately 500 and 575 nt long. Between-population differences in numbers of bands amplified were found for *Wiseana fuliginea, W. mimica, W. copularis* 'southern' and *W. copularis* 'northern' (Appendix 2).

Populations either had a single 450 nt band amplified or 450 and 400 nt bands. The shorter of the two bands from two individuals from the Mackenzie Country population of *W. mimica* was sequenced and had a 24 bp GC-rich section missing compared with individuals from the *W. mimica* population at Cass and the remainder of the *Wiseana* taxa.

We are confident that the correct band was sequenced from the populations that amplified two bands. For example, the *W. copularis* 'southern' Christchurch population that amplified two bands produced a sequence that was identical to that from the *W. copularis* 'southern' population at Ohai that amplified only one band. All other taxa produced a single band.

The Nucleotide Sequence - A high degree of similarity between aligned nucleotides (Hillis, 1994) of closely related taxa has been found in previous studies. Paskewitz *et al.* (1993) reported 98-99% similarity for the *Anopheles gambiae* species complex and Severini *et al.* (1996) reported 97% for the *Culex pipiens* complex. We also found a high percentage sequence similarity, with the aligned *Wiseana* sequences having 94% of their nucleotides in common (Appendix 3). Guanine (G) and cytosine (C) content was 57% within the genus *Wiseana*. Values of 50-60% have been reported in other insect studies (Wesson, *et al.*, 1992; Paskewitz, *et al.*, 1993, Severini *et al.*, 1996). Wesson *et al.* (1992) found that the G and C bases occurred between blocks of similar sequence but in our sequences these bases occurred throughout the region. The sequences from the ITS2 region for *Wiseana cervinata* 'northern' and *W. copularis* 'southern' were identical as were those from *Wiseana fuliginea* and *W. mimica*, and *Wiseana cervinata* 'southern' and *W. copularis* 'northern'. All gaps that were greater than two nucleotides long corresponded to unpaired loop regions in the secondary structure. In total, 20 of the 38 variable characters were parsimony informative in the *Wiseana* data set.

Addition of the remainder of the taxa from the New Zealand *'Oxycanus'* lineages, i.e., *Cladoxycanus*, *Dioxycanus* spp., *Dumbletonius* spp and *Heloxycanus*, introduced larger gaps into the aligned sequence because all taxa had longer sequence than the *Wiseana* taxa. Alignment similarity was reduced to 73% and GC content to 36%. Seventy two of the 135 variable characters were parsimony informative (Appendix 4).

The full data set included taxa from the two other New Zealand hepialid lineages *Aenetus* and *Aoraia* and taxa from the Australian genera *Jeana*, *Oxycanus* and *Trictena*.

Aligned sequence similarity and GC content were reduced to 29 and 14% respectively. Three hundred and ten of the 385 variable characters were parsimony informative (Appendix 5).

An approximately equal number of transition and transversion substitutions was observed in each data set. Similar results have been reported by Vogler and DeSalle (1994) for the ITS1 region in tiger beetles. No transition bias was evident in the ITS2 data, unlike the mtDNA region where there are often more transitions than transversions (DeSalle *et al.*, 1987; Brown *et al.*, 1994).

Distance Estimates - Corrected divergences using the Kimura (1980) two-parameter model were calculated for all pairwise combinations of taxa (Table 1). Divergences within the genus *Wiseana* ranged from 10% between *Wiseana umbraculata* and *W. signata* 'northern' to 0.25% between *W. cervinata* 'northern' and 'southern'. The largest divergence was 84% between *Jeana timeata* and *Aoraia lenis*. Divergence between *Cladoxycanus minos* and *Wiseana signata* 'southern' was 25% and between *Dumbletonius unimaculatus* and *Wiseana cervinata* 'northern' 18%. Odorico and Miller (1997) report divergences ranging from 0-39% for the ITS2 region of five species of *Acropora* corals, while Campbell *et al.* (1993) report ingroup-outgroup divergences ranging from 57-70% for *Nasonia* wasps.

The Search - A heuristic search of the unordered and unweighted *Wiseana* sequence resulted in 99 maximum parsimony trees: tree length (TL) 41, consistency index (CI) 0.91 and retention index (RI) 0.92. The G_1 statistic of -2.16 is considered to be highly skewed because of the effect of so many taxa with identical sequence (Swofford *et al.*, 1996). The majority rule consensus phylogram with bootstrap proportions is shown in Figure 2. Three major clades are evident; the *W. cervinata, W. copularis, W. fuliginea, W. jocosa, W. mimica* clade; the *W. signata* clade and the *W. umbraculata* clade.

The data set comprising *Wiseana* and the remainder of the *'Oxycanus'* lineages produced 75 maximum parsimony trees: TL 190, CI 0.89, RI 0.90 and G_1 -1.0. The majority rule consensus phylogram with bootstrap proportions is shown in Figure 3. Inspection of the 75 maximum parsimony trees showed the order of branching to be (*Cladoxycanus, Dumbletonius characterifer,* ((*Heloxycanus, Dioxycanus*), (*Dumbletonius unimaculatus, Wiseana*))). All alternative arrangements occurred within the genus *Wiseana*. Maximum likelihood analysis produced 105 trees under the Kimura (1980) two-parameter model, with a likelihood value of 1675.19.

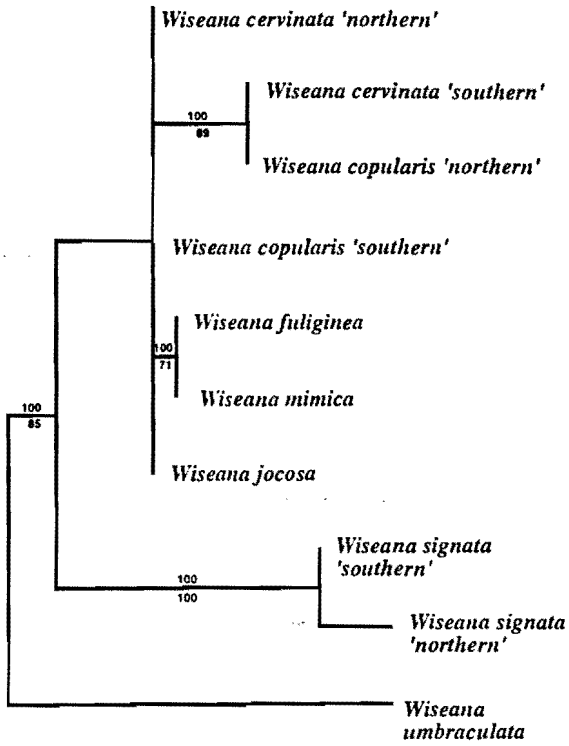

Figure 2: Majority rule consensus phylogram of the 99 most parsimonious trees from the analysis of ITS2 sequence data from New Zealand hepialids in the genus *Wiseana* using mid-point rooting. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule values are shown above the branches and bootstrap proportions (>50%) below.

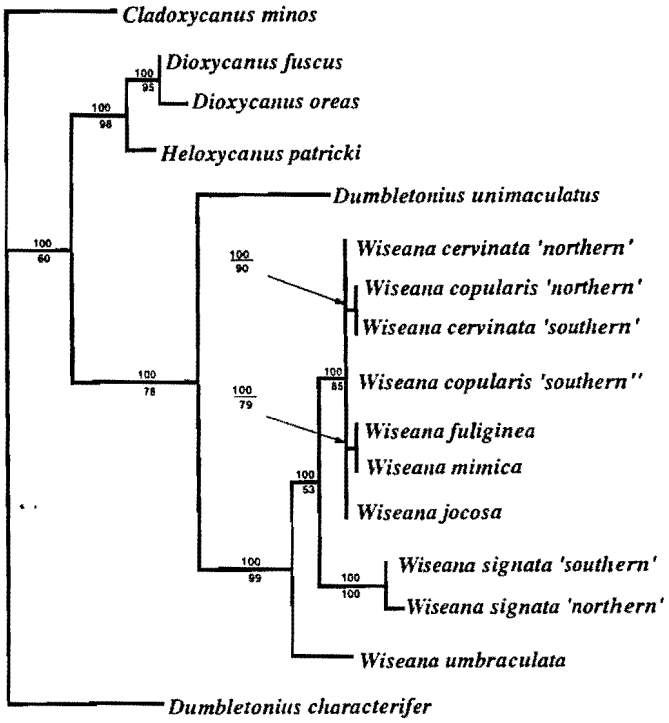

Figure 3: Majority rule consensus phylogram of the 75 most parsimonius trees for the New Zealand *'Oxycanus'* lineage *s.str.* from the analysis of ITS2 sequence data. Branch lengths are proportional to morphological character state change. Majority rule values are shown above the branches and bootstrap proportions (>50%) below.

Table 1: Percentage divergences of nrDNA ITS2 sequence data from New Zealand hepialid moths, corrected for multiple hits using the Kimura (1980) two-parameter model.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
1. *Aenetus virescens*																		
2. *Aoraia ensyii*	0.626																	
3. *A. lenis*	0.657	0.071																
4. *Cladoxycanus minos*	0.315	0.638	0.680															
5. *Dioxycanus fuscus*	0.345	0.704	0.737	0.082														
6. *D. oreas*	0.360	0.723	0.750	0.096	0.010													
7. *Dumbletonius characterifer*	0.377	0.714	0.709	0.270	0.284	0.299												
8. *D. unimaculatus*	0.454	0.701	0.681	0.316	0.295	0.303	0.161											
9. *Heloxycanus patricki*	0.358	0.712	0.762	0.090	0.026	0.037	0.291	0.312										
10. *Jeana timeata*	0.508	0.813	0.846	0.469	0.429	0.433	0.416	0.410	0.466									
11. *Oxycanus australis*	0.415	0.786	0.820	0.371	0.368	0.375	0.276	0.321	0.382	0.441								
12. *O. diremptus*	0.415	0.787	0.821	0.371	0.368	0.375	0.269	0.321	0.382	0.434	0.0							
13. *O. sordidus*	0.433	0.775	0.784	0.363	0.362	0.374	0.240	0.298	0.367	0.434	0.129	0.128						
14. *Trictena argentata*	0.530	0.325	0.329	0.566	0.557	0.575	0.602	0.613	0.568	0.745	0.678	0.678	0.692					
15. *T. atripalpis*	0.573	0.369	0.372	0.622	0.639	0.657	0.682	0.673	0.663	0.812	0.753	0.754	0.751	0.013				
16. *Wiseana cervinata* 'northern'	0.423	0.700	0.708	0.271	0.287	0.298	0.191	0.085	0.308	0.388	0.334	0.325	0.271	0.583	0.680			
17. *W. cervinata* 'southern'	0.422	0.712	0.720	0.272	0.288	0.303	0.196	0.192	0.310	0.388	0.343	0.334	0.272	0.586	0.681	0.002		
18. *W. copularis* 'southern'	0.420	0.702	0.710	0.272	0.287	0.299	0.192	0.185	0.309	0.389	0.335	0.326	0.272	0.586	0.680	0.00	0.002	
19. *W. copularis* 'northern'	0.422	0.712	0.720	0.272	0.288	0.303	0.196	0.192	0.310	0.388	0.343	0.334	0.272	0.586	0.681	0.002	0.00	0.002

Table 1 continued: Percentage divergences of nrDNA ITS2 sequence data from New Zealand hepialid moths, corrected for multiple hits using the Kimura (1980) two-parameter model.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
20. *W. fuliginea*	0.418	0.696	0.705	0.263	0.289	0.301	0.168	0.189	0.304	0.380	0.316	0.316	0.258	0.588	0.685	0.005	0.007	0.005
21. *W. jocosa*	0.418	0.690	0.699	0.267	0.286	0.298	0.172	0.186	0.309	0.375	0.321	0.321	0.263	0.575	0.671	0.000	0.002	0.000
22. *W. mimica*	0.418	0.696	0.705	0.263	0.289	0.301	0.168	0.189	0.304	0.380	0.316	0.316	0.258	0.588	0.685	0.005	0.007	0.005
23. *W. signata* 'southern'	0.396	0.686	0.708	0.253	0.286	0.298	0.176	0.185	0.305	0.376	0.301	0.302	0.257	0.599	0.668	0.071	0.073	0.071
24. *W. signata* 'northern'	0.393	0.686	0.708	0.267	0.297	0.308	0.178	0.187	0.315	0.382	0.303	0.304	0.263	0.593	0.662	0.084	0.086	0.084
25. *W. umbraculata*	0.459	0.712	0.725	0.298	0.310	0.322	0.190	0.199	0.328	0.398	0.333	0.328	0.263	0.584	0.671	0.059	0.062	0.059

Table 1 continued.

	19.	20.	21.	22.	23.	24.
20. *W. fuliginea*	0.007					
21. *W. jocosa*	0.002	0.005				
22. *W. mimica*	0.007	0.000	0.005			
23. *W. signata* 'southern'	0.073	0.071	0.071	0.071		
24. *W. signata* 'northern'	0.086	0.084	0.084	0.084	0.007	
25. *W. umbraculata*	0.062	0.049	0.044	0.049	0.093	0.101

The order of branching was identical to that recovered in the MP trees and all alternative arrangements occurred within *Wiseana*.

The complete data set included all the taxa in the New Zealand *'Oxycanus'* lineages, *Aenetus virescens* from the New Zealand *Aenetus* lineage, *Aoraia ensyii* and *A. lenis* from the New Zealand *Aoraia* lineage and the Australian taxa *Jeana timeata, Oxycanus australis, O. diremptus, O. sordidus, Trictena argentata* and *T. atripalpis*. Seventy six trees were produced: TL 893, CI 0.70, RI 0.80 and G_1 -0.96. All alternative arrangements occurred within the genus *Wiseana*. The majority rule consensus phylogram with bootstrap proportions is shown in Figure 4. The *Aenetus, Aoraia* and *Trictena* taxa fell outside the three major clades recovered. The three clades comprised: (*Cladoxycanus*, (*Heloxycanus, Dioxycanus*)); (*Oxycanus, Jeana*) and (*Dumbletonius, Wiseana*).

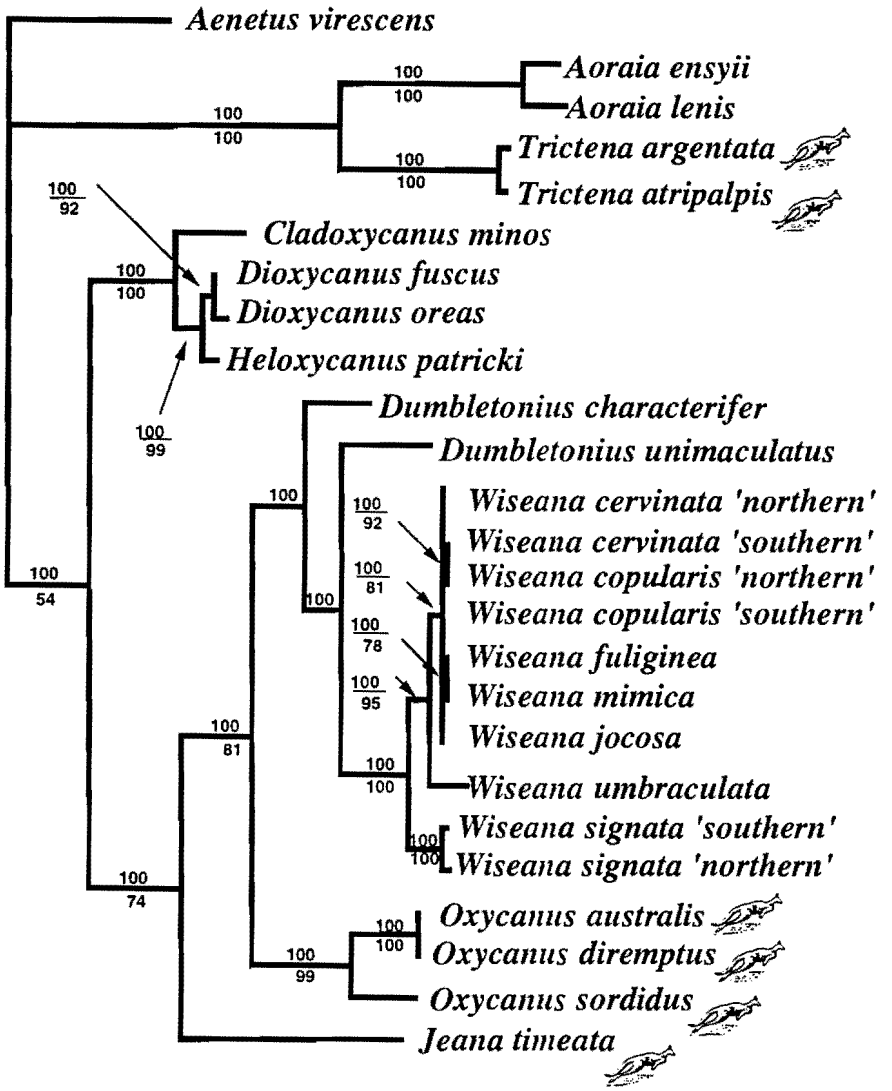

Figure 4: Majority rule consensus phylogram of the 76 most parsimonious trees from the analysis of ITS2 sequence data from New Zealand and Australian hepialids. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule values are shown above the branches and bootstrap proportions (>50%) below.

Discussion

***Wiseana* data -** Lack of informative characters from the analysis of morphological (Chapter 2) and molecular (Chapter 3) data sets to support branching order within the genus *Wiseana* suggested recent and rapid evolution. This hypothesis was supported by the results of the analysis of the nrDNA ITS2 region (Figure 2). There were few nucleotide substitutions even though this is regarded as a rapidly evolving region (Hillis *et al.*, 1996; Severini *et al.*, 1996). Three groups of the *Wiseana* taxa had identical sequences. The sequence from *Wiseana cervinata* 'northern' and *W. copularis* 'southern' was identical, as was the sequence from *W. cervinata* 'southern' and *W. copularis* 'northern' and that from *W. fuliginea* and *W. mimica*.

Although *Wiseana cervinata* 'northern' and *W. copularis* 'southern' had identical ITS2 sequence, each had a unique mitochondrial DNA haplotype and can be distinguished morphologically. In the mtDNA phylogeny, *W. cervinata* 'northern' and *W. cervinata* 'southern' haplotypes occurred as an unresolved polytomy with the remainder of the *Wiseana* taxa. The *Wiseana copularis* 'northern' haploytype always occurred in a clade with the *W. copularis* 'southern' haplotype. *Wiseana cervinata* males have antennae with short triangular rami and rounded apices, while *W. copularis* males have long, rectangular rami with truncate apices. Scanning electron microscope (SEM) images show that males of each of these taxa can be identified by the forewing discal cell, white scale shape (Figures 5, 6 & 7). *Wiseana cervinata* scales taper to a broad, truncate apex, while scales from *W. copularis* are parallel sided, tapering to a narrow, rounded apex.

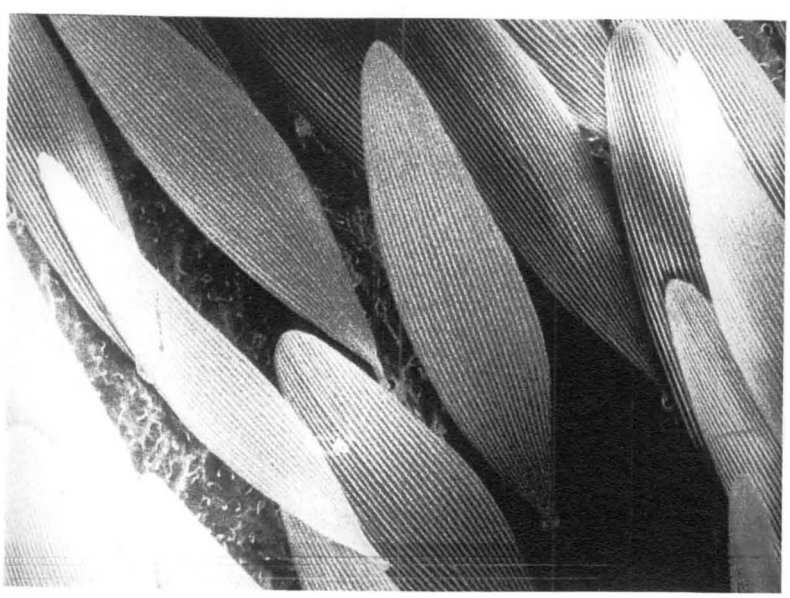

Figure 5: Scanning electron microscope (SEM) photograph of male *Wiseana cervinata* 'southern' (MC) forewing discal cell, white scale shape.

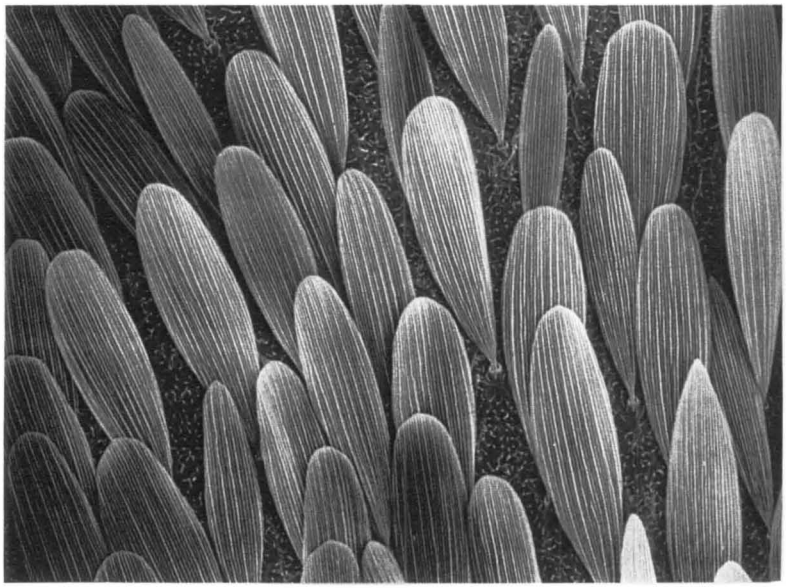

Figure 6: Scanning electron microscope (SEM) photograph of male *W. cervinata* 'northern' (TK) forewing discal cell, white scale shape.

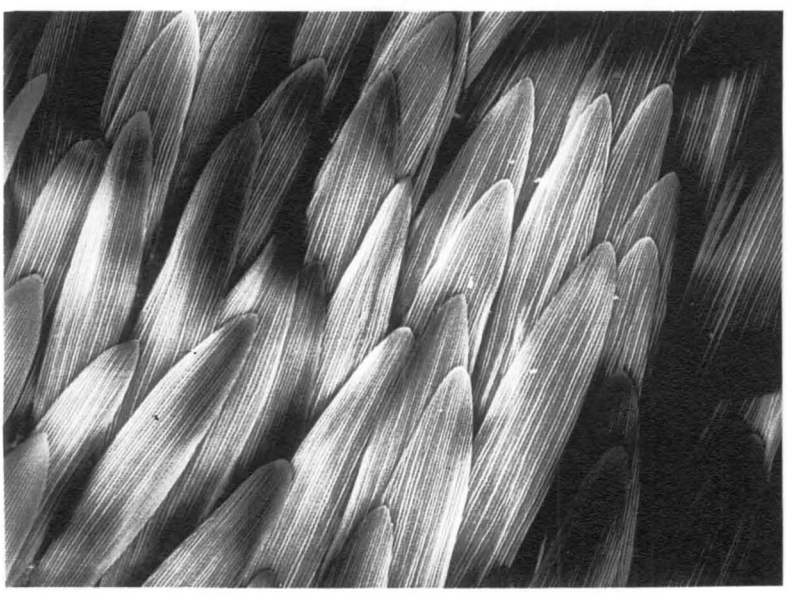

Figure 7: Scanning electron microscope (SEM) photograph of male *Wiseana copularis* 'southern' (MC) forewing discal cell, white scale shape.

Wiseana fuliginea and *W. mimica* had identical ITS2 sequence. There were two nucleotide differences between these taxa and *W. jocosa*. Analysis of mtDNA always placed *W. fuliginea* in a clade with *W. mimica*. Estimated time of divergence of *W. fuliginea* and *W. mimica* based on mtDNA data was less than 100,000 years (Chapter 3), making them the most recently separated of the *Wiseana* taxa. *Wiseana fuliginea* and *W. mimica* males are distinguished by antennal rami shape and forewing discal cell, white scale shape. *Wiseana fuliginea* has short trianglar rami and *W. mimica* long triangular rami. *Wiseana fuliginea* has long thin scales that taper slowly to a long, sharp apex and *W. mimica* has scales oval in mid-section tapering abruptly to an acute apex (Figures 8 & 9).

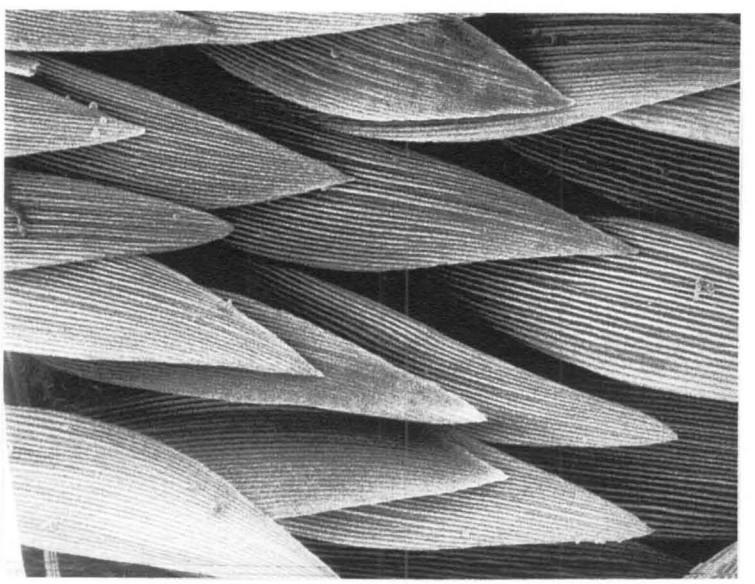

Figure 8: Scanning electron microscope (SEM) photograph of male *Wiseana fuliginea* (MC) forewing discal cell, white scale shape.

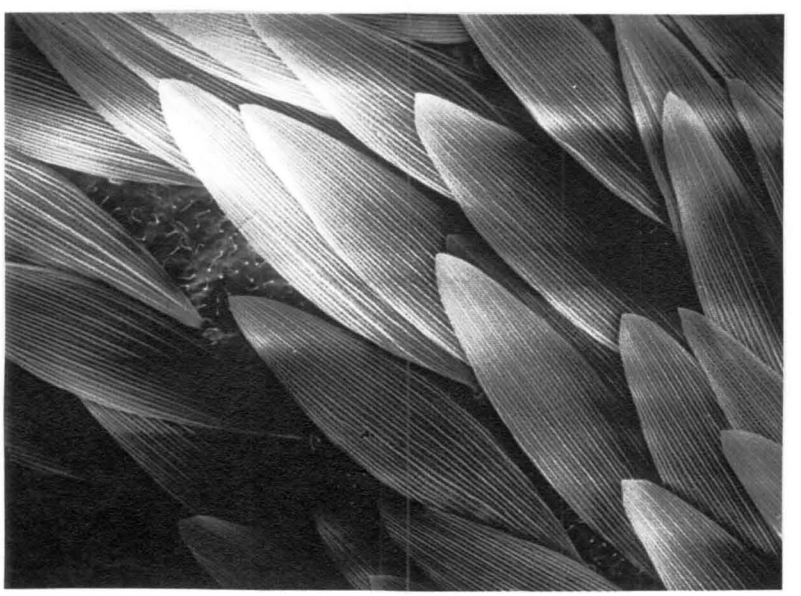

Figure 9: Scanning electron microscope (SEM) photograph of male *Wiseana mimica* (MK) forewing discal cell, white scale shape.

Wiseana cervinata 'southern' and *W. copularis* 'northern' had identical ITS2 sequence and shared two synapomorphies. Possibly, *W. cervinata* 'southern' and *W. copularis* 'northern', have retained polymorphic variation that was present in the common ancestor before cladogenesis (ancestral polymorphisms). It is unlikely that this would have been observed in the mtDNA as the mtDNA alleles go to fixation by genetic drift four times faster than any comparable nuclear allele, so there is less chance of capturing ancestral alleles (Birkey *et al.*, 1983). However, Brower and DeSalle (1994) considered that phylogenetic relationships are very difficult to recover in rapidly evolved taxa, "*regardless of the existence of ancestral polymorphisms*".

It is also possible that a hybridization event has taken place between *W. cervinata* 'southern' and *W. copularis* 'northern' taxa. These taxa occur sympatrically in the Nelson region on South Island (pers obs), but most other combinations of the known *Wiseana* taxa also occur sympatrically. For example, Herbert (1995) reported *Wiseana cervinata* in sympatry with all other *Wiseana* taxa. There is no morphological evidence of recent or on-going hybridization between *W. cervinata* 'southern' and *W. copularis* 'northern' and the mtDNA phylogeny does not support this hypothesis. Mitochondrial DNA always placed the *W. cervinata* 'southern' with the *W. cervinata* 'northern' haplotype and the *W. copularis* 'northern' with the *W. copularis* 'southern' haplotype.

The only relationships resolved using the ITS2 sequence were those between *W. signata* 'southern', *W. signata* 'northern' and *W. umbraculata*. The relationship recovered was always (*W. umbraculata*, (*W. signata* 'southern', *W. signata* 'northern')). The same relationship was recovered with the mtDNA. The two *W. signata* mtDNA haplotypes have not been distinguished morphologically. In the analysis of morphological data *Wiseana signata* was always recovered in a clade with *W. umbraculata* (Chapter 2).

Compared with protein coding gene regions (Simon *et al.*, 1994), less is known about the evolution of DNA in non-coding regions (Brower and DeSalle, 1994). Maintenance of secondary structure is considered to be a constraint on nucleotide substitutions in the ITS regions (Wesson *et al.*, 1992). All *Wiseana* taxa had very similar secondary structure (Figure 10) with extra nucleotides being added in the unpaired loop regions. Cornel *et al.* (1996) found many species-specific differences between five cryptic *Anopheles quadrimaculatus* species to be in the unpaired regions and this was also the case in our study for *Wiseana signata* 'southern' and 'northern' and *W. umbraculata*.

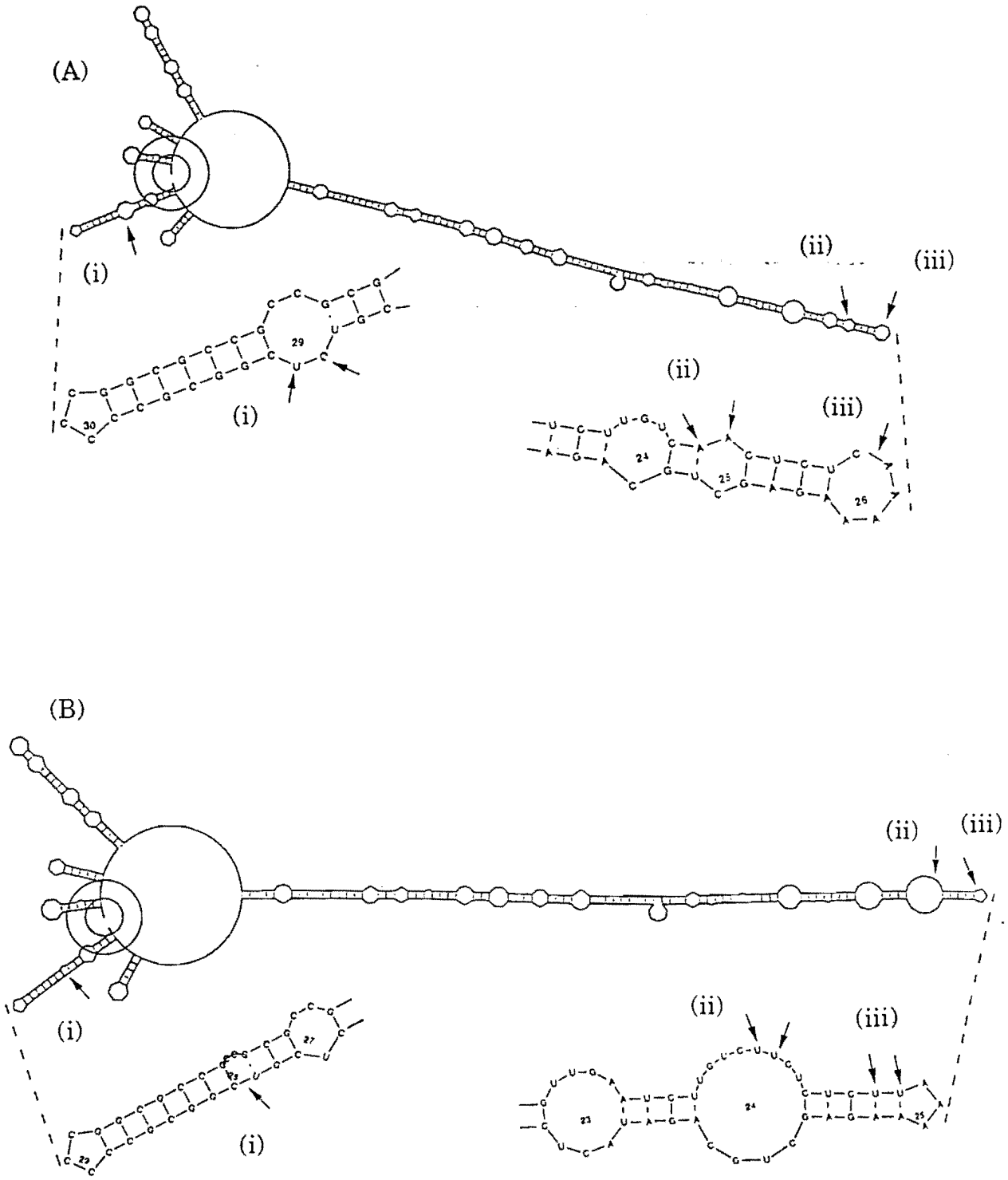

Figure 10: Inferred secondary structure of the ITS2 region from (A) *Wiseana signata* 'southern' and (B) *W. signata* 'northern' calculated using RNAdraw (Matzura and Wennborg, 1996). Species-specific differences in the unpaired regions are indicated by arrows.

Repeat motifs are commonly found in ITS2 sequences (Campbell *et al.*, 1993; Cornel *et al.*, 1996). Cornel *et al.* (1996) cautioned that species-specific differences from regions with subrepeats should not be relied upon as they may not reflect phylogenetic relationships. Instead, indels in or close to subrepeats may arise to compensate for accumulating mutations that result from slippage. There were three regions in the ITS2 sequences of *Wiseana* taxa where repeat motifs occurred. Extra length in the *W. cervinata* 'southern' and 'northern' and in *W. copularis* 'southern' and 'northern' sequence was due to two CGCC repeats between positions 68 and 75. *Wiseana cervinata* 'southern' and *W. copularis* 'northern' had a GTCG repeat at positions 81-84. All taxa except *W. signata* 'southern' and 'northern' and *W. umbraculata* had a TG motif at positions 326-327, repeated four times between positions 328-335. The cause of repeat motifs is unknown but two hypotheses have been put forward; the infidelity of the *Taq* polymerase enzyme may cause repetitive sequences to increase in length (Schlötterer and Tautz, 1991) and repetitive motifs may result from slippage-strand mispairing during replication (Tautz *et al.*, 1988; Gonzalez *et al*, 1990).

In summary, the ITS2 region adds no new information regarding relationships within the genus *Wiseana*, but does support the mtDNA result of the two *W. signata* taxa forming a clade with *W. umbraculata*. The remaining *Wiseana* taxa had very similar sequence which supports the hypothesis of very recent and rapid divergence

New Zealand *'Oxycanus'* lineages - Addition of the remainder of the taxa from the New Zealand *'Oxycanus'* lineages (Figure 3) recovered a phylogeny congruent with that recovered from the mtDNA COI & II gene regions. *Cladoxycanus* and *Dumbletonius characterifer* were the basal taxa and on all maximum parsimony and all maximum likelihood trees formed a polytomy with the remaining taxa. *Cladoxycanus* has several morphological characters that distinguish it from the *'Oxycanus'* lineage *s. str.*, including no arolium on the adult pretarsus, no sclerotized bridge on the apices of the pseudotegumen and labial palpus basal segments with rami. However, *Cladoxycanus* also shares seven morphological synapomorphies with the *'Oxycanus* lineage *s. str* taxa. This analysis adds no new information regarding the relationship of *Cladoxycanus* to the remaining *'Oxycanus'* lineage *s. str.* taxa. As with the mtDNA, *Dumbletonius characterifer* is never recovered in a clade with *D. unimaculatus*, suggesting that the genus is not monophyletic. The branching order of the remaining taxa was ((*Heloxycanus, Dioxycanus*), (*Dumbletonius unimaculatus, Wiseana*)). The (*Heloxycanus, Dioxycanus*) clade was supported by seven synapomorphies and 98% bootstrap value. It was also recovered in both the morphological and mtDNA analyses.

Dumbletonius unimaculatus was recovered in a clade with *Wiseana* in this and the mtDNA analyses, but the morphological data support both *Dumbletonius* species in a clade together, with the clade being sister group to the *Wiseana* clade.

No outgroup was included in the analysis of the New Zealand *'Oxycanus'* lineages, as the morphological and mtDNA results suggest that the other two New Zealand hepialid lineages are relatively distant from the *'Oxycanus'* lineages. Both morphological and mtDNA analyses suggest that taxa from the Australian genera *Oxycanus* and *Jeana* may be the closest relatives to the New Zealand *'Oxycanus'* lineages. Morphological data placed the Australian taxa as sister group to the New Zealand *'Oxycanus'* lineage *s. str.*, while the mtDNA data placed the Australian taxa outside *Cladoxycanus*, i.e., outside both the New Zealand *'Oxycanus'* lineages. However in this analysis, addition of *Aenetus virescens* from the *Aenetus* lineage and exemplars from the *Aoraia* lineage to the New Zealand *'Oxycanus'* lineages data set resulted in *Dumbletonius characterifer* and *D. unimaculatus* joining the (*Cladoxycanus*, (*Heloxycanus*, *Dioxycanus*)) clade basally (Figure 11). This phylogeny had not been recovered from previous analyses of ITS2, mtDNA or morphological data sets.

New Zealand and Australian data - The phylogeny recovered from ITS2 sequence data from all New Zealand hepialid lineages and representatives from the Australian genera *Jeana, Oxycanus* and *Trictena* is shown in Figure 4. The New Zealand taxa *Aenetus* and *Aoraia* and Australian taxa *Trictena* fell outside the New Zealand *'Oxycanus'* lineages and the Australian *Oxycanus* and *Jeana*. In previous morphological and mtDNA analyses, *Aenetus*, *Aoraia* and *Trictena* have been used as outgroups as no close relationship has ever been inferred between them and the *'Oxycanus'* lineages (Dumbleton, 1966; Dugdale, 1994). Contrary to phylogenies produced from previous morphological and mtDNA analyses, the ITS2 data suggested that the New Zealand *'Oxycanus'* lineages are not monophyletic. *Oxycanus* and *Jeana* taxa are sister group to the clade comprising *Dumbletonius characterifer, D. unimaculatus* and *Wiseana*, while the (*Cladoxycanus*, (*Heloxycanus*, *Dioxycanus*)) clade occurred basal to these.

Hillis and Dixon (1991) considered that regions with 70-100% homology and pairwise sequences that differ by less than 30% are most useful for phylogenetic reconstruction. In this analysis, similarity between the aligned sequences was low at 29% and remained low even when all gaps were removed (B. Brown, unpublished data).

Pairwise sequence divergences were large and ranged from: 46-47% between *Jeana* and *Cladoxycanus* or *Heloxycanus;* 24% between *Dumbletonius characterifer* and *Oxycanus sordidus* and 26-34% between *Wiseana* taxa and *Oxycanus* taxa. Given this, and the fact that this region has been used most successfully to recover phylogenies from very closely related species complexes, it is considered that the signal in the data may not represent phylogenetic signal and that some branches with more divergent taxa may be joining on to the tree randomly. This hypothesis is supported by the recovery of new, incongruent topologies each time more divergent taxa were added to the tree. However, topologies congruent with those from mtDNA analyses were recovered when deep branches such as those leading to *Aenetus, Aoraia, Jeana, Oxycanus* and *Trictena* were excluded.

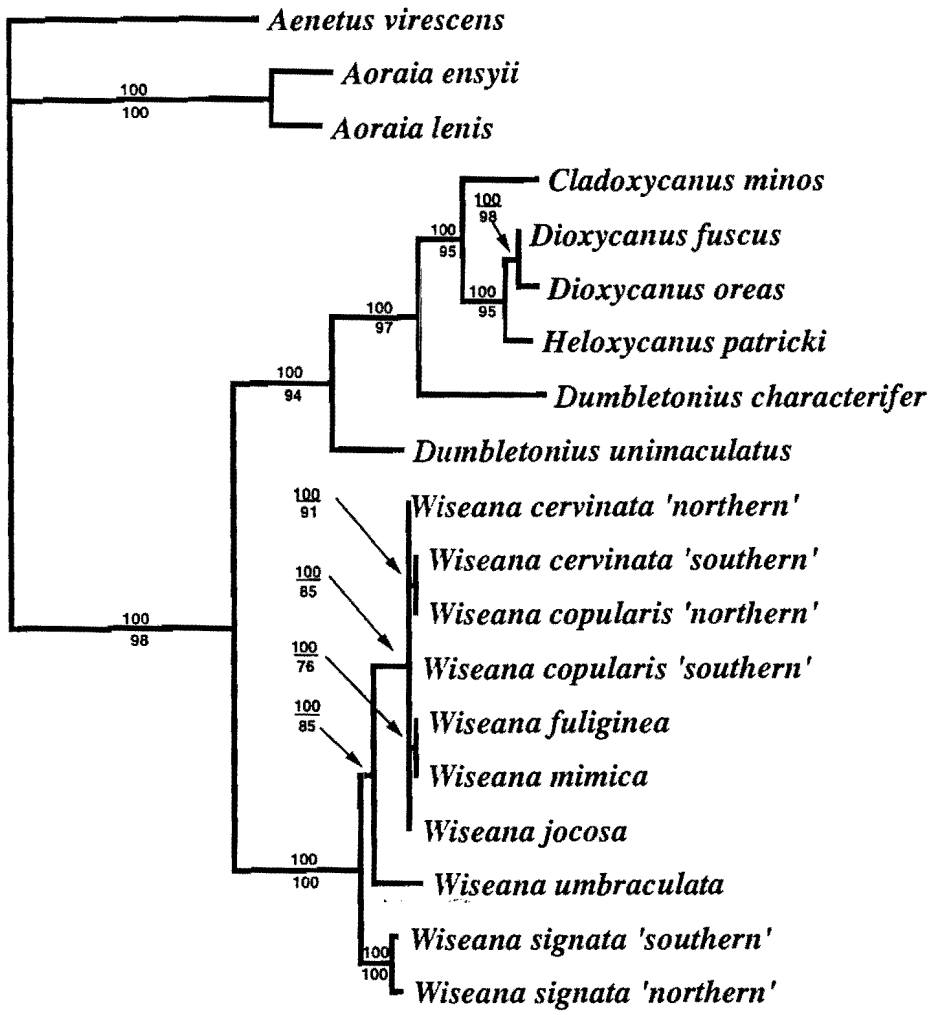

Figure 11: Majority rule consensus phylogram of the 75 most parsimonious trees from the analysis of ITS2 sequence data from New Zealand hepialids from the *Aenetus, Aoraia, 'Oxycanus' Cladoxycanus* and *'Oxycanus' s.str.* lineages. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule values are shown above the branches and bootstrap proportions (>50%) below.

Conclusions

A key issue in the successful recovery of phylogeny from molecular data is the choice of gene region (Graybeal, 1994). It is important that the region has sufficient variation to give phylogenetic signal but not so much variation that the sequences cannot be aligned or phylogenetic signal is lost through multiple substitutions (saturation) or overpowered by random noise (homoplasy) (Brower and DeSalle, 1994; Simon *et al*, 1994).

Use of the ITS2 region to resolve relationships within the New Zealand hepialids highlights the problem (or perhaps the impossibility) of finding one region with suitable variation to resolve relationships within a group where there are both very recent and more ancient divergences. The ITS2 region appears to be evolving too slowly for most *Wiseana* taxa, although relationship between *Wiseana signata* and *W. umbraculata* was resolved and agrees with results from other independent data sets. The phylogeny recovered for the New Zealand *'Oxycanus'* lineage *s. str.* was also congruent with that from mtDNA. The relationship of *Cladoxycanus minos* to the *'Oxycanus'* lineage *s. str.* and of the New Zealand hepialids to Australian hepialids was not able to be resolved using the ITS2 region.

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Appendix 1: Primers used to amplify the ITS2 region of hepialid moths from New Zealand and Australia.

(1) FFA/ITS4

Aenetus virescens
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
Dumbletonius unimaculatus
Heloxycanus patricki
Jeana timeata
Oxycanus australis
Oxycanus diremptus
Oxycanus sordidus
Wiseana cervinata 'southern' & 'northern'
Wiseana jocosa
Wiseana mimica

(2) HA/HB

Aoraia ensyii
Aoraia lenis
Trictena argentata
Trictena atripalpis
Wiseana copularis 'southern' & 'northern'
Wiseana fuliginea
Wiseana signata 'southern' & 'northern'
Wiseana umbraculata

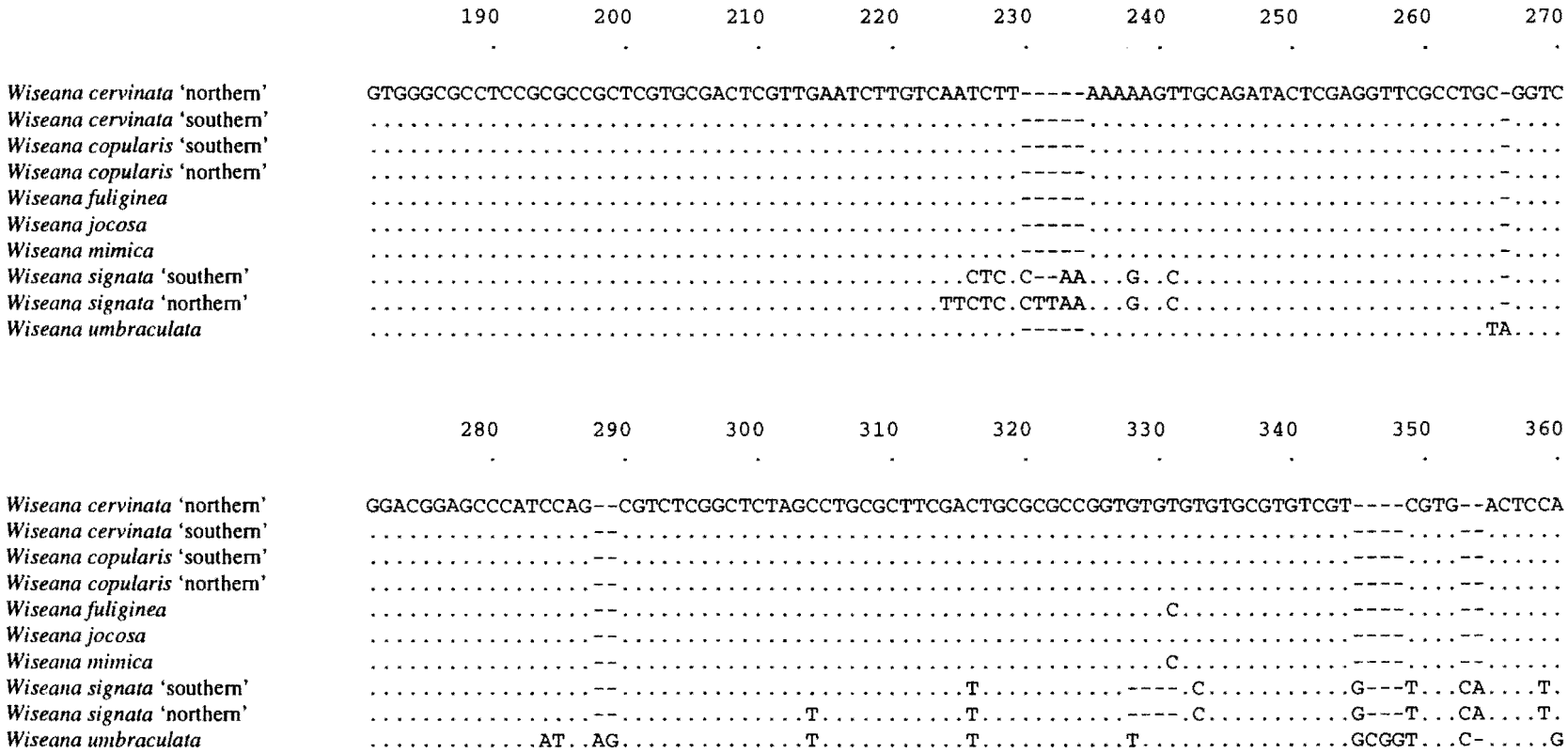
Appendix 2: Populations of New Zealand Hepialids showing within and between population differences in the number of bands amplified in the PCR reaction.

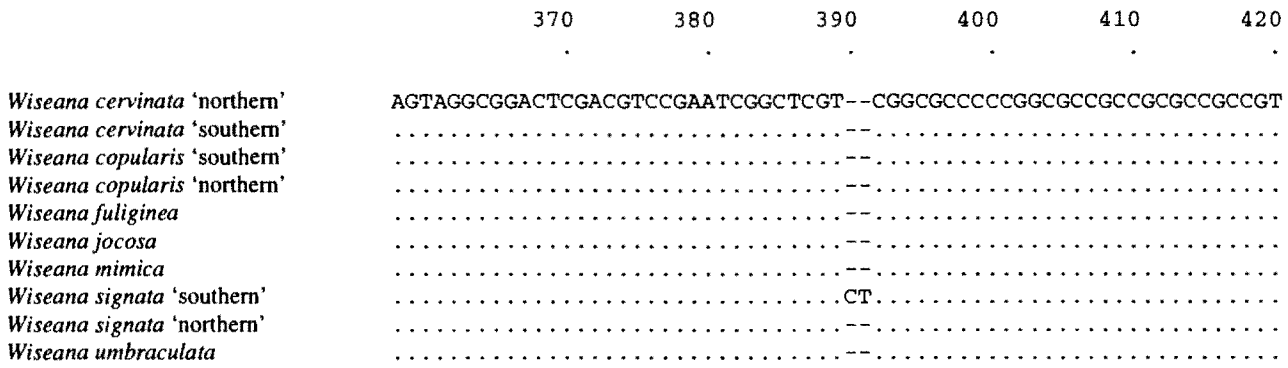
(1) Within population differences	Species	Location	Number of bands
	Dumbletonius unimaculatus	Waitakere Ranges, Auckland	1
	Dumbletonius unimaculatus	Waitakere Ranges, Auckland	2
(2) Between population differences			
	Wiseana copularis 'southern'	Ohai, Southland	1
	Wiseana copularis 'southern'	Christchurch	2
	Wiseana copularis 'northern'	Opouri Saddle, Cobb Ridge	1
	Wiseana copularis 'northern'	Greens Beach, Westland, Pohara, Nelson	2
	Wiseana fuliginea	Birdlings Flat, Canterbury	2
		Dunedin	1
	Wiseana mimica	Mackenzie Country	2
		Cass, Canterbury	1

Appendix 3: Aligned nucleotide sequence from the nrDNA ITS2 region for ten *Wiseana* taxa. Dots indicate nucleotides matching top line in each block.

```
                                10        20        30        40        50        60        70        80        90
                                .         .         .         .         .         .         .         .         .
Wiseana cervinata 'northern'    CGGCTGTAATGAAACAATGCCACATTGCTGCGCTCGCGCAGCTTCTGATCGTTCGGTCGAGAGCGCCCGCCCGCCGGTCG----CTTCTC
Wiseana cervinata 'southern'    ..........................................................................GTCG......
Wiseana copularis 'southern'    ..........................................................................----......
Wiseana copularis 'northern'    ..........................................................................GTCG......
Wiseana fuliginea               ..........................................................-------.....----......
Wiseana jocosa                  ..........................................................-------.....----......
Wiseana mimica                  ..........................................................-------.....----......
Wiseana signata 'southern'      ..........................................................T-------.....----......
Wiseana signata 'northern'      ..........................................................T-------.....----......
Wiseana umbraculata             .......G..................................................C.....-..T.---.CG..CGCT......

                                100       110       120       130       140       150       160       170       180
                                .         .         .         .         .         .         .         .
Wiseana cervinata 'northern'    GTCCCGTCGGTTTAAAAATAATTC---AAACTCACTTTCGACACGCACGCGGCTAGCGAACGCACGCTCGTCGCGCTCGGCTTCCGAGAC
Wiseana cervinata 'southern'    ....................G.TTGA....................................................................
Wiseana copularis 'southern'    ....................---.....................................................................
Wiseana copularis 'northern'    ....................G.TTGA....................................................................
Wiseana fuliginea               ....................--C..-....................................................................
Wiseana jocosa                  ....................---.....................................................................
Wiseana mimica                  ....................--C..-....................................................................
Wiseana signata 'southern'      ..................TT..A-ACT...A.....................--.....................................
Wiseana signata 'northern'      ..................TT..A-ACT...A.....................--.....................................
Wiseana umbraculata             ....................A--A.C.AA.....................C.....................................
```


190 200 210 220 230 240 250 260 270
Wiseana cervinata 'northern' GTGGGCGCCTCCGCGCCGCTCGTGCGACTCGTTGAATCTTGTCAATCTT-----AAAAAGTTGCAGATACTCGAGGTTCGCCTGC-GGTC
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata
280 290 300 310 320 330 340 350 360
Wiseana cervinata 'northern' GGACGGAGCCCATCCAG--CGTCTCGGCTCTAGCCTGCGCTTCGACTGCGCGCCGGTGTGTGTGTGCGTGTCGT----CGTG--ACTCCA
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata


370 380 390 400 410 420
Wiseana cervinata 'northern' AGTAGGCGGACTCGACGTCCGAATCGGCTCGT--CGGCGCCCCCGGCGCCGCCGCGCCGCCGT
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern' CT
Wiseana signata 'northern'
Wiseana umbraculata

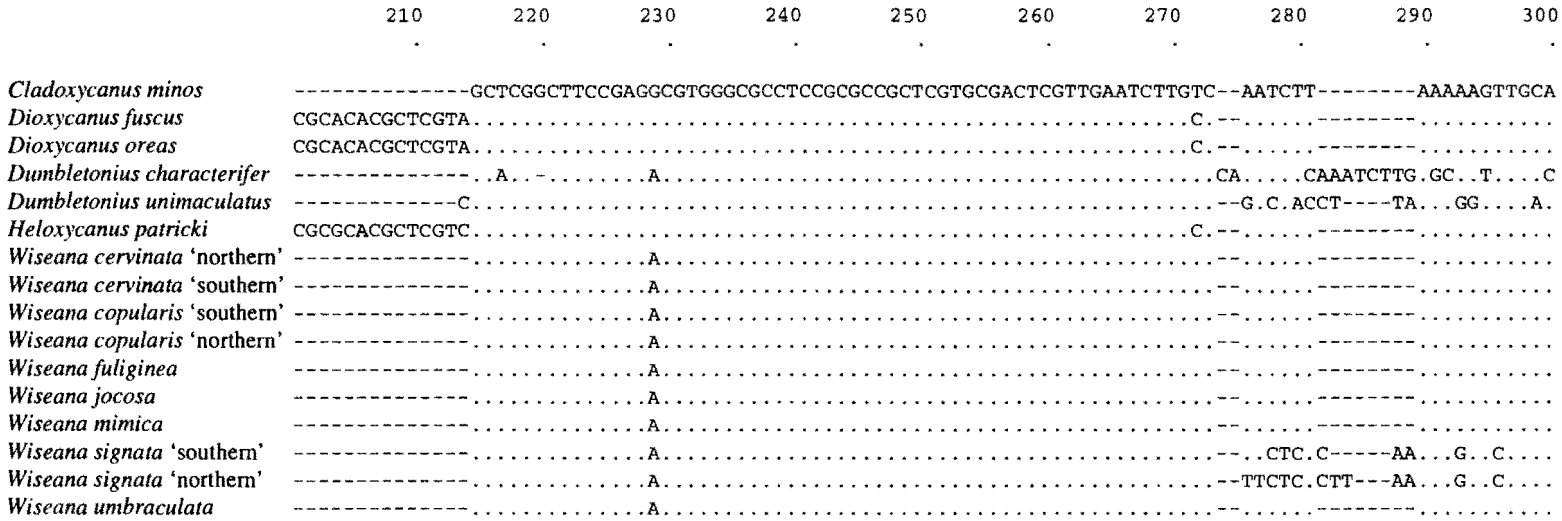
Appendix 4: Aligned nucleotide sequence from the nrDNA ITS2 region for 16 New Zealand hepialid taxa from the *'Oxycanus' Cladoxycanus* and *'Oxycanus' s.str.* lineages. Dots indicate nucleotides matching top line in each block.

```
                                10        20        30        40        50        60        70        80        90       100
                                .         .         .         .         .         .         .         .         .         .

Cladoxycanus minos              CGGCTGTAATGAAACAATGCCACACTGCTC---GCGGGCGCTAACGCGCTCGCGCAGCTTCTGATCGTTCGGTCGAGAACGCGCTGC------T-TCGGT
Dioxycanus fuscus               ............................---..C.....................................--......G..------.-.....
Dioxycanus oreas                ............................---..C.....................................--......G..------.-G....
Dumbletonius characterifer      ..........................GCTC..C.......A.................................G.A...AC.AGCGCGGCT....C
Dumbletonius unimaculatus       ............................---..T.....................................GT..C--------------T.C
Heloxycanus patricki            ............................---..C.....................................--......G..------.-.....
Wiseana cervinata 'northern'    ......................T....----------------.....................................G...C.GC.-CGC-----....
Wiseana cervinata 'southern'    ......................T....----------------.....................................G...C.GC.-CGC-----....
Wiseana copularis 'southern'    ......................T....----------------.....................................G...C.GC.-CGC-----....
Wiseana copularis 'northern'    ......................T....----------------.....................................G...C.GC.-CGC-----....
Wiseana fuliginea               ......................T....----------------.....................................G...C--------------...
Wiseana jocosa                  ......................T....----------------.....................................G...C--------------...
Wiseana mimica                  ......................T....----------------.....................................G...C--------------...
Wiseana signata 'southern'      ......................T....----------------.....................................G...T--------------...
Wiseana signata 'northern'      ......................T....----------------.....................................G...T--------------...
Wiseana umbraculata             .......G..............T....----------------.....................C.......G...CGCT.----------.CG

                                110       120       130       140       150       160       170       180       190       200
                                .         .         .         .         .         .         .         .         .         .

Cladoxycanus minos              CGCG----------CGCCTCGTCCCGTCGGTTTAAAAAT--TTC-----AAACACTTTCGACATGCACGGCAAGCGCACACGCTCG-TCGC---------
Dioxycanus fuscus               ....----------..........................--..A------.....A.................C....G.GT..G..C....ACACGCAAG
Dioxycanus oreas                ..T.----------..........................--..A------.....A.................C....G.GT..G..C....ACACGCAAG
Dumbletonius characterifer      ....CTCGCTCGCC.........T..............T-...------.....................G..-------.GG.....-....---------
Dumbletonius unimaculatus       .T-------------CT......T..............--...-AGAA.............CATG.ACGGCAA..G.....G.--...T---------
Heloxycanus patricki            ....----------..........................--..A------.....................C....G.GA..G..CG...ACACGCAAG
Wiseana cervinata 'northern'    ..-------------.TT.....................AA...---AA.CT...........C.....CGGCTA..GA....A..C...T---CGC---
Wiseana cervinata 'southern'    ..-.-TCG-------.TT.....................AAG.TTGAAA.CT...........C.....CGGCTA..GA....A..C...T---CGC---
Wiseana copularis 'southern'    ..-------------.TT.....................AA...---AA.CT...........C.....CGGCTA..GA....A..C...T---CGC---
Wiseana copularis 'northern'    ..-.-TCG-------.TT.....................AAG.TTGAAA.CT...........C.....CGGCTA..GA....A..C...T---CGC---
Wiseana fuliginea               ..-------------.TT.....................AA...---CA.CT...........C.....CGGCTA..GA....A..C...T---CGC---
Wiseana jocosa                  ..-------------.TT.....................AA...---AA.CT...........C.....CGGCTA..GA....A..C...T---CGC---
Wiseana mimica                  ..-------------.TT.....................AA...---CA.CT...........C.....CGGCTA..GA....A..C...T---CGC---
Wiseana signata 'southern'      ..-------------.TT.....................TT..A-ACTA.C...........C.....--GCTA..GA....A..C...T---CGC---
Wiseana signata 'northern'      ..-------------.TT.....................TT..A-ACTA.C...........C.....--GCTA..GA....A..C...T---CGC---
Wiseana umbraculata             ....CT---------.TT.....................AA..A--AAC.............C.....CGGCCA..GA....A..C...T---CGC---
```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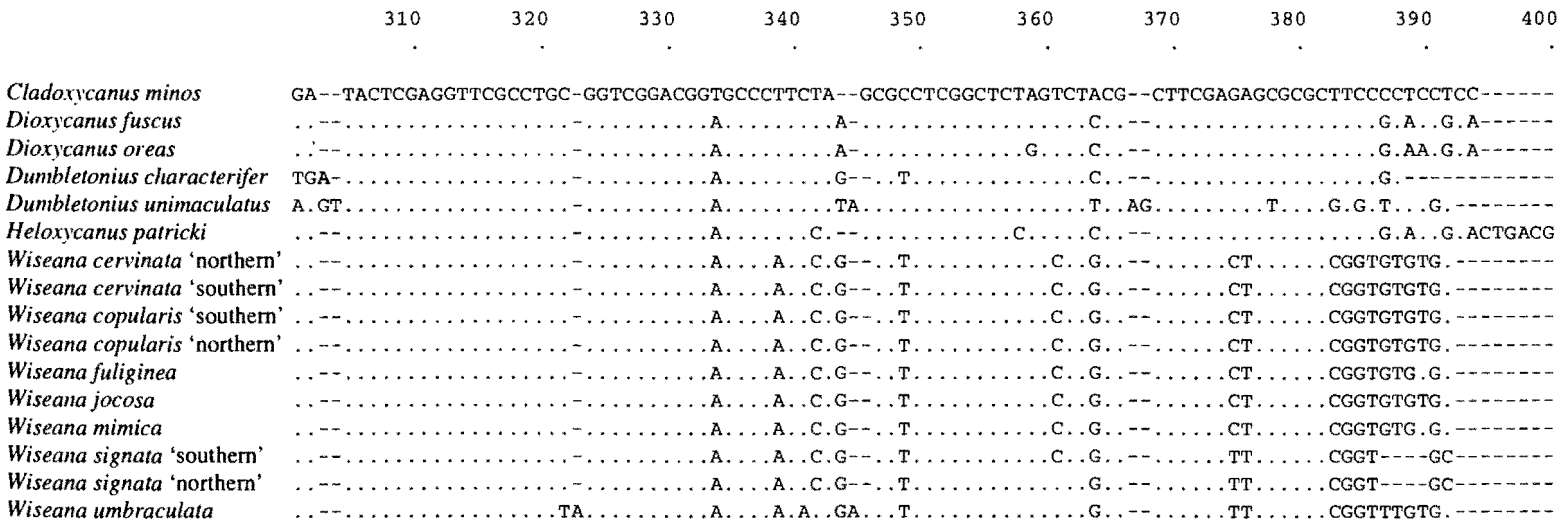
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
Dumbletonius unimaculatus
Heloxycanus patricki
Wiseana cervinata 'northern'
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata

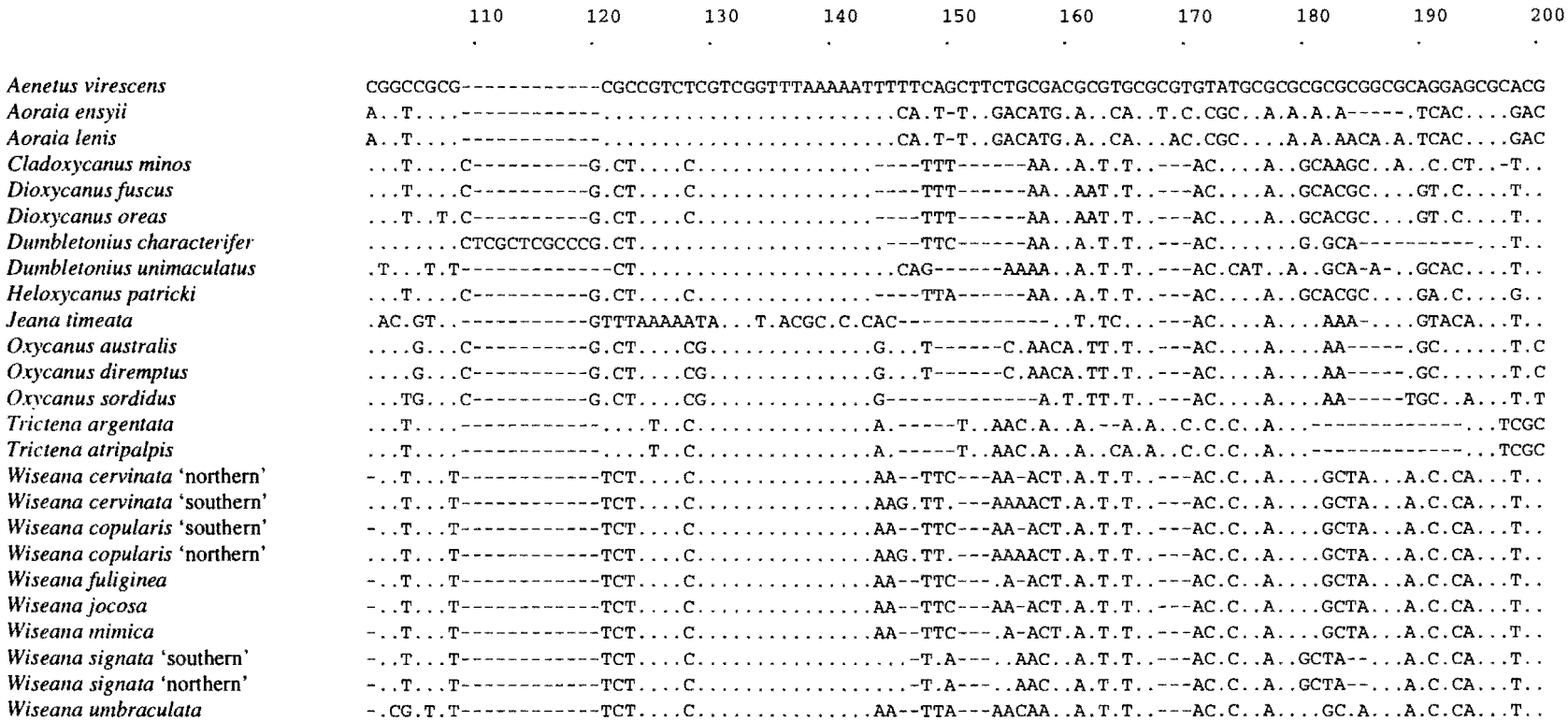

310 320 330 340 350 360 370 380 390 400
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
Dumbletonius unimaculatus
Heloxycanus patricki
Wiseana cervinata 'northern'
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata

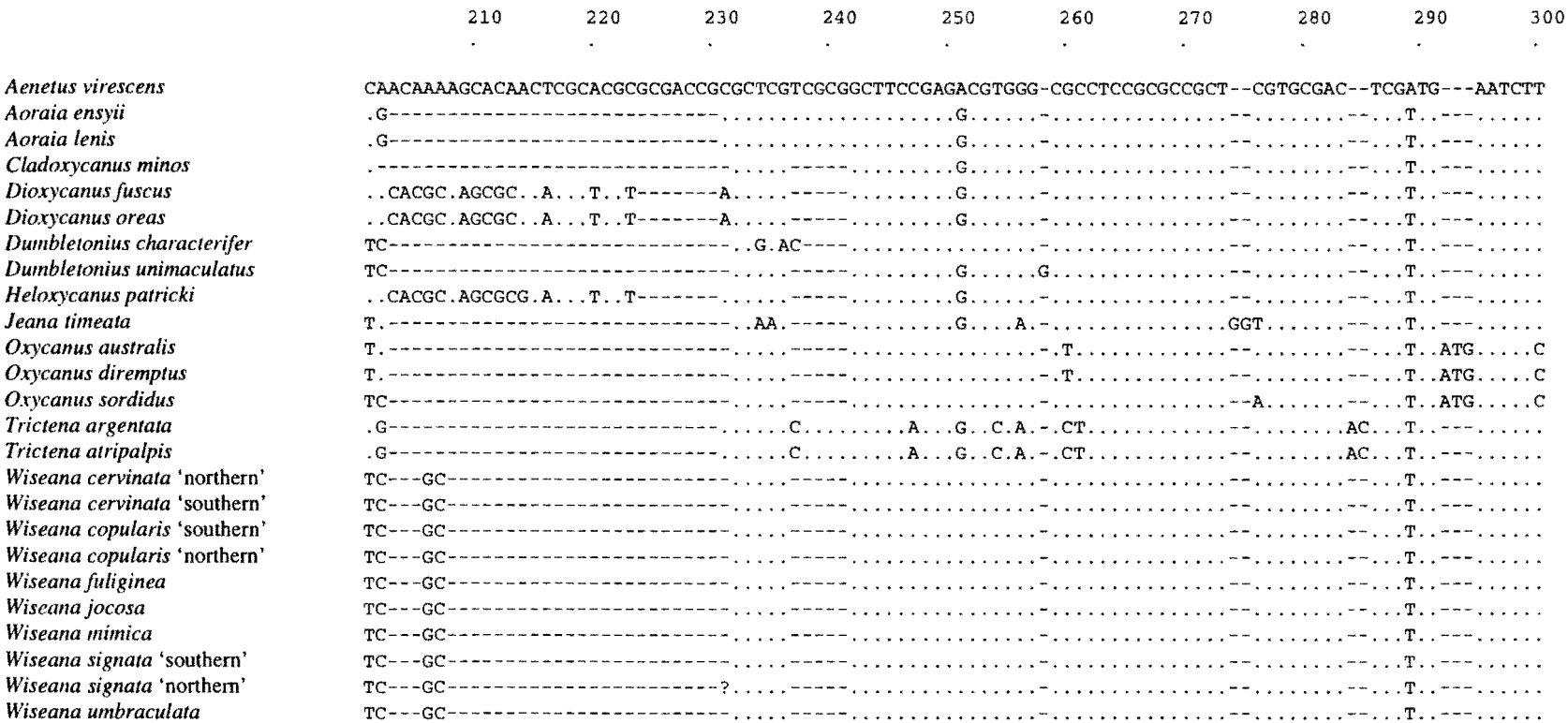
```
                                410       420       430       440       450       460       470       480       490       500
                                .         .         .         .         .         .         .         .         .         .
Cladoxycanus minos              ------------GCCTCTTTGGCGGTGGTTGATGTGTGCGT-----GTCGCTAAGTAGGCGGACTCGACGTCCGAATCGGCTCGT--CGGCGCCCCCGG
Dioxycanus fuscus               ----GTGTGTGGA.TG.GCG..T..CCTCG.CGC.C..C...TTCAA.....CG.....................................--..........
Dioxycanus oreas                ----GTGTGTGGA.TG.GCG..T..CCTCGACGC.C..C...TTCAA.....CG.....................................--..........
Dumbletonius characterifer      ----------------.GCAA.T..TC--------------------GAC.C.....................................--..........
Dumbletonius unimaculatus       ----------------GCGC.T..C-------------------------.C.....................................--..........
Heloxycanus patricki            TGTCGTGTGCGGA.TG.GCGC.T..CCTCG.CGC.C..C..CTGCAA.....CG.....................................--..........
Wiseana cervinata 'northern'    -------------.--.GCG..T..T----------C.T.-------A.T.C.....................................--..........
Wiseana cervinata 'southern'    -------------.--.GCG..T..T----------C.T.-------A.T.C.....................................--..........
Wiseana copularis 'southern'    -------------.--.GCG..T..T----------C.T.-------A.T.C.....................................--..........
Wiseana copularis 'northern'    -------------.--.GCG..T..T----------C.T.-------A.T.C.....................................--..........
Wiseana fuliginea               -------------.--.GCG..T..T----------C.T.-------A.T.C.....................................--..........
Wiseana jocosa                  -------------.--.GCG..T..T----------C.T.-------A.T.C.....................................--..........
Wiseana mimica                  -------------.--.GCG..T..T----------C.T.-------A.T.C.....................................--..........
Wiseana signata 'southern'      -------------.--.GCG..T..TG---------..T.C-----AA.T.....................................CT..........
Wiseana signata 'northern'      -------------.--.GCG..T..TG---------..T.C-----AA.T.....................................--..........
Wiseana umbraculata             -------------.--.GCG..T..TGC.-------...T.C------A.T.CG...................................--..........

                                510
                                .
Cladoxycanus minos              CGCCGCCGCGCCGCCGT
Dioxycanus fuscus               .................
Dioxycanus oreas                .................
Dumbletonius characterifer      .................
Dumbletonius unimaculatus       .................
Heloxycanus patricki            .................
Wiseana cervinata 'northern'    .................
Wiseana cervinata 'southern'    .................
Wiseana copularis 'southern'    .................
Wiseana copularis 'northern'    .................
Wiseana fuliginea               .................
Wiseana jocosa                  .................
Wiseana mimica                  .................
Wiseana signata 'southern'      .................
Wiseana signata 'northern'      .................
Wiseana umbraculata             .................
```

Appendix 5: Aligned nucleotide sequence from the nrDNA ITS2 region for 19 New Zealand and six Australian hepialid taxa. Dots indicate nucleotides matching top line in each block.

```
                                10        20        30        40        50        60        70        80        90        100
                                .         .         .         .         .         .         .         .         .         .
Aenetus virescens               CGGCTGTAATGAAACAATGCCACACTGC---TCGTCGGCGCTCAC--GCGTC--GGCGCAGCCTCTGACCGTTCGGTCG--ACCGCGGC---------CT
Aoraia ensyii                   ..............................---...A......CTGACG...C.--.........T.................--G.....----------T.
Aoraia lenis                    ..............................---...A......CTCACG...C.--.........T............A.....--G.....----------T.
Cladoxycanus minos              ............................T---..CG......A..--...CTCG--......T.....T..........AG.A....CTG-------CT.
Dioxycanus fuscus               ............................T---..C.......A..--...CTCG--......T.....T..........A--A....CGG-------CT.
Dioxycanus oreas                ............................T---..C.......A..--...CTCG--......T.....T..........A--A....CGG-------CTG
Dumbletonius characterifer      ............................TGC..C.......A.A--...CTCG--......T.....T..........AGGAA...A.GAGCGCGGCT.
Dumbletonius unimaculatus       ............................T---..CT......A..--...CTCG--......T.....T..........A--GA.T.----------C
Heloxycanus patricki            ............................T---..C.......A..--...CTCG--......T.....T..........A--A....CGG-------CT.
Jeana timeata                   ..............................---..TG..CTC..GGA----.CG--T.TT....T.............GT-------...A.GTCGTCGTCT.
Oxycanus australis              ..............CGA.............---...CG......G.AGG...C.--C...T...TC.......C..C.CTC-G.A....C.GCTCT---CTC
Oxycanus diremptus              ..............CGA.............---...CG......G.AGG...C.--C...T...TC.......C..C.CTC-G.A....C.G-------CTC
Oxycanus sordidus               ..............CGA.............---...CT.....CG.A--...C.--C.......TC.......C..C.CTC-G.A....T.G-------CT.
Trictena argentata              ...............T...........TGC...C.......TCGGC..TCG--C.......T.....................--.....A.---------T.
Trictena atripalpis             ...............T...........TGC...C.......TC.GC..T----C.......T.....................--.....A.---------T.
Wiseana cervinata 'northern'    ........................T...T-------------------...CTCG--......T.....T..........AG.G...CCGCCCGCC-----
Wiseana cervinata 'southern'    ........................T...T-------------------...CTCG--......T.....T..........AG.G...CCGCCCGCCG--G.
Wiseana copularis 'southern'    ........................T...T-------------------...-TCG--......T.....T..........AG.G...CCGCCCGCC-----
Wiseana copularis 'northern'    ........................T...T-------------------...CTCG--......T.....T..........AG.G...CCGCCCGCCG--G.
Wiseana fuliginea               ........................T...T-------------------...CTCG--......T.....T..........AG.G...C----------
Wiseana jocosa                  ........................T...T-------------------...CTCG--......T.....T..........AG.G...C----------
Wiseana mimica                  ........................T...T-------------------...CTCG--......T.....T..........AG.G...C----------
Wiseana signata 'southern'      ........................T...T-------------------...CTCG--......T.....T..........AG.G...T----------
Wiseana signata 'northern'      ........................T...T-------------------...CTCG--......T.....T..........AG.G...T----------
Wiseana umbraculata             .......G................T...T-------------------...CTCG--......T.....T.....C....AG.G...C..TCGCGC-----
```


110 120 130 140 150 160 170 180 190 200
Aenetus virescens
Aoraia ensyii
Aoraia lenis
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
Dumbletonius unimaculatus
Heloxycanus patricki
Jeana timeata
Oxycanus australis
Oxycanus diremptus
Oxycanus sordidus
Trictena argentata
Trictena atripalpis
Wiseana cervinata 'northern'
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata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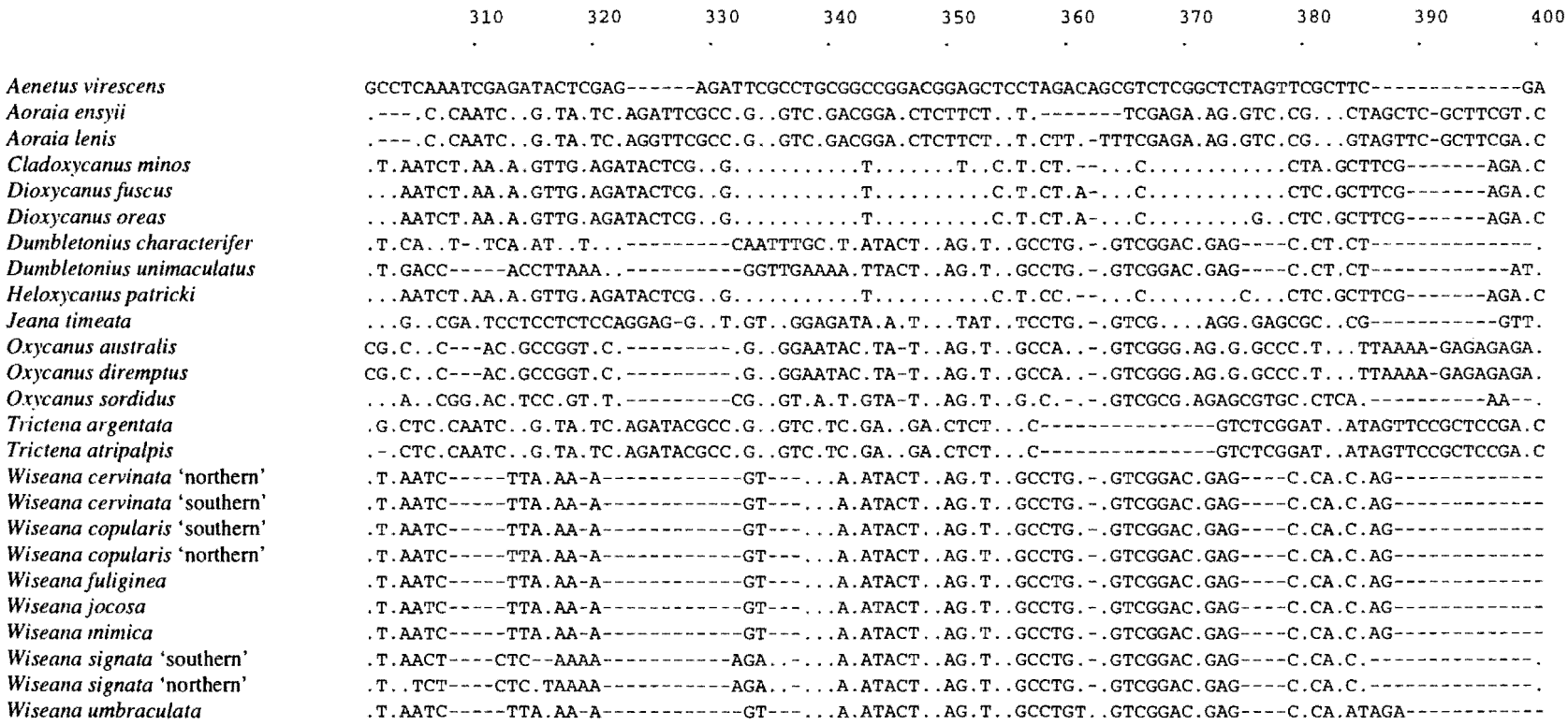
Aoraia ensyii .G----------.....G.....-.....--.....--...T..---.....
Aoraia lenis .G----------.....G.....-.....--.....--...T..---.....
Cladoxycanus minos .----------.....-----.....G.....-.....--.....--...T..---.....
Dioxycanus fuscus ..CACGC.AGCGC..A...T..T-------A.....-----.....G.....-.....--.....--...T..---.....
Dioxycanus oreas ..CACGC.AGCGC..A...T..T-------A.....-----.....G.....-.....--.....--...T..---.....
Dumbletonius characterifer TC----------..G.AC----.....-.....--.....--...T..---.....
Dumbletonius unimaculatus TC----------.....-----.....G.....G.....--.....--...T..---.....
Heloxycanus patricki ..CACGC.AGCGCG.A...T..T-------.....-----.....G.....-.....--.....--...T..---.....
Jeana timeata T.----------..AA.-----.....G....A.-.....GGT.....--...T..---.....
Oxycanus australis T.----------.....-----.....-.T.....--.....--...T..ATG.....C
Oxycanus diremptus T.----------.....-----.....-.T.....--.....--...T..ATG.....C
Oxycanus sordidus TC----------.....-----.....-.....--A.....--...T..ATG.....C
Trictena argentata .G----------.....C.....A...G..C.A.-.CT.....--.....AC...T..---.....
Trictena atripalpis .G----------.....C.....A...G..C.A.-.CT.....--.....AC...T..---.....
Wiseana cervinata 'northern' TC---GC----------.....-----.....-.....--.....--...T..---.....
Wiseana cervinata 'southern' TC---GC----------.....-----.....-.....--.....--...T..---.....
Wiseana copularis 'southern' TC---GC----------.....-----.....-.....--.....--...T..---.....
Wiseana copularis 'northern' TC---GC----------.....-----.....-.....--.....--...T..---.....
Wiseana fuliginea TC---GC----------.....-----.....-.....--.....--...T..---.....
Wiseana jocosa TC---GC----------.....-----.....-.....--.....--...T..---.....
Wiseana mimica TC---GC----------.....-----.....-.....--.....--...T..---.....
Wiseana signata 'southern' TC---GC----------.....-----.....-.....--.....--...T..---.....
Wiseana signata 'northern' TC---GC----------?.....-----.....-.....--.....--...T..---.....
Wiseana umbraculata TC---GC----------.....-----.....-.....--.....--...T..---.....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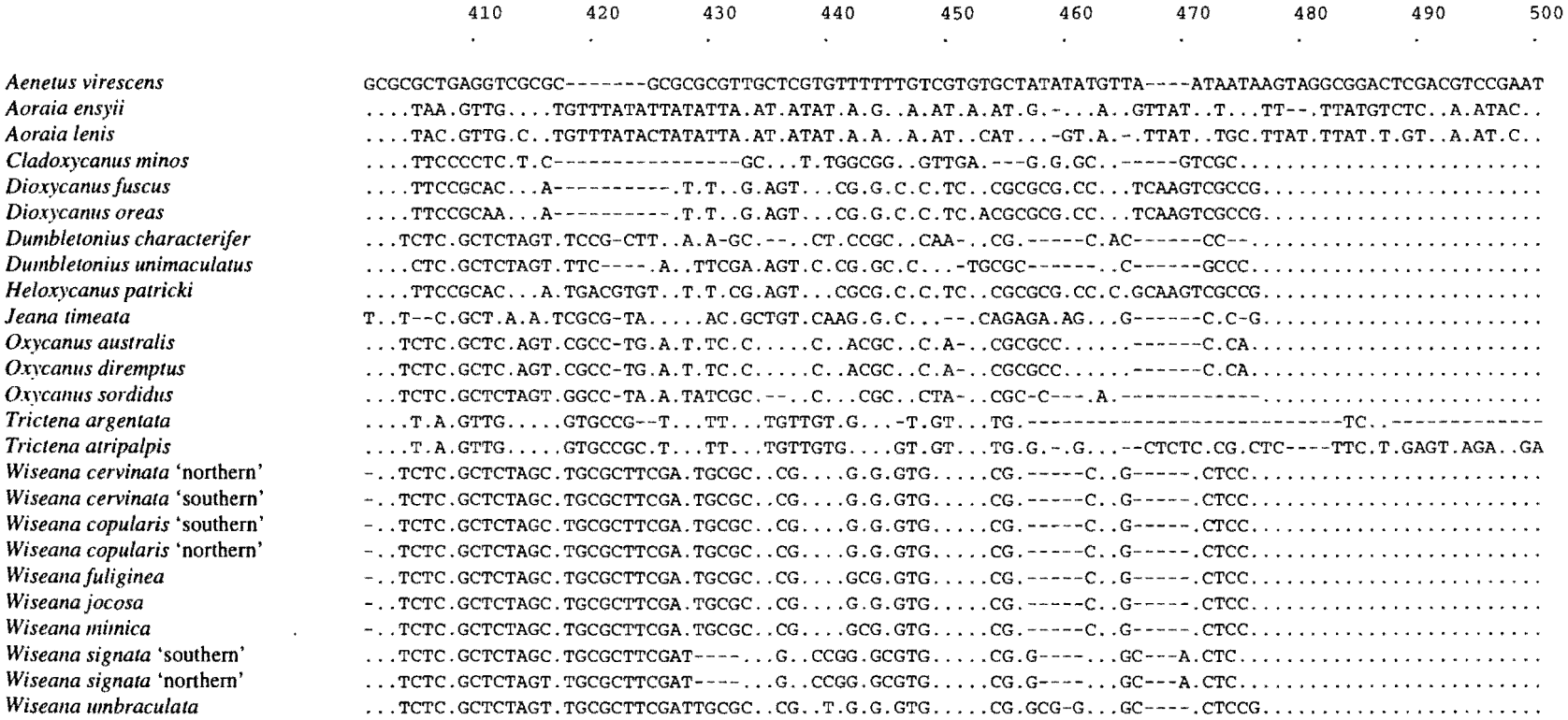
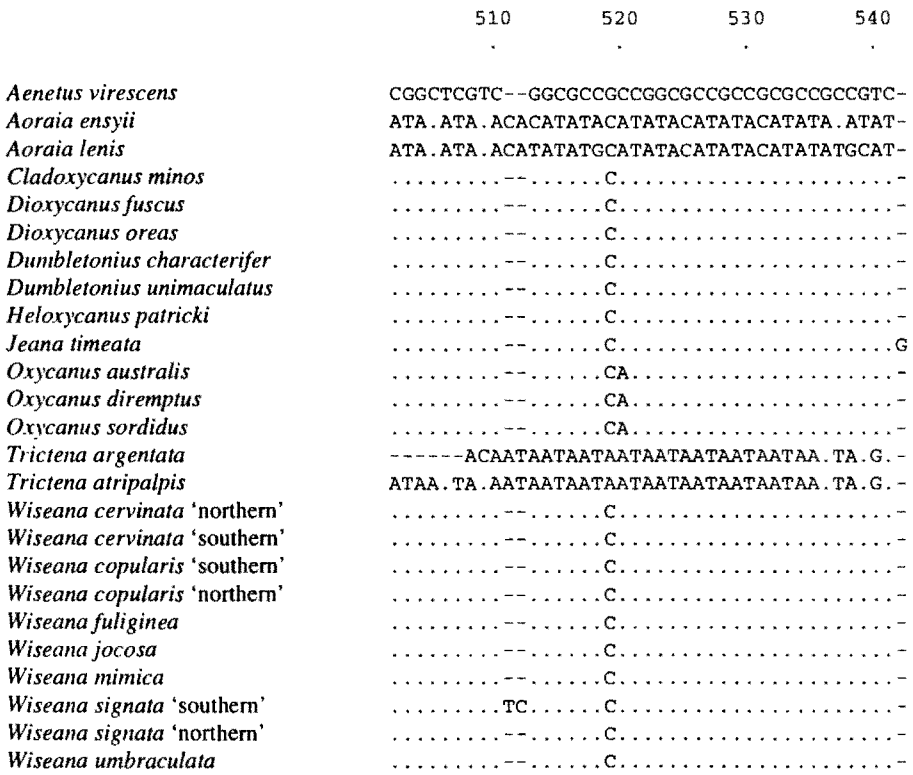
Aenetus virescens GCCTCAAATCGAGATACTCGAG------AGATTCGCCTGCGGCCGGACGGAGCTCCTAGACAGCGTCTCGGCTCTAGTTCGCTTC----------GA
Aoraia ensyii .---.C.CAATC..G.TA.TC.AGATTCGCC.G..GTC.GACGGA.CTCTTCT..T.-------TCGAGA.AG.GTC.CG...CTAGCTC-GCTTCGT.C
Aoraia lenis .---.C.CAATC..G.TA.TC.AGGTTCGCC.G..GTC.GACGGA.CTCTTCT..T.CTT.-TTTCGAGA.AG.GTC.CG...GTAGTTC-GCTTCGA.C
Cladoxycanus minos .T.AATCT.AA.A.GTTG.AGATACTCG..G.....T.....T..C.T.CT.--...C.....CTA.GCTTCG-------AGA.C
Dioxycanus fuscus ...AATCT.AA.A.GTTG.AGATACTCG..G.....T.....C.T.CT.A-...C.....CTC.GCTTCG-------AGA.C
Dioxycanus oreas ...AATCT.AA.A.GTTG.AGATACTCG..G.....T.....C.T.CT.A-...C.....G..CTC.GCTTCG-------AGA.C
Dumbletonius characterifer .T.CA..T-.TCA.AT..T...----------CAATTTGC.T.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CT.CT----------.
Dumbletonius unimaculatus .T.GACC-----ACCTTAAA..----------GGTTGAAAA.TTACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CT.CT----------AT.
Heloxycanus patricki ...AATCT.AA.A.GTTG.AGATACTCG..G.....T.....C.T.CC.--...C.....C...CTC.GCTTCG-------AGA.C
Jeana timeata ...G..CGA.TCCTCCTCTCCAGGAG-G..T.GT..GGAGATA.A.T...TAT..TCCTG.-.GTCG....AGG.GAGCGC..CG----------GTT.
Oxycanus australis CG.C..C---AC.GCCGGT.C.---------.G..GGAATAC.TA-T..AG.T..GCCA..-.GTCGGG.AG.G.GCCC.T...TTAAAA-GAGAGAGA.
Oxycanus diremptus CG.C..C---AC.GCCGGT.C.---------.G..GGAATAC.TA-T..AG.T..GCCA..-.GTCGGG.AG.G.GCCC.T...TTAAAA-GAGAGAGA.
Oxycanus sordidus ...A..CGG.AC.TCC.GT.T.---------CG..GT.A.T.GTA-T..AG.T..G.C.-.-.GTCGCG.AGAGCGTGC.CTCA.----------AA--.
Trictena argentata .G.CTC.CAATC..G.TA.TC.AGATACGCC.G..GTC.TC.GA..GA.CTCT...C----------GTCTCGGAT..ATAGTTCCGCTCCGA.C
Trictena atripalpis .-.CTC.CAATC..G.TA.TC.AGATACGCC.G..GTC.TC.GA..GA.CTCT...C----------GTCTCGGAT..ATAGTTCCGCTCCGA.C
Wiseana cervinata 'northern' .T.AATC-----TTA.AA-A----------GT---...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.AG----------
Wiseana cervinata 'southern' .T.AATC-----TTA.AA-A----------GT---...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.AG----------
Wiseana copularis 'southern' .T.AATC-----TTA.AA-A----------GT---...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.AG----------
Wiseana copularis 'northern' .T.AATC-----TTA.AA-A----------GT---...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.AG----------
Wiseana fuliginea .T.AATC-----TTA.AA-A----------GT---...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.AG----------
Wiseana jocosa .T.AATC-----TTA.AA-A----------GT---...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.AG----------
Wiseana mimica .T.AATC-----TTA.AA-A----------GT---...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.AG----------
Wiseana signata 'southern' .T.AACT----CTC--AAAA----------AGA..-...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.----------.
Wiseana signata 'northern' .T..TCT----CTC.TAAAA----------AGA..-...A.ATACT..AG.T..GCCTG.-.GTCGGAC.GAG----C.CA.C.----------.
Wiseana umbraculata .T.AATC-----TTA.AA-A----------GT---...A.ATACT..AG.T..GCCTGT..GTCGGAC.GAG----C.CA.ATAGA----------


410 420 430 440 450 460 470 480 490 500
Aenetus virescens GCGCGCTGAGGTCGCGC-------GCGCGCGTTGCTCGTGTTTTTTGTCGTGTGCTATATATGTTA----ATAATAAGTAGGCGGACTCGACGTCCGAAT
Aoraia ensyii ....TAA.GTTG....TGTTTATATTATATTA.AT.ATAT.A.G..A.AT.A.AT.G.-...A..GTTAT..T...TT--.TTATGTCTC..A.ATAC..
Aoraia lenis ....TAC.GTTG.C..TGTTTATACTATATTA.AT.ATAT.A.A..A.AT..CAT...-GT.A.-.TTAT..TGC.TTAT.TTAT.T.GT..A.AT.C..
Cladoxycanus minos ....TTCCCCTC.T.C----------------GC...T.TGGCGG..GTTGA.---G.G.GC..-----GTCGC.....
Dioxycanus fuscus ....TTCCGCAC...A----------.T.T..G.AGT...CG.G.C.C.TC..CGCGCG.CC...TCAAGTCGCCG.....
Dioxycanus oreas ....TTCCGCAA...A----------.T.T..G.AGT...CG.G.C.C.TC.ACGCGCG.CC...TCAAGTCGCCG.....
Dumbletonius characterifer ...TCTC.GCTCTAGT.TCCG-CTT..A.A-GC.--..CT.CCGC..CAA-..CG.-----C.AC------CC--.....
Dumbletonius unimaculatus ....CTC.GCTCTAGT.TTC----.A..TTCGA.AGT.C.CG.GC.C...-TGCGC------..C------GCCC.....
Heloxycanus patricki ....TTCCGCAC...A.TGACGTGT..T.T.CG.AGT...CGCG.C.C.TC..CGCGCG.CC.C.GCAAGTCGCCG.....
Jeana timeata T..T--C.GCT.A.A.TCGCG-TA.....AC.GCTGT.CAAG.G.C...--.CAGAGA.AG...G------C.C-G.....
Oxycanus australis ...TCTC.GCTC.AGT.CGCC-TG.A.T.TC.C.....C..ACGC..C.A-..CGCGCC.....------C.CA.....
Oxycanus diremptus ...TCTC.GCTC.AGT.CGCC-TG.A.T.TC.C.....C..ACGC..C.A-..CGCGCC.....------C.CA.....
Oxycanus sordidus ...TCTC.GCTCTAGT.GGCC-TA.A.TATCGC.--..C...CGC..CTA-..CGC-C---.A.----------.....
Trictena argentata ....T.A.GTTG.....GTGCCG--T...TT...TGTTGT.G...-T.GT...TG.----------TC..----------
Trictena atripalpis ....T.A.GTTG.....GTGCCGC.T...TT...TGTTGTG....GT.GT...TG.G.-.G...--CTCTC.CG.CTC----TTC.T.GAGT.AGA..GA
Wiseana cervinata 'northern' -..TCTC.GCTCTAGC.TGCGCTTCGA.TGCGC..CG....G.G.GTG.....CG.-----C..G-----.CTCC.....
Wiseana cervinata 'southern' -..TCTC.GCTCTAGC.TGCGCTTCGA.TGCGC..CG....G.G.GTG.....CG.-----C..G-----.CTCC.....
Wiseana copularis 'southern' -..TCTC.GCTCTAGC.TGCGCTTCGA.TGCGC..CG....G.G.GTG.....CG.-----C..G-----.CTCC.....
Wiseana copularis 'northern' -..TCTC.GCTCTAGC.TGCGCTTCGA.TGCGC..CG....G.G.GTG.....CG.-----C..G-----.CTCC.....
Wiseana fuliginea -..TCTC.GCTCTAGC.TGCGCTTCGA.TGCGC..CG....GCG.GTG.....CG.-----C..G-----.CTCC.....
Wiseana jocosa -..TCTC.GCTCTAGC.TGCGCTTCGA.TGCGC..CG....G.G.GTG.....CG.-----C..G-----.CTCC.....
Wiseana mimica -..TCTC.GCTCTAGC.TGCGCTTCGA.TGCGC..CG....GCG.GTG.....CG.-----C..G-----.CTCC.....
Wiseana signata 'southern' ...TCTC.GCTCTAGC.TGCGCTTCGAT----...G..CCGG.GCGTG.....CG.G----...GC---A.CTC.....
Wiseana signata 'northern' ...TCTC.GCTCTAGT.TGCGCTTCGAT----...G..CCGG.GCGTG.....CG.G----...GC---A.CTC.....
Wiseana umbraculata ...TCTC.GCTCTAGT.TGCGCTTCGATTGCGC..CG..T.G.G.GTG.....CG.GCG-G...GC----.CTCCG.....


510
520
530
540
Aenetus virescens
Aoraia ensyii
Aoraia lenis
Cladoxycanus minos
Dioxycanus fuscus
Dioxycanus oreas
Dumbletonius characterifer
Dumbletonius unimaculatus
Heloxycanus patricki
Jeana timeata
Oxycanus australis
Oxycanus diremptus
Oxycanus sordidus
Trictena argentata
Trictena atripalpis
Wiseana cervinata 'northern'
Wiseana cervinata 'southern'
Wiseana copularis 'southern'
Wiseana copularis 'northern'
Wiseana fuliginea
Wiseana jocosa
Wiseana mimica
Wiseana signata 'southern'
Wiseana signata 'northern'
Wiseana umbraculata

Chapter 5

Phylogenetic relationships of the '*Oxycanus*' lineages of hepialid moths from New Zealand inferred from analysis of combined morphology, mtDNA and nrDNA sequence data

B. Brown, R.M. Emberson and A.M. Paterson

Abstract

Morphological character data and sequence data from the mitochondrial DNA cytochrome oxidase subunit I and II (COI & II) gene regions and from the nuclear ribosomal DNA internal transcribed spacer-2 (ITS2) were obtained for 19 New Zealand hepialid (Lepidoptera: Hepialidae) taxa. We compared the structure of the phylogenetic trees produced from the cladistic analysis of the separate data sets with those produced from total evidence and taxonomic congruence methods.

The total evidence phylogeny produced from the combined morphology, COI & II and ITS2 data sets was congruent with that produced from the ITS2 data set alone. As with all separate analyses, *Aenetus* and *Aoraia* taxa were recovered as separate basal branches. *Cladoxycanus* was recovered in a clade comprising *Dumbletonius*, *Dioxycanus* and *Heloxycanus* taxa. This arrangement conflicted with the phylogenies recovered from the separate morphology and COI & II data sets, where *Cladoxycanus* was recovered as a separate branch.

The total evidence phylogeny produced from the combined morphology and COI & II data sets was congruent with that produced from the morphology data set alone. *Aenetus* and *Aoraia* and *Cladoxycanus* were recovered as separate basal branches. Placement of the *Dumbletonius* taxa in a clade together conflicts with the COI & II and ITS2 ('*Oxycanus*' lineages only) analyses, where *Dumbletonius characterifer* was recovered as either the basal taxon in the '*Oxycanus*' lineage *s. str.* or in an unresolved basal polytomy with *Cladoxycanus*, and *D. unimaculatus* was recovered in a clade with *Wiseana* taxa.

The most resolved phylogeny for the genus *Wiseana* was recovered from the combined morphology, COI & II, ITS2 and allozyme data sets, although the placement of *W. jocosa* and *W. copularis* taxa was uncertain.

Taxonomic congruence produced a more conservative estimate of New Zealand hepialid relationships with only the (*Heloxycanus, Dioxycanus*), (*Wiseana copularis* 'southern', *W. copularis* 'northern') and (*W. fuliginea, W. mimica*) clades being resolved.

Key words - New Zealand, hepialids, *'Oxycanus'* lineages, parsimony, total evidence, taxonomic congruence, morphology, COI & II, ITS2.

Status - Prepared for submission to the Biological Journal of the Linnean Society

Introduction

The recovery of organismal phylogeny is one of the goals of systematics (Penny & Hendy, 1986; Miyamoto & Cracraft, 1991; Lanyon, 1993; De Queiroz *et al*, 1995). That there exists only one true phylogeny is not disputed (Brady, 1985; Bull *et al.*, 1993), but whether it is actually recoverable (Miller *et al.*, 1997), what methods are most appropriate (Kluge, 1989; Miyamoto & Fitch, 1995) and how to know when you have recovered it (Penny & Hendy, 1986; Miyamoto & Cracraft, 1991; Kim, 1993; Kim & Jansen, 1994) are contentious issues.

Phylogeny may be inferred from many sources: morphology, proteins, DNA, ecology, behaviour and life history (Kluge, 1989; Vane-Wright *et al.*, 1992; Paterson *et al.*, 1995; Weller *et al.*, 1996). Although it has been concluded that no one source of characters should be any more homoplasious than any other (Sanderson & Donoghue, 1989; De Queiroz & Wimberger, 1993; Miller & Wenzel, 1995) and that morphological and molecular data are both useful (Brown *et al.*, 1994; Smith & Sytsma, 1994; Miller, 1996), how to proceed when different data sets produce conflicting phylogenies is not clear. Followers of one school of thought, taxonomic congruence *sensu* Mickevich (1978), recommend analysis of each data set/data partition separately and summary of areas of agreement on a consensus tree (De Queiroz, 1993; Miyamoto & Fitch, 1995). Advantages of this method are that congruence among the different data sets can be observed and if present, is strong evidence that the true phylogeny has been recovered (Penny & Hendy, 1986; Swofford, 1991; Kim & Jansen, 1994). The main disadvantage of this method is that the consensus tree is usually less resolved than any of the separate data sets (Nixon & Carpenter, 1996). Followers of the other school of thought, total evidence *sensu* Kluge,(1989) believe that data partitions are arbitrary (Kluge & Wolf, 1993; De Salle & Brower, 1997; Miller *et al.*, 1997) and that combining all data into one set, followed by parsimony analysis will produce the most reliable estimate of phylogeny (Kluge, 1989; Chippendale & Wiens, 1994; Brower, 1996). Total evidence trees tend to be more resolved than those produced from taxonomic congruence, but, if only the total evidence method is used, the relative contributions of the characters from the different data sets are difficult to measure (Swofford, 1991). Part of the problem of knowing how to summarize the information produced from different data sets, is the assessment and elimination of incongruence between the data sets (Swofford, 1991; Patterson *et al.*, 1993; Cunningham, 1997). A further issue is determining the level of incongruence above which combining data sets results in a less accurate phylogeny than that implied by the individual data sets (Bull *et al.*, 1993; De Queiroz, 1993; Sullivan, 1996).

We have reconstructed phylogenies for hepialid moths from New Zealand using morphological characters (Chapter 2) and DNA sequence characters from the mtDNA COI & II (Chapter 3) and ITS2 (Chapter 4) regions. Each data set produced a slightly different topology. Relationships within the genus *Wiseana*, which has several pest species, were poorly resolved.

Our aims in this study were to: (i) estimate the phylogeny for New Zealand hepialid moths from morphological and molecular data sets using total evidence and taxonomic congruence methods, (ii) compare the phylogenies recovered using total evidence and taxonomic congruence methods with those produced from separate analyses of the morphological, COI & II and ITS2 data sets, (iii) investigate whether parsimony methods remain consistent under the total evidence approach and (iv) assess the amount of support for the clades recovered under the total evidence approach using spectral analysis.

Material and Methods

Separate analyses - Details of specimen collection and full details of morphological characters, the data matrix and analysis are presented in Chapter 2. Details of DNA extraction, PCR, nucleotide sequencing, sequence alignment, the data matrix and sequence analysis for the COI & II and ITS2 data sets are presented in Chapters 3 & 4 respectively.

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

Assessment of incongruence/hetergeneity among data sets - Assessment of the level of incongruence between data sets is recommended before combining (Huelsenbeck *et al.*, 1996; Cunningham, 1997) so that the sources of incongruence can be investigated and eliminated. The partition homogeneity test, also known as the incongruence length difference (ILD) test (Mickevich & Farris, 1981; Farris *et al.*, 1994), was calculated in PAUP*4.0 61d-64d (Swofford, 1998). A random distribution of the sum of tree lengths is calculated by randomly repartitioning the morphological, COI & II and ITS2 data sets into three data sets the same size as the originals and calculating the sum of the tree lengths multiple times. The sum of tree lengths for the random distribution was calculated 2000 times using a combined morphology, COI & II and ITS2 data set with all invariant characters removed as recommended (Cunningham, 1997).

The random distribution is compared with the sum of tree lengths for the original data sets. If there is a high probability that shorter trees are found within the randomised data sets, compared with the original separate data sets, then the separate data sets are regarded as being congruent (Mason-Gamer & Kellogg, 1996).

Combined analysis - All manipulations of the separate data sets to produce the combined data sets were carried out using MacClade version 3.1 (Maddison & Maddison, 1992). *Aoraia rufivena* was not included in the combined morphological, COI & II and ITS2 data set because it could not be amplified using the ITS2 primers. Additional haplotypes for *W. cervinata, W. copularis* and *W. signata* were identified from the COI & II and ITS2 data sets. There was only one discernible morphological difference between the haplotypes. The adult male forewing discal cell, white scale shape (Character 20, Chapter 2) for the *W. cervinata* 'southern' haplotype was narrow with a truncate apex, while that from the *W. cervinata* 'northern' haplotype was broad with a truncate apex. Apart from this character, the haplotypes were assigned the same morphological character state as had been assigned to the species in Chapter 2.

All combined data sets were analysed using parsimony because the presence of morphological data precluded the use of maximum likelihood. Maximum likelihood may be preferred to analyse molecular data as the method is known to be consistent (Yang, 1996), a model of evolution incorporating branch length, substitution process and among site-rate heterogeneity can be specified, and the method is more robust to violations in the assumptions of the specified model (Swofford *et al.*, 1996) compared with parsimony. In the separate analyses of both the COI & II and ITS2 data sets, parsimony and maximum likelihood methods produced congruent topologies. Therefore, using only parsimony in the combined analyses should not be disadvantageous in this case.

Wiseana - This genus has a history of taxonomic instability due to overlapping intra and interspecific morphological characteristics (Dumbleton, 1966; MacArthur, 1986; Dugdale, 1994; Herbert, 1995). The larvae of four species, *W. cervinata, W. copularis, W. fuliginea* and *W. mimica*, are pests of improved pasture, defoliating the vegetation (Barratt *et al.*, 1990; Dugdale, 1994; Herbert, 1995). A fully resolved phylogeny for *Wiseana* was not recovered from any of the separate analyses. A total evidence data set for only *Wiseana* included morphological, COI & II and ITS2 characters and 16 allozyme characters from adults and larvae taken from the work of Herbert (1995) (Appendix 1).

Dumbletonius unimaculatus was used as the outgroup as it was sister taxon to the genus *Wiseana* in the separate COI & II and ITS2 (*'Oxycanus'* lineages only) analyses.

Taxonomic Congruence - A majority rule consensus tree was constructed from the strict consensus trees calculated for each separate data set, using PAUP 3.1 (Swofford, 1993).

Phylogenetic Analysis - We searched for maximum parsimony (MP) trees in the unordered, equally weighted combined data sets with PAUP 3.1 using the heuristic search option and 10 stepwise addition replicates. Bootstrap proportions (Felsenstein, 1985), measuring the frequency of a branch's occurence in the resampling of pseudoreplicates from the data set, were calculated in PAUP 3.1, as were other tree statistics such as confidence interval (CI) (Kluge & Farris, 1969), retention index (RI) (Farris, 1989) and skewness (G_1) (Hillis & Huelsenbeck, 1992). Support for specified clades was assessed using spectral analysis (Hendy & Penny, 1993). Spectral analysis allows testing of alternative hypotheses of relationship without necessarily inferring a phylogenetic tree. The 'spectrum' generated by this method represents the estimated support for or conflict against any possible grouping of taxa. *'Spectrum'* PPC Version 2.0 (Charleston, 1997) converts nucleotide sequence data from a single gene into purines and pyrimidines and assesses all possible bipartitions (splits) in the data. The length of a split represents the expected number of character-state changes per site (i.e., the length of a branch). The closer the data match the estimated branch lengths, the higher the support value. A minimum support value was set at 0.0001. Clades recovered in the total evidence analyses were searched for in the COI & II data set and levels of support recorded. The COI & II data set was used because it produced the most resolved molecular estimate of New Zealand hepialid phylogeny.

Results

Separate analyses - *Aenetus* and *Aoraia* taxa were recovered basally in all analyses. Disagreement between the morphological, COI & II and ITS2 phylogenies occurred over the placement of *Dumbletonius characterifer* and *D. unimaculatus* and the arrangements within the genus *Wiseana*. In the morphological phylogeny (Figure 1), *D. characterifer* and *D. unimaculatus* were recovered in one clade. They are recovered separately in the COI & II phylogeny (Figure 2), with *D. characterifer* being the basal taxon of the *'Oxycanus'* lineage *s. str.* and *D. unimaculatus* being recovered in a clade with the genus *Wiseana*. The ITS2 topology (Figure 3) differs substantially from those recovered from the morphological and COI & II data sets, with two major clades recovered. One comprised (*Dumbletonius unimaculatus, D. characterifer, Cladoxycanus, Heloxycanus, Dioxycanus*) and the other comprised the genus *Wiseana* alone. Analysis of the ITS2 data for the *'Oxycanus'* lineages alone, produced a phylogeny congruent with that recovered from COI & II data, except that the relationship of *Cladoxycanus minos* and *Dumbletonius characterifer* was unresolved, as were relationships within the genus *Wiseana* (Figure 4).

Wiseana - Relationships within the genus *Wiseana* were not fully resolved using any of the character sets. In the morphological data set (Figure 1), most characters were invariant or autapomorphic. *Wiseana cervinata, W. copularis, W. fuliginea, W. jocosa* and *W. mimica* formed one clade while *W. signata* and *W. umbraculata* formed another. The COI & II data set (Figure 2) recovered additional haplotypes for *W. cervinata*, *W. copularis* and *W. signata* and resolved some relationships at the tips of branches. Figure 2 shows the synapomorphies supporting clades within the genus *Wiseana*. It was hypothesised in Chapter 3, that the genus had evolved recently and/or rapidly. Relationships within *Wiseana* were also poorly resolved using ITS2 sequence data (Figure 4), with *W. cervinata* 'northern' and *W. copularis* 'southern', *W. cervinata* 'southern' and *W. copularis* 'northern', and *W. fuliginea* and *W. mimica* having identical sequence. The *Wiseana jocosa* sequence differed from the *W. fuliginea* and *W. mimica* sequence by two nucleotides. Only the relationships between *W. signata* 'southern' and *W. signata* 'northern' and *W. umbraculata* were resolved using ITS2.

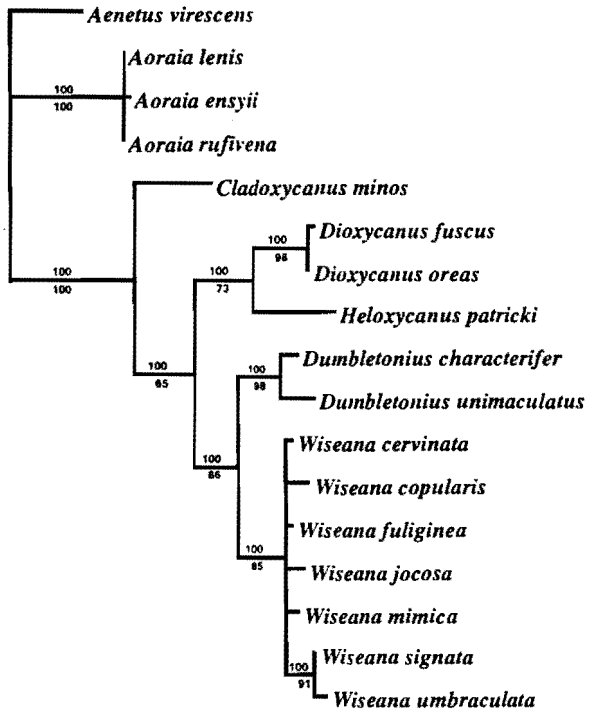


Fig. 1: Majority rule consensus phylogram of the 116 most parsimonious trees from the morphological data for the New Zealand Hepialidae indicating unambiguous synapomorphies. Branch lengths are proportional to morphological character state change. Majority rule consensus values are shown above the branches and bootstrap proportions (>50%) below.

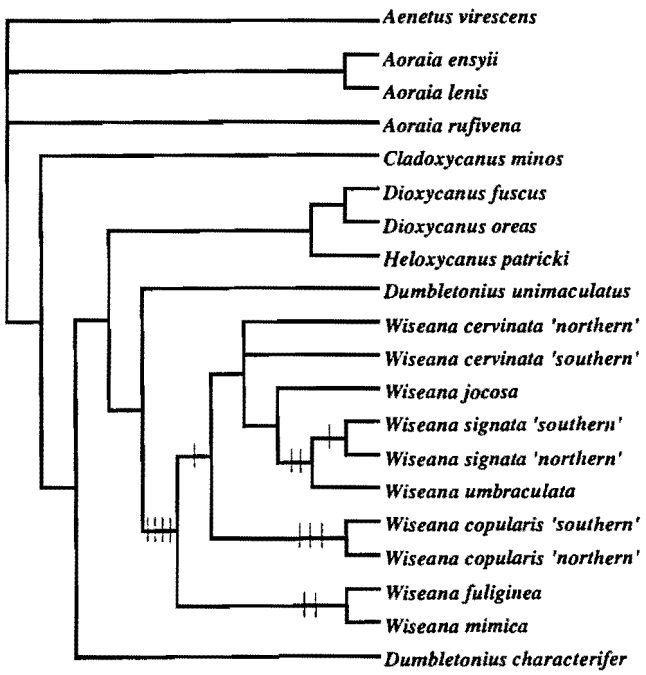


Figure 2: Majority rule consensus tree of the six most parsimonious trees from the analysis of mtDNA COI & II sequence data from New Zealand hepialids. Unambiguous synapomorphies supporting clades in the genus *Wiseana* are shown on the branches.

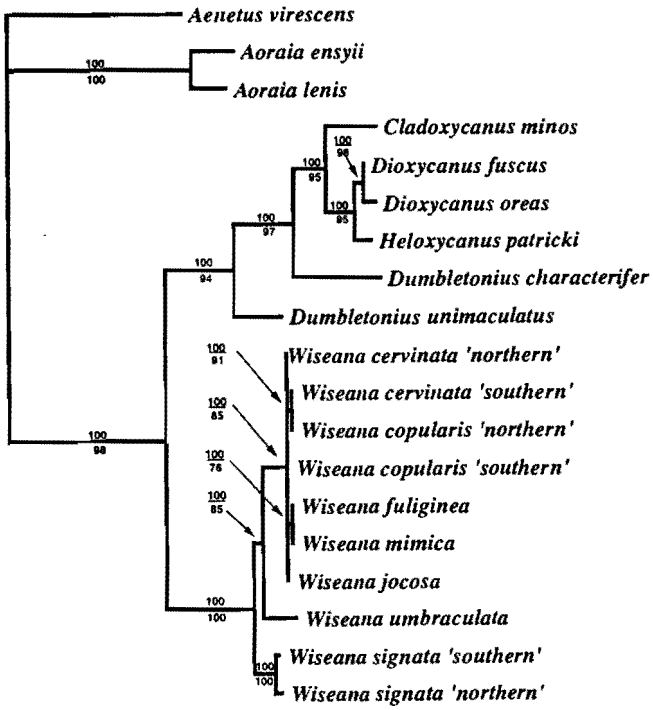


Figure 3: Majority rule consensus phylogram of the 75 most parsimonius trees from the analysis of ITS2 sequence data from New Zealand hepialids. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule consensus values are shown above the branches and bootstrap proportions (>50%) below.

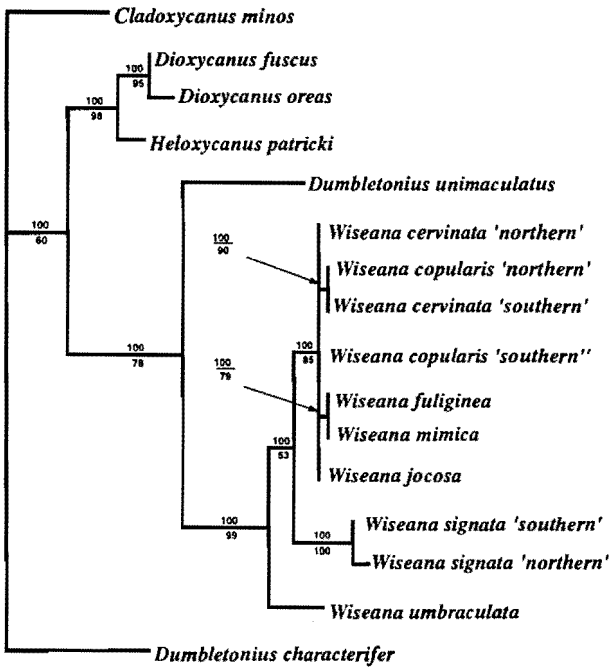


Figure 4: Majority rule consensus phylogram of the 75 most parsimonius trees from the analysis of ITS2 sequence data from New Zealand *'Oxycanus'* lineage of hepialid moths. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule consensus values are shown above the branches and bootstrap proportions (>50%) below.

Total evidence - morphological, COI & II and ITS2 data sets - Assessment of incongruence between the data sets is recommended before combining (Cunningham, 1997) so that appropriate remedial action may be taken. Weak incongruence may be due to sampling error (Bull *et al.*, 1993; Sullivan, 1996). Strong incongruence may indicate the data sets have different evolutionary histories or that the assumptions of the phylogenetic methods used, have been violated (Swofford, 1991). Strong incongruence may lead to a reduction in phylogenetic accuracy or unresolved trees when the different data sets are combined (Bull *et al.*, 1993). Application of the ILD test to the partitioned morphological, COI & II and ITS2 data sets showed there to be no significant incongruence ($p>0.01$) and therefore combining the data sets should not reduce accuracy (Cunningham, 1997).

A heuristic search of the combined morphological, COI & II and ITS2 data sets for the New Zealand hepialid taxa produced nine MP trees, TL 857, CI 0.72, RI 0.78, G_1 -1.04. The majority rule consensus tree with bootstrap proportions is presented in Figure 5. *Aenetus* and *Aoraia* taxa occurred as two branches, separate from the remaining taxa, which fell into two major clades. One clade comprised *Dumbletonius unimaculatus, D. characterifer, Heloxycanus, Dioxycanus* and *Cladoxycanus.* There was strong bootstrap support for this clade and all eight synapomorphies came from the ITS2 data set. The other major clade comprised the *Wiseana* taxa and all alternative arrangements of taxa in the nine MP trees recovered occurred within this genus.

Total evidence - morphology and COI & II - A heuristic search of the combined morphological and COI & II data sets produced four MP trees, TL 344, CI 0.68, RI 0.71, G_1 -1.1. The majority rule consensus tree with bootstrap proportions is shown in Figure 6. The synapomorphies supporting the major clades came from both the morphological and COI & II data sets. The topology, apart from within the genus *Wiseana*, was congruent with the phylogeny recovered from the morphology data set alone.

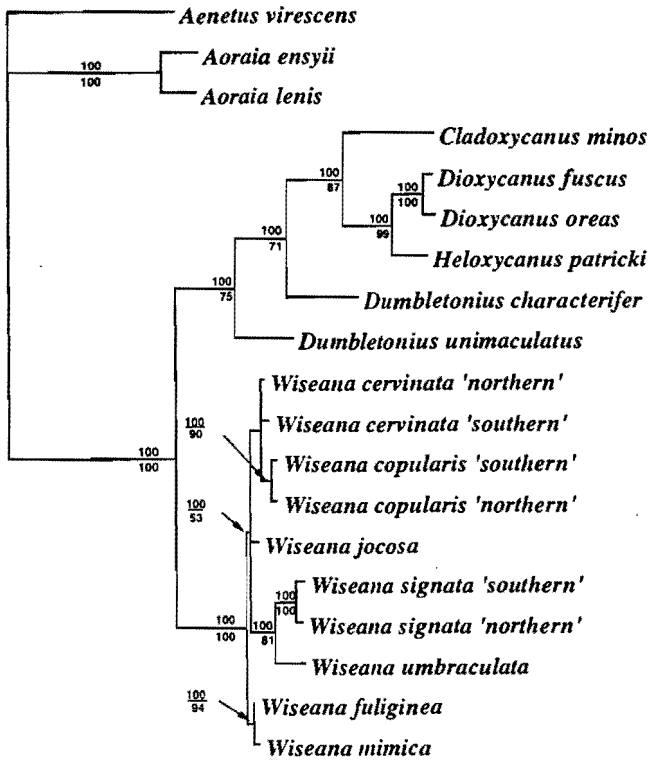


Figure 5: Majority rule consensus phylogram of the nine most parsimonius trees from the analysis of the combined morphology, COI & II and ITS2 data sets for New Zealand hepialid moths. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule consensus values are shown above the branches and bootstrap proportions (>50%) below.

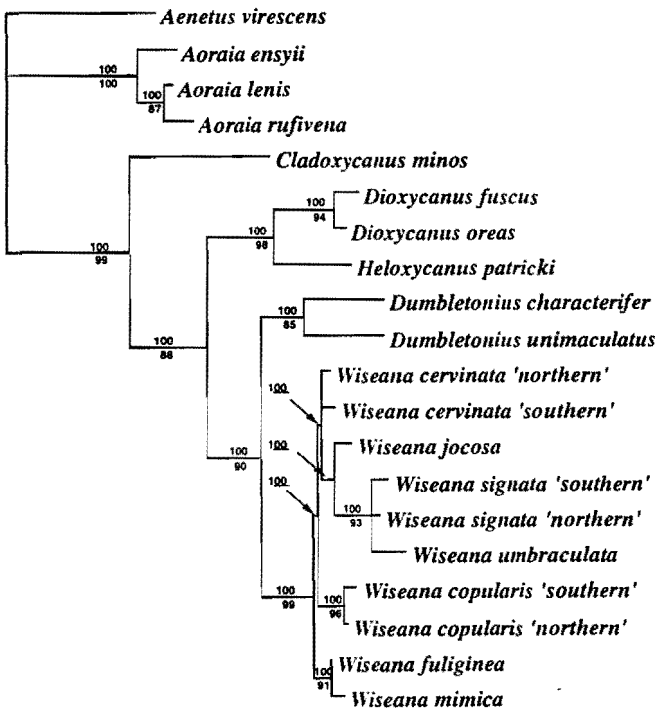


Figure 6: Majority rule consensus phylogram of the four most parsimonius trees from the analysis of the combined morphology and COI & II data sets for New Zealand hepialid moths. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule consensus values are shown above the branches and bootstrap proportions (>50%) below.

Wiseana - A heuristic search of the combined morphological, COI & II, ITS2 and allozyme data sets produced one MP tree, TL 221, CI 0.78, RI 0.78, G_1 -1.39. The majority rule consensus tree with bootstrap proportions and unambiguous synapomorphies is shown in Figure 7. *Wiseana cervinata* 'southern' and *W. cervinata* 'northern' were recovered in a clade together, as were *W. copularis* 'southern' and *W. copularis* 'northern' and *W. fuliginea* and *W. mimica*. *Wiseana signata* 'southern' and *W. signata* 'northern' occur in a basal clade with *W. umbraculata*. All nodes, apart from those joining the (*W. cervinata, W. jocosa, W. copularis*) clade were strongly supported by synapomorphies and bootstrap values.

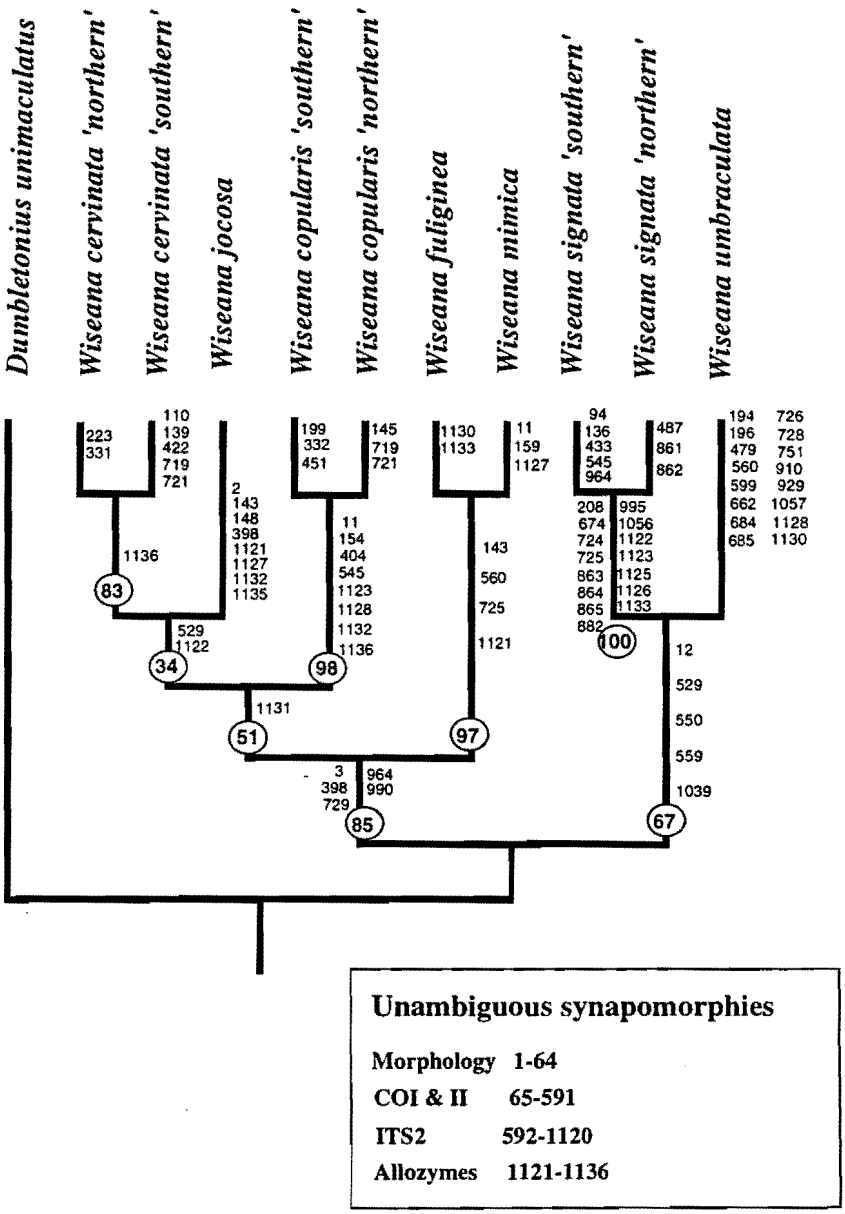


Figure 7: The most parsimonius tree from the analysis of the combined morphology, COI & II, ITS2 and allozyme data sets for the genus *Wiseana* (Lepidoptera: Hepialidae) from New Zealand. Unambiguous synapomorphies are indicated on the branches. Bootstrap proportions (>50%) are shown in circles on the branches.

Taxonomic congruence - The majority rule consensus tree produced from the strict consensus tree of the morphological, COI & II and ITS2 data sets is presented in Figure 8. *Aenetus* and *Aoraia* taxa are recovered as separate clades. Of the remaining taxa, only the (*Heloxycanus, Dioxycanus*), (*W. copularis* 'southern', *W. copularis* 'northern'), (*W. fuliginea, W. mimica*) and (*W. signata* 'southern', *W. signata* 'northern', *W. umbraculata*) clades were resolved. Spectral analysis showed there to be very high support for the (*Heloxycanus, Dioxycanus*) clade (support = 0.01), in comparison with, for example, a *W. cervinata* 'southern', *W. cervinata* 'northern', *W. copularis* 'southern', W. copularis 'northern' clade. There was high support for the (*W. copularis* 'southern', *W. copularis* 'northern') clade (support = 0.0047), the (*W. fuliginea, W. mimica*) clade (suppport = 0.005) and the (*W. signata* 'southern', *W. signata* 'northern', *W. umbraculata*) clade (support = 0.0039).

The majority rule consensus tree produced from the strict consensus tree of the morphological and COI & II data sets (not shown) was identical to that described for the morphological, COI & II and ITS2 data sets except *W. fuliginea* and *W. mimica* did not occur in a clade together.

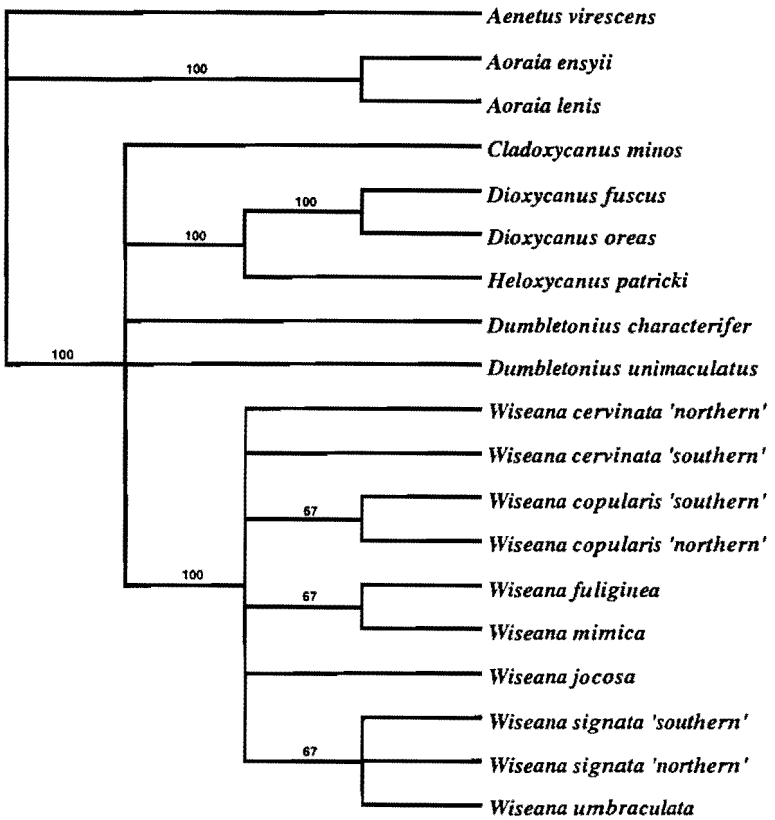


Figure 8: Majority rule consensus tree generated from the strict consensus trees of the morphology, COI & II and ITS2 data sets for New Zealand hepialid moths. Majority rule consensus values are shown above the lines.

Discussion

Current practice is to carry out separate analyses of each data set before combining data (Miyamoto & Fitch, 1995; Graybeal, 1997; Mitchell & Wagstaff, 1997). This enables the amount of phylogenetic signal in each data set to be assessed via the skewness statistic (G_1) (Hillis & Huelsenbeck, 1992). The G_1 statistics recovered for the morphological and COI & II data sets indicated good phylogenetic structure in the data, but the value for the ITS2 data (-2.16) is believed to be highly skewed, due to the large number of taxa with identical sequence (Swofford *et al.*, 1996).

Combined morphological, COI & II and ITS2 data sets - Combining the morphological, COI & II and ITS2 data sets produced a topology only part of which had been recovered previously (Figure 5). Separate branches for the *Aenetus* and *Aoraia* taxa had been recovered in all separate analyses. The (*Dumbletonius, Cladoxycanus, Heloxycanus, Dioxycanus*) clade was recovered in both this and the separate ITS2 analysis. All synapomorphies supporting this clade were from the ITS2 data set. Although there was strong bootstrap support for the arrangements within this clade, this does not imply statistical support (Sanderson, 1989) or accuracy (Hillis & Bull, 1993). Spectral analysis found little support for the (*Dumbletonius, Cladoxycanus, Heloxycanus, Dioxycanus*) clade (support = 0.0005). To have *Cladoxycanus* within this clade implies that the seven morphological synapomorphies identified for *Dumbletonius, Dioxycanus, Heloxycanus* and *Wiseana* (Characters 17, 22, 26, 27, 34, 63 and 64, in Chapter 2) were incorrectly assigned homology or have arisen more than once. After re-examination of these characters, we can find no reason to dismiss them as being homoplasious. For example, the hindwing veins Sc and R_1 are not fused apically in *Aenetus*, *Aoraia* or *Cladoxycanus*, but are fused apically in the remaining taxa (Character 17). The placement of *Cladoxycanus* in this analysis conflicts with both the morphological and COI & II data sets which support *Cladoxycanus* as a terminal taxon separate from, and basal to the remaining hepialid taxa. In the ITS2 (*'Oxycanus'* lineages only) maximum parsimony and maximum likelihood analyses, *Cladoxycanus* and *Dumbletonius characterifer* were recovered basally as a polytomy with the remaining taxa.

The arrangement within *Wiseana* had not been completely recovered previously. The (*W. fuliginea, W. mimica*) clade had been recovered in all separate analyses, but its basal placement within the genus had been seen only in the COI & II topology. The recovery of the *W. signata* haplotypes in a clade with *W. umbraculata* had been seen in the morphological and COI & II topologies, but in the separate ITS2 phylogeny, *W. umbraculata* joined a clade comprising all *Wiseana* taxa apart from the *Wiseana signata* haplotypes. Spectral analysis showed there to be no support for this clade (support = -0.0006) and no support for the (*W. cervinata, W. copularis*) clade (support = 0.0000). Spectral values may underestimate support for a particular grouping because information is considered from only a single gene (in this case the COI & II gene region) and data are converted into purines and pyrimidines with a corresponding loss of information.

Does the combined morphology, COI & II and ITS2 data set produce an accurate estimate of New Zealand hepialid phylogeny? Huelsenbeck *et al.* (1996) highlighted the situation where an inconsistent method will converge on an incorrect phylogeny as more data are added. Parsimony methods may become inconsistent if there are different rates of evolution in different data sets. The ITS2 region is usually employed to resolve relationships between very recently evolved species complexes because it is evolving rapidly (Wesson *et al.*, 1992; Severini *et al.*, 1996). If the ITS2 region is evolving at a higher rate than either the morphological characters or the COI & II region for hepialid taxa other than *Wiseana*, then adding it to the morphological and COI & II data sets may have caused the parsimony method to converge on a phylogeny that had not been recovered from any previous analyses - one which may be, in fact, incorrect.

Combined morphological and COI & II data set - A combined morphological and COI & II data set was also investigated because of our lack of confidence in the ITS2 data set with regards the placement of *Cladoxycanus* and the *Dumbletonius* taxa. The topology recovered (Figure 6), apart for that within *Wiseana*, was identical to that from the morphological data set. This result confirms the findings of others (e.g., Weller *et al.*, 1996; Miller *et al.*, 1997; Sperling *et al.*, 1997) that morphological characters contribute strongly to the structure of the phylogeny in combined data sets. The recovery of the two *Dumbletonius* taxa in a clade together was supported by five morphological and two COI & II characters.

We have two competing hypotheses for the placement of *Dumbletonius characterifer* and *D. unimaculatus*. *Dumbletonius characterifer* and *D. unimaculatus* are recovered separately in the COI & II phylogeny (Figure 2) and the ITS2 phylogeny (*'Oxycanus'* taxa only) (Figure 4) with *D. characterifer* recovered basally and *D. unimaculatus* recovered in a clade with the genus *Wiseana*. In the ITS2 analyses, *D. characterifer* and *Cladoxycanus* were recovered as an unresolved polytomy.

We re-examined the five morphological characters that support the placement of the *Dumbletonius* taxa in a clade together. Both taxa have a sclerotized apical segment on the maxillary palpi (Character 3), as do *Wiseana signata* and *W. umbraculata*. All other taxa had unsclerotized apical segments. Both taxa had an obscurely bilobed prelabium as did *Aoraia* taxa (Character 6). All other taxa had either a strongly bilobed or simple prelabium. Both *Dumbletonius* taxa had very large twin processes on the male genitalia (Character 27), to support the membrane associated with the anus. *Aenetus, Aoraia* and *Cladoxycanus* had no twin processes and in all other taxa the twin processes were either small or large. On tergum 7 (T7) of the female genitalic region, *Dumbletonius* and *Dioxycanus* taxa had cuticular processes present (Character 38). These were absent from all other taxa. The antrum floor of the female genitalia of *Dumbletonius* taxa was strongly sclerotized while other taxa had either membranous, thick and folded or sclerotized tissue (Character 48). We can identify no reason why these characters might be convergent as a result of the forest environment the *Dumbletonius* taxa share.

Alternatively, characters 3, 6, 27, 38 and 48 may be plesiomorphic. They may have been retained in *Dumbletonius* taxa, while changes in character state occurred along other branches. Mapping the characters on to a phylogeny derived from the COI & II data set, where *Dumbletonius characterifer* and *D. unimaculatus* are recovered separately, indicated that no independent gains were required and there was no increase in evolutionary events. This scenario seems more plausible than hypothesising five independent gains for each *Dumbletonius* taxon.

The autapomorphies each taxon exhibits may result from selection pressure for mate recognition. *Dumbletonius unimaculatus* has yellow/pink hindwing scales while *D. characterifer* has brown (Character 21). The male pseudotegumen shape (Character 28), saccus flange (Character 34), mid-posterior processes (Character 30) and ventral posterior processes (Character 31) all on the male genitalia may allow mating with only conspecific females. A study of male and female genitalia and mating behaviour of these taxa may shed light on the function of these characters.

Spectral analysis showed there to be little support for the (*Dumbletonius characterifer, D. unimaculatus*) clade (support = 0.0003) seen in the combined morphology and COI & II analyses and the separate morphological analyses. There was higher support for a (*Dumbletonius unimaculatus, Wiseana*) clade (support = 0.0032) as recovered in both the COI & II and ITS2 (*'Oxycanus'* lineages only) analyses. Not only did independent data sets recover congruent topologies, but for both data sets, maximum parsimony and maximum likelihood methods also recovered congruent topologies. Recovery of congruent topologies from independent data sets is regarded as strong evidence that the true phylogeny has been recovered (Penny & Hendy, 1986; Swofford, 1991). In addition, congruent phylogenies produced for a data set by different methods give confidence in the accuracy of the methods (Kim, 1993; Hillis, 1995; Miyamoto and Fitch, 1995). This result suggests that the genus *Dumbletonius* is not a monophyletic group.

Future work might include searching for additional haplotypes in the *D. characterifer* populations as this taxon has a North Island and South Island distribution. All specimens in this study came from Mangahuia, on North Island. Northern and southern haplotypes have been identified for *Wiseana cervinata*, *W. copularis* and *W. signata* (Chapter 3). The effect of adding additional *Dumbletonius* haplotypes would be to break up long branches and increase the accuracy of the phylogeny (Hillis, 1998; Graybeal, 1998). An alternative possibility would be to sequence longer sections of the COI & II gene regions to increase the potential number of synapomorphies available.

***Wiseana* - Combined morphological, COI & II, ITS2 and allozyme -**

The estimation of relationships within the genus *Wiseana* using all currently available character information combined into one data set produced the most resolved phylogeny ever recovered (Figure 7). In addition, only one maximum parsimony tree was found. This is in contrast to all other analyses, both separate and combined, where the recovery of multiple maximum parsimony and maximum likelihood trees was entirely due to the instability of *Wiseana* taxa. In all other analyses, the *W. cervinata* 'southern' and 'northern' relationship had been unresolved. They were recovered in a clade together in this analysis supported by an allozyme character from Herbert (1995). Spectral analysis indicated moderate support (support = 0.0012) for this clade. Morphological (Chapter 2), behavioural (Herbert, 1995) and life history (Dugdale, 1994) differences have been identified for these taxa and support the hypothesis that *W. cervinata* as currently described, is in fact, two species. The recovery of *W. jocosa* in a clade with the *W. cervinata* taxa had no bootstrap support or spectral support (support = 0.0000). They share no obvious biological characters, although Herbert (1995) found *W. cervinata* 'northern' and *W. jocosa* males to have more rounded forewing scales compared with *W. cervinata* 'southern'. In the morphological analyses *W. jocosa* was recovered in a clade with *W. fuliginea* and *W. mimica* (Chapter 2). In the COI & II and combined (morphology, COI & II and ITS2) analyses *W. jocosa* was recovered in a clade with *W. signata* and *W. umbraculata* taxa. Spectral analyses indicated little support for either of these hypotheses.

The *W. copularis* 'northern' and 'southern' taxa have been found in the same clade in all analyses. Life history differences are unknown, but morphological differences in male genitalia were noted (Dugdale, 1994; pers obs). The clade (*W. cervinata* 'northern', *W. cervinata* 'southern', *W. jocosa*, *W. copularis* 'northern', *W. copularis* 'southern') was supported by one synapomorphy from Herbert (1995) and a low bootstrap value. There was no spectral support for this clade (support = 0.0000). *Wiseana fuliginea* and *W. mimica* were recovered in a clade together in all analyses. Spectral support for this clade was moderate (support = 0.005). Herbert (1995) found *W. fuliginea* and *W. mimica* to be very similar biochemically. In the COI & II and ITS2 nucleotide sequences one and zero differences were found respectively. Corrected pairwise sequence divergences for the COI & II data indicated 0.19% difference between *W. fuliginea* and *W. mimica*, whereas *Wiseana signata*, *W. copularis* and *W. cervinata* haplotypes differed by 1.1%, 0.9% and 0.7% respectively, which suggests that the (*W. fuliginea, W. mimica*) divergence was relatively more recent.

There was moderate spectral support for the (*W. cervinata* 'northern', *W. cervinata* 'southern', *W. jocosa, W. copularis* 'northern', *W. copularis* 'southern', *W. fuliginea, W. mimica*) clade (support = 0.001) but the exact placement of the *W. jocosa* and *W. copularis* taxa is unknown. The *W. signata* 'southern' and 'northern' taxa have not been identified morphologically or biologically, but they occurred in a clade together in all analyses, except in a minority of COI & II trees where the *W. signata* 'southern' haplotype was recovered with *W. umbraculata*. There was moderate spectral support for the *W. signata* clade (support = 0.0019) and, as expected, higher support for the (*W. signata, W. umbraculata*) clade (support = 0.0039). In all separate analyses a (*W. signata, W.umbraculata*) clade was identified. *Wiseana signata* and *W. umbraculata* taxa are larger than other *Wiseana* taxa (Dugdale, 1994; Herbert, 1995) and all have pallid antennae.

The combined analysis for *Wiseana* provided more synapomorphies than had been available in all other analyses. Characters 729, 964 and 990 from the ITS2 data set were particularily useful in stabilising the (*W. cervinata* 'northern', *W. cervinata* 'southern', *W. jocosa, W. copularis* 'northern', *W. copularis* 'southern', *W. fuliginea, W. mimica*) clade.

Taxonomic congruence - It has been argued that not all possible information is revealed in separate analysis of data sets (Miller *et al.*, 1997; Sperling *et al.*, 1997) and that the smaller, separate data sets are subject to more sampling error compared with combined data sets (Huelsenbeck *et al.*, 1996). However, De Queiroz *et al.* (1995) argued that because the consensus topology shows areas of agreement from the trees of each separate analysis, it is likely that the areas of agreement represent real clades. As expected, the topology produced from the consensus of the strict consensus tree from each of the individual data sets was less resolved than either of the total evidence trees. However, this result supports *Aenetus* and *Aoraia* as separate clades and separate from the remainder of the New Zealand hepialid taxa. The monophyly of the *Heloxycanus* and *Dioxycanus* clade is strongly supported, as is that of the genus *Wiseana*.

To combine or not to combine - The total evidence approach of combining data sets generally produces a more robust and informative hypothesis of relationships (Vane-Wright *et al.*, 1992; Weller *et al.*, 1996; Miller *et al.*, 1997) in comparison with the taxonomic congruence approach. Certainly taxonomic congruence produced a less resolved estimate of relationships for the New Zealand hepialid fauna compared to the total evidence method. However, we question whether the more resolved total evidence phylogeny for the New Zealand hepialid fauna is necessarily more accurate, as the combined morphology, COI & II and ITS2 analysis produced a previously unseen topology. Two conflicting, but well supported phylogenies were produced for New Zealand hepialids. The morphology, and combined morphology and COI & II data sets supported *Dumbletonius* taxa in a clade together, while the COI & II, and ITS2 (*'Oxycanus'* lineages only) data sets supported a (*Dumbletonius characterifer, Dioxycanus, Heloxycanus, Dumbletonius unimaculatus, Wiseana*) topology.

Estimating phylogeny from multiple data sets is not just about which method gives the most resolved phylogeny. The behaviour of the method of analysis, e.g., parsimony, as more data are added and the effects of violations of the assumptions of the method, must also be considered. It seems that separate analyses are essential so that the effects of combining data sets can be assessed. We support what workers are doing in practice, which is to carry out separate analyses, assess incongruence before combining, give greater weight to accuracy rather than resolution and use congruence between data sets as evidence that the true phylogeny has been recovered.

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Appendix 1: Coded character-states of allozyme loci for *Wiseana* adults and larvae, with *Dioxycanus oreas* as outgroup (from Herbert, 1995; Table 7.7).

	10
Dioxycanus oreas	2005012016200004
Wiseana cervinata	2332112123113321
Wiseana copularis	2122112224133223
Wiseana fuliginea	1134112121212222
Wiseana jocosa	1332111123123112
Wiseana mimica	1134111122213222
Wiseana signata	2211222112241222
Wiseana umbraculata	2131112315253222

Chapter 6

Morphological character evolution in hepialid moths (Lepidoptera: Hepialidae) from New Zealand

B. Brown, R.M. Emberson and A.M. Paterson

Abstract

Mapping morphological characters on a molecular-based phylogeny enabled examination of character evolution and an historical perspective into evolutionary processes, both of which are important aspects of systematic research and comparative biology. In this study, 64 morphological characters from hepialid moths in New Zealand were mapped on a phylogenetic tree reconstructed from mitochondrial DNA COI & II sequence data. Morphological characters hypothesised to be synapomorphies for the New Zealand *'Oxycanus'* lineages and *'Oxycanus' s. str.* lineage alone were confirmed to be homologous when mapped on to the COI & II phylogeny. The direction of character state transformation was determined for five characters, with members of the *Aenetus* and *Aoraia* lineages exhibiting hypothesised ancestral states. Male genitalic characters were less homoplasious than other character partitions and covaried significantly with phylogeny.

Key words - Hepialidae, *'Oxycanus' Cladoxycanus* lineage, *'Oxycanus'* lineage *s. str.*, phylogeny, mtDNA, morphology, character mapping.

Status - Prepared for submission to the Biological Journal of the Linnean Society

Introduction

The goals of systematics include gaining an understanding of the historical hierarchy and evolutionary processes that underpin the diversity seen in natural systems (Cranston *et al.*, 1994; Savage, 1995). Achieving these goals is now possible because of the development and application of the cladistic methodology (Hennig, 1966) which has allowed the estimation of phylogeny from morphological, protein, molecular, ecological, behavioural and life history characters. Concerns that homoplasy might render some character types less useful has been unfounded, with morphological characters shown to be no more homoplasious than molecular ones (Sanderson & Donoghue, 1989) and behavioural and ecological characters no more homoplasious than morphological ones (De Queiroz & Wimberger, 1993; Miller & Wenzel, 1995).

Although the estimation of phylogeny is the main use of data derived from molecular data sets (Hillis *et al.*, 1996), many questions regarding the evolution of other types of characters can be answered. Morphological, ecological, behavioural and life history characters can be mapped on an independent molecular phylogeny to test whether they reflect phylogenetic relationship or have evolved multiple times. For example, does 'oxycanus' wing venation, as seen in some New Zealand hepialid moths, reflect phylogeny or has it evolved many times independently. The identification of a shared character among extant taxa implies that the common ancestor of those taxa also had that character.

Mapping allows the determination of character state transformations (Grandcolas *et al.*, 1994; Hart *et al.*, 1997), determination of the phylogenetic origins of processes such as pollination mutualism (Pellmyr *et al.*, 1996) and the coevolution between insects and their host plants (Brown *et al.*, 1994; Funk *et al.*, 1995; Menken, 1996) or between host organisms and their parasites (Paterson & Gray, 1997). Evolution of geographic position (Graybeal, 1997), life history and behaviour can also be traced using a phylogenetic framework (Langtimm & Dewsbury, 1991; Packer 1991; Paterson *et al.*, 1995).

Some types of character may be better predictors of relationships than others. For example, Miller (1991) found larval characters in the Notodontidae (Lepidoptera) to reflect relationships better than those from adults, but found the opposite to be true in the Josiini tribe (Lepidoptera: Notodontidae) (Miller, 1996).

Male genitalic characters are generally useful in describing taxa (Dugdale, 1994; Chapter 2), but are they good predictors of relationship for the New Zealand hepialid moths, or do they retain little phylogenetic information because of selection pressure to maintain mating compatibility with conspecific females? All adult Hepialidae have non-functional mouthparts (Nielsen & Kristensen, 1989). Are non-functional mouthparts free to vary and indicate recent relationships, in a similar way to third position codons in molecular data?

The aims of this study were to: (i) trace the evolution of morphological characters from New Zealand hepialid moths by mapping the characters on a phylogeny estimated from the mitochondrial DNA (mtDNA) cytochrome oxidase subunit I and II (COI & II) gene regions, (ii) determine if the morphological characters hypothesised to be synapomorphies for the New Zealand *'Oxycanus'* lineages are homologous, (iii) determine if there are groups of morphological characters that are better predictors of relationship than others and (iv) determine the character state polarity for morphological characters.

Materials and methods

There has been some debate as to whether characters whose evolution is under study should be included in the data set used to reconstruct their phylogeny. It has been argued that inclusion leads to an underestimation of the number of homoplasious character-state transformations (Coddington, 1988; Brooks & McLennan, 1991). It has also been argued that characters under study should not be included so that the problem of circularity is avoided (Brooks & McLennan, 1991; Vane-Wright *et al.*, 1992; Stearns, 1994). Deleport (1993) concluded that circularity was not a problem, as phylogeny construction is a separate process from phylogeny use, and that it is the independence of the evolutionary hypothesis being tested that is important, not the independence of the characters themselves. Zrzavy (1997) considered that characters under study should be included to strengthen the null hypothesis, i.e. that all similarities are homologous until proven otherwise. Common practice, however, is not to include the characters under study in the building of the phylogeny (e.g., Harrison & Bogdanowicz, 1995; Paterson *et al.*, 1995; Graybeal, 1997). In this study, no morphological characters were used to reconstruct the phylogenies used for character mapping.

The COI & II phylogeny was chosen to map the morphological characters on because it was derived from a data set independent of the morphological data set and represents our most resolved molecular estimate of New Zealand hepialid phylogeny. Details of specimen collection, DNA extraction and nucleotide sequencing of the COI & II data set have been described in Chapter 3. A heuristic search of the unordered and unweighted COI & II nucleotide sequence was undertaken using PAUP Version 3.1 (Swofford, 1993). Maximum likelihood (ML) trees were recovered using Phylip 3.4 (Felsenstein, 1991), under the Kimura two-parameter model (Kimaru, 1980), which compensates for rate heterogeneity across sites.

The evolution of characters in a 64-character morphological data set (Appendix 1) was traced on to the COI & II phylogeny using MacClade Version 3.1 (Maddison & Maddison, 1992). Full descriptions of the characters can be found in Chapter 2. The morphological data were partitioned into seven categories: adult male mouthparts, male antennae, male wings, male genitalia, female genitalia, larvae and pupae. How well the data fit the tree can be measured by either the consistency index (CI) (Kluge & Farris, 1969) or the retention index (RI) (Farris, 1989). The retention index was preferred because it is not influenced by the number of taxa or the number of autapomorphic characters, whereas the consistency index is. A retention index value of one indicates a lack of homoplasy and perfect fit to the tree. The Kruskal-Wallis test was used to determine if the retention indices of particular groupings of characters e.g. adult versus immature life stages or male versus female genitalia, were significantly different. Retention indices were calculated in PAUP 3.1.

Results

The heuristic search of the COI & II data set produced six maximum parsimony (MP) trees: tree length (TL) 182, consistency index (CI) 0.55, retention index (RI) 0.65 and G_1 statistic -0.79. The majority rule consensus tree (Figure 1) shows *Cladoxycanus* and *Dumbletonius characterifer* as a polytomy with the remainder of the *'Oxycanus' s. str.* taxa. *Cladoxycanus* was recovered as a separate branch, basal to *D. characterifer* and the remainder of the *'Oxycanus' s. str.* taxa, in two of the six maximum parsimony trees and all of the maximum likelihood trees. Morphological evidence (Chapter 2) also supported *Cladoxycanus* as the basal taxon for the *'Oxycanus'* lineages. It was concluded (Chapter 3), that the recovery of *Dumbletonius characterifer* in a clade with *Cladoxycanus minos* in two of the six MP trees was a case of long branch attraction (Hendy & Penny, 1989), because the taxa were recovered as separate branches under maximum likelihood. The *Wiseana signata* 'southern' haplotype occurred in a clade with *W. umbraculata* in three of the six MP trees. However, the two *Wiseana signata* haplotypes occurred in a clade together in the remaining three MP trees and in all the maximum likelihood trees. In all trees recovered from the maximum parsimony and maximum likelihood analyses of the ITS2 region (Chapter 4) the *W. signata* haplotypes were recovered in a clade together. Thus for character mapping, we have selected Tree Five from the COI & II analyses, with *Cladoxycanus* as a separate branch, *Dumbletonius characterifer* as the basal taxon of the *'Oxycanus'* lineage *s. str.* and the *W. signata* haplotypes in a clade together.

Inferences made when mapping characters on a phylogeny are only as accurate as the phylogenies on which they are based (Hillis *et al.*, 1996; Ryan, 1996). We place confidence in our estimate of phylogeny based on COI & II nucleotide sequences because of the congruence between the phylogeny recovered from the maximum parsimony and maximum likelihood analyses.

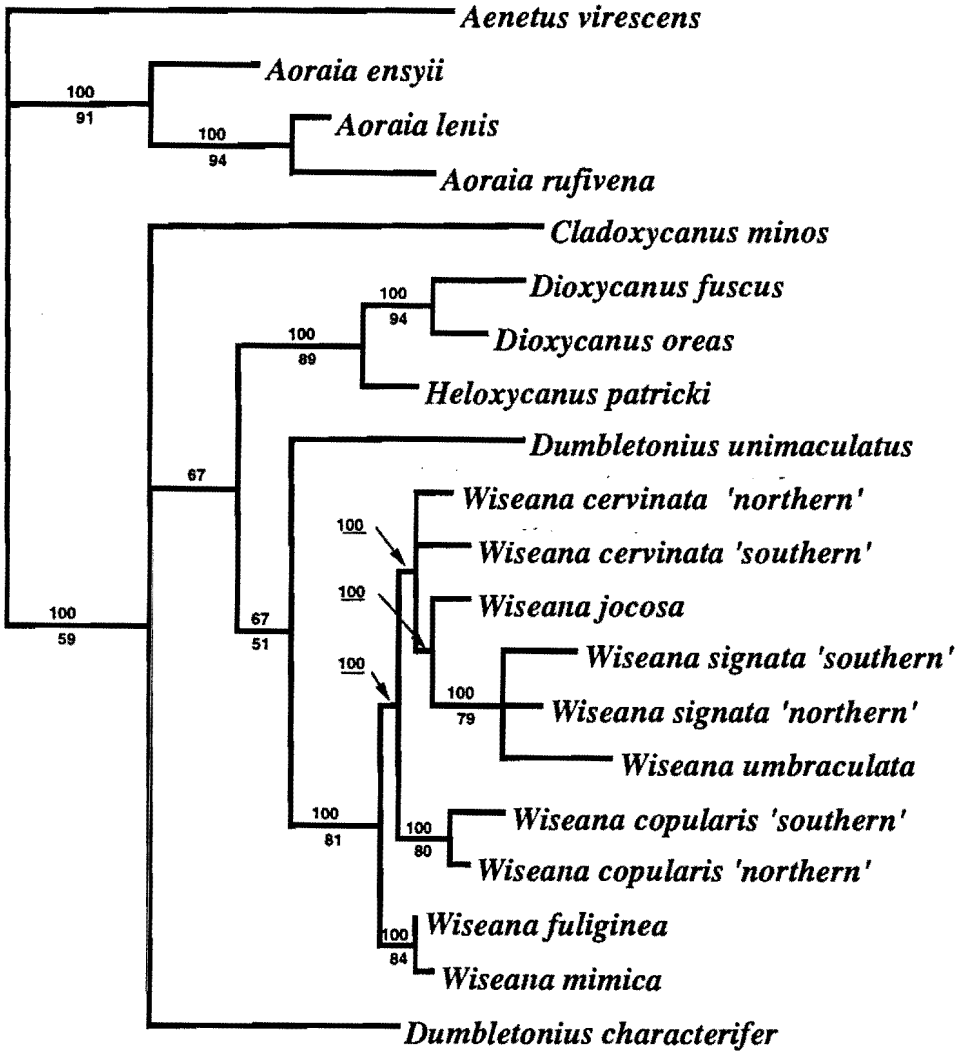

Figure 1: Majority rule consensus phylogram of the six most parsimonious trees from the analysis of mtDNA COI & II sequence data from New Zealand hepialids. Branch lengths are proportional to the number of unambiguous nucleotide changes. Majority rule values are shown above the branches and bootstrap proportions (>50%) below.

Morphological character partitions - The morphological characters in this study were taken from male and female specimens, different parts of the adult body and from different life stages. The Kruskal-Wallis test indicated that some character partitions had significantly less homoplasy when compared with another character partition, i.e., less homoplasy had to be inferred for characters to fit to the tree. If the characters fit the tree better, then this implies that they are better predictors of relationship. Adult characters were found to be better predictors of relationship compared with characters from immature stages ($K = 557$, $df = 1$, $p < 0.001$), genitalia were better predictors of relationship than other adult body characters ($K = 570$, $df = 1$, $p < 0.001$) and male genitalia were better predictors than female genitalia ($K = 109$, $df = 1$, $p < 0.001$) (Table 1).

	Adult male mouthparts	Male antennae	Male wings	Male genitalia	Female genitalia	Larvae	Pupae	Overall
Average RI	0.46	0.75	0.5	0.72	0.72	0.66	0.66	0.65
Characters*	1-9	11-15	16-21	24-37	38-50	51-56	57-64	

Table 1: Average homoplasy, as measured by the retention index (RI) (Farris, 1989), for morphological character partitions from New Zealand hepialid moths, mapped on to the COI & II phylogeny. (* Appendix 1).

Discussion

Confirmation of homology - A cladistic analysis of a morphological data set (Chapter 2) identified eight synapomorphies supporting taxa in the *'Oxycanus' Cladoxycanus* and seven supporting taxa in the *'Oxycanus' s. str.* lineage. The eight characters supporting the *'Oxycanus' Cladoxycanus* lineage were: (Character 8) labial palpi inserted directly into the prelabium, (Character 16) the forewing veins R_4 and R_5 arise separately from a combined R_{2+3} stem ('oxycanus' wing venation), (Character 36) trulleum sclerite in the genitalia of adult males broadly rectangular, strongly sclerotized and widened centrally or deeply concave, (Character 37) valvae on the male genitalia unarmed, (Character 50) apical tuft present on the T8 caudal margin in females, (Character 51) stemmata on the larval head capsule form two parallel arcs, (Character 55) prosternum on the larval abdominal segments small and separate from the V_1 pinaculum and (Character 56) larval metathoracic seta L_3 not on the SD_1 or SD_2 pinaculum. Mapping these characters on to the COI & II phylogeny indicated they had arisen only once within the New Zealand hepialid fauna and were indeed homologous (Figure 2A). The COI & II phylogeny for New Zealand taxa and Australian hepialid exemplars indicated that the Australian *Oxycanus* and *Jeana* taxa may be sister group to the New Zealand *'Oxycanus'* lineages. If this is the case, then direct labial palpi insertion, 'oxycanus' wing venation and unarmed valvae on the male genitalia may have arisen in the ancestor *Oxycanus* and *Jeana* and the New Zealand *'Oxycanus'* lineages.

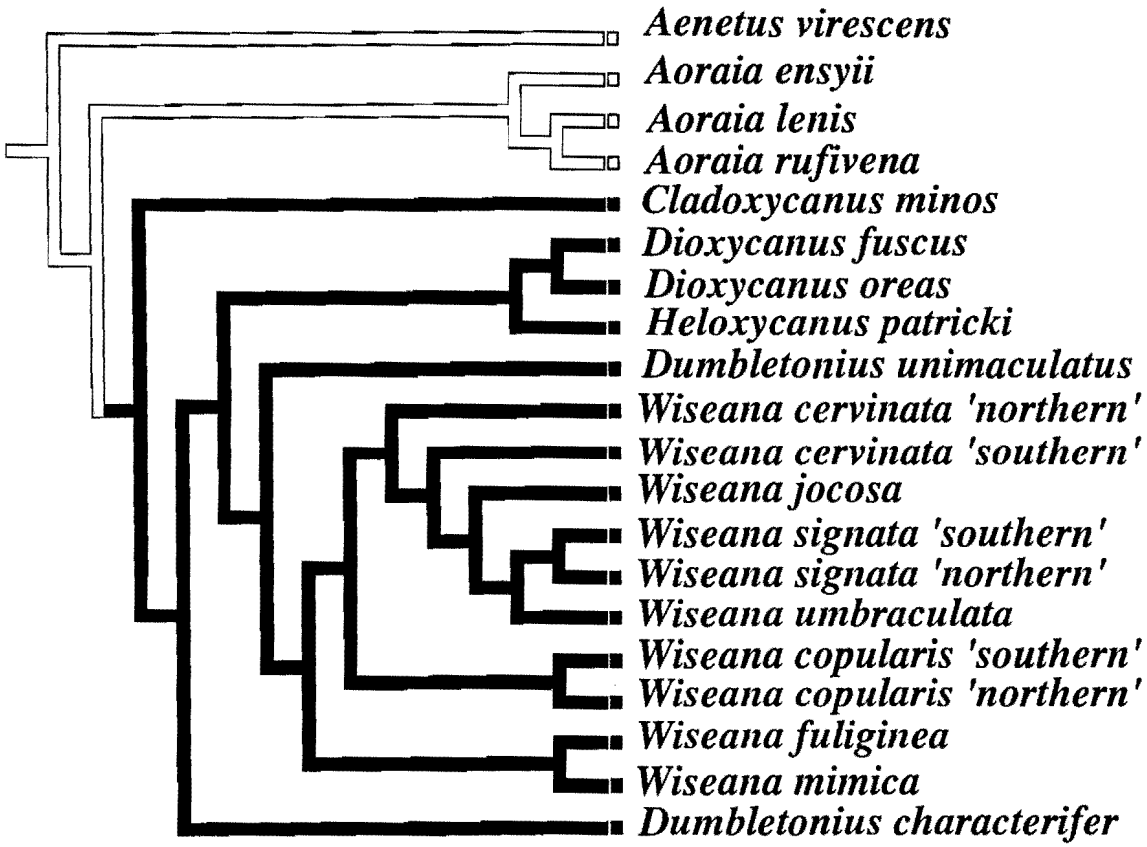

Figure 2: (A) Adult male wing venation for New Zealand hepialid moths (Character 16) mapped on to the COI & II phylogeny showing a unique origin for the 'oxycanus' wing venation pattern (shaded lines). An identical pattern was seen for characters 8, 36, 37, 50, 51, 55 and 56. (B) Adult male prelabium palpal insertion for New Zealand hepialid moths (Character 8) mapped on to the COI & II phylogeny showing hypothesised ancestral character states for *Aenetus* and *Aoraia* taxa (unshaded lines). A similar hypothesis was made for characters 46, 48, 50 and 56.

Seven characters hypothesised to be synapomorphies for taxa in the *'Oxycanus'* lineage *s. str.* were also found to be homologous (Figure 3). These characters were: (Character 17) hindwing veins Sc and R_1 fused apically, (Character 22) episternal tooth on the adult male prothorax slender and reaching the base of the laterocervicale, (Character 26) dorsal hood (*sensu* Dugdale, 1994) present on the male genitalia, (Character 27) twin processes (*sensu* Dugdale, 1994) present on the male genitalia, (Character 34) small flange present on the posterior margin of the vinculum/saccus complex of the male genitalia, (Character 63) seta D_2 absent from the pupal abdominal segment A1 and (Character 64) short carina present sublaterally on pupal abdominal segments A4-6 anteroventral of the spiracle. Twin processes and the flange on the vinculum/saccus complex are also found in the Australian *Oxycanus* and *Jeana* taxa examined in this study. These characters may have arisen twice independently, arise in the ancestor of *Oxycanus*, *Jeana* and New Zealand *'Oxycanus'* lineages and been lost from *Cladoxycanus,* or the structures may not be homologous.

Reduction in labial palpi segment number and lack of the vom Rath's organs on the final palpi segments occurs several times in the Exoporia (i.e., Superfamilies Mnesarchaeoidea and Hepialoidea) (Nielsen & Kristensen, 1989). *Dioxycanus* and *Heloxycanus* taxa have labial palpi with only two segments and lack vom Rath's organs (Characters 4 & 5). We consider that these losses reflect relationship. The presence of microsensillae on two lateral mounds situated proximally on the antennae of adult males (Character 14) also supports this hypothesis.

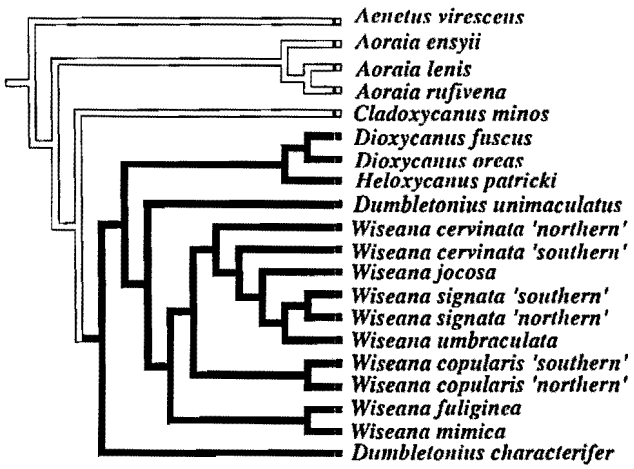

Figure 3: Adult male wing venation for New Zealand hepialid moths (Character 17) mapped on the COI & II phylogeny showing a unique origin for the apical fusion of the Sc and R_1 hindwing veins. Shaded lines indicate taxa with apical fusion of the Sc and R_1 hindwing veins. An identical pattern was seen for characters 22, 26, 27, 34, 63 and 64.

Identification of ancestral character states - Mapping morphological characters on the COI & II phylogeny allows the determination of ancestral character states (Miles & Dunham, 1993; Swofford *et al.*, 1996). *Aenetus* and *Aoraia* taxa exhibit what we hypothesise to be ancestral character states for five characters (Figure 2B). In both taxa the prelabium is raised, whereas in all other taxa the labial palpi are inserted directly into the labium (Character 8). In female *Aenetus* and *Aoraia*, the ovipore is simple and the antrum floor membranous, while in all other taxa the ovipore is bilobed or strongly bilobed and the antrum floor sclerotized (Characters 46 & 48). Female *Aenetus* and *Aoraia* have no apical tuft of long scales on T8, but the tuft is present in all other taxa (Character 50). On the larvae of *Aenetus* and *Aoraia*, the metathoracic seta L3 is on the SD_1 or SD_2 pinaculum and in other taxa is not on these pinacula (Character 56).

Independent evolutionary events - Mapping the morphological characters on the COI & II tree introduces an historical perspective that enables similarity to be separated into that which represents inheritance from a common ancestor or that due to repeated and independent evolutionary events (McLennan, 1994). The latter is hypothesised for the following characters. In character 29, small, irregular teeth on the pseudotegumen dorsal margin in both *Aoraia* taxa and *Dumbletonius characterifer* have arisen independently. Open intergenital lobes (Character 47) in the female genitalia is believed to be the pleisiomorphic state in the Hepialidae (Nielsen and Kristensen, 1989). The lobes were found to be open in *Aenetus* and *Aoraia*, both hypothesised to be basal taxa within the New Zealand Hepialidae (Chapter 2). The open lobes observed in the females of the genus *Dioxycanus* are believed to have occurred independently. Character 13 indicates that the presence of scales on the dorsal surface of the proximal antennal flagellomeres is the ancestral state and there have been two independent losses in *Dioxycanus* and *Wiseana* taxa (Figure 4).

Similarity in character state between taxa or changes in character state may be induced by physical factors rather than indicate relationship. For example, the higher than wide clypeus shape seen in *Aenetus* (Character 10) may be due to the lateral compression of the central anterior head features by the enormous compound eyes. The change to a strip-like clypeus as seen in *Cladoxycanus* and *Heloxycanus* may be due a reduction in the head capsule size in these taxa.

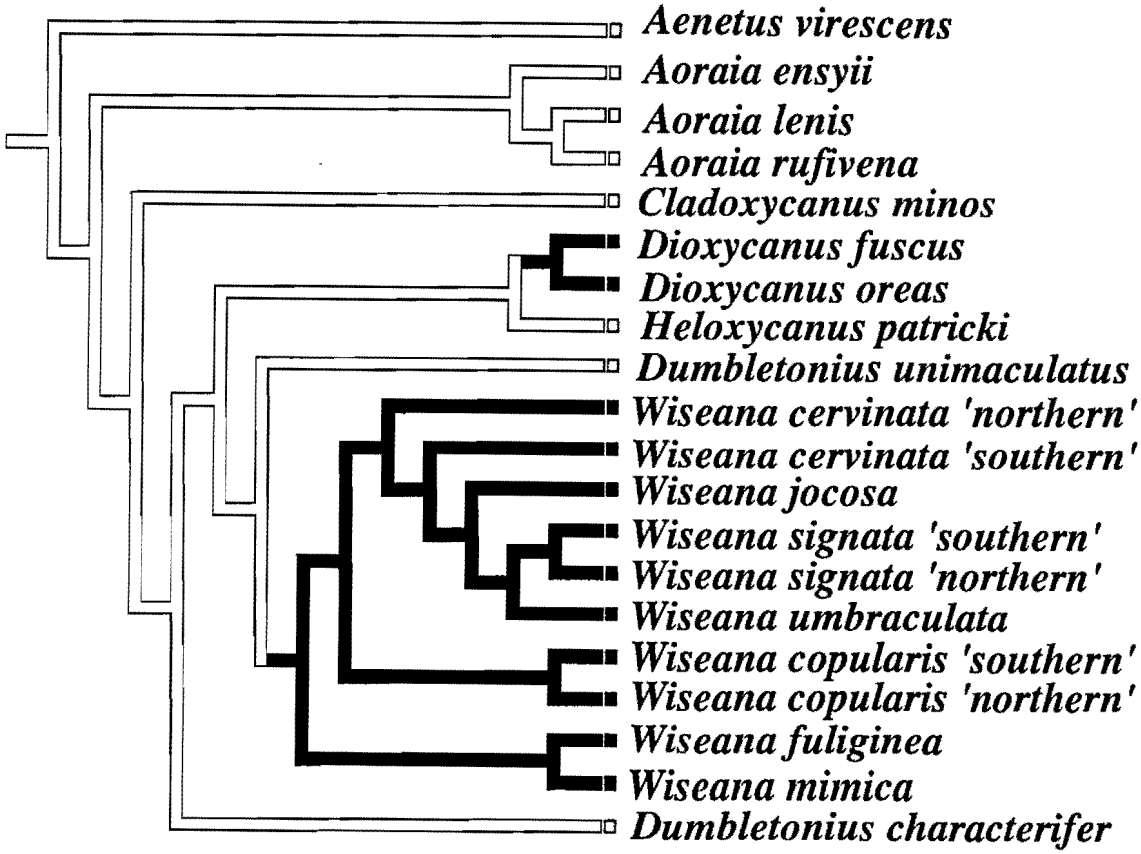

Figure 4: Hypothesised evolution of dorsal antennal scales for New Zealand hepialid moths (Character 13) mapped on the COI & II phylogeny. Shading indicates loss of scales on the dorsal surface of the proximal antennal flagellomeres.

Character state evolution - For some characters, several alternatives in character state evolution can be hypothesised and evaluated. For example, absence of the mid-posterior processes (*sensu* Dugdale, 1994) (Character 30) may have been the ancestral state as seen in *Aenetus* (Figure 5). There may have been four independent gains of these processes in *Aoraia*, *Dumbletonius characterifer, Dioxycanus* and *Wiseana* or alternatively, the processes may have been gained once in the ancestor of the group excluding *Aenetus*, and lost three times in *Cladoxycanus*, *Dumbletonius unimaculatus* and *Heloxycanus*. We consider the latter hypothesis with losses of highly sclerotized processes to be more likely than independent gains.

Character 33 is another example of a complex character. The ancestral state may have been absent in *Aenetus* (Figure 6). The flap over the phallus opening (supraphallic papilla) may have been gained twice, in *Aoraia* and *Dumbletonius characterifer* or alternatively it may have arisen once, in the common ancestor of *Aoraia* and the *'Oxycanus'* lineages, and been lost once in *Cladoxycanus*.

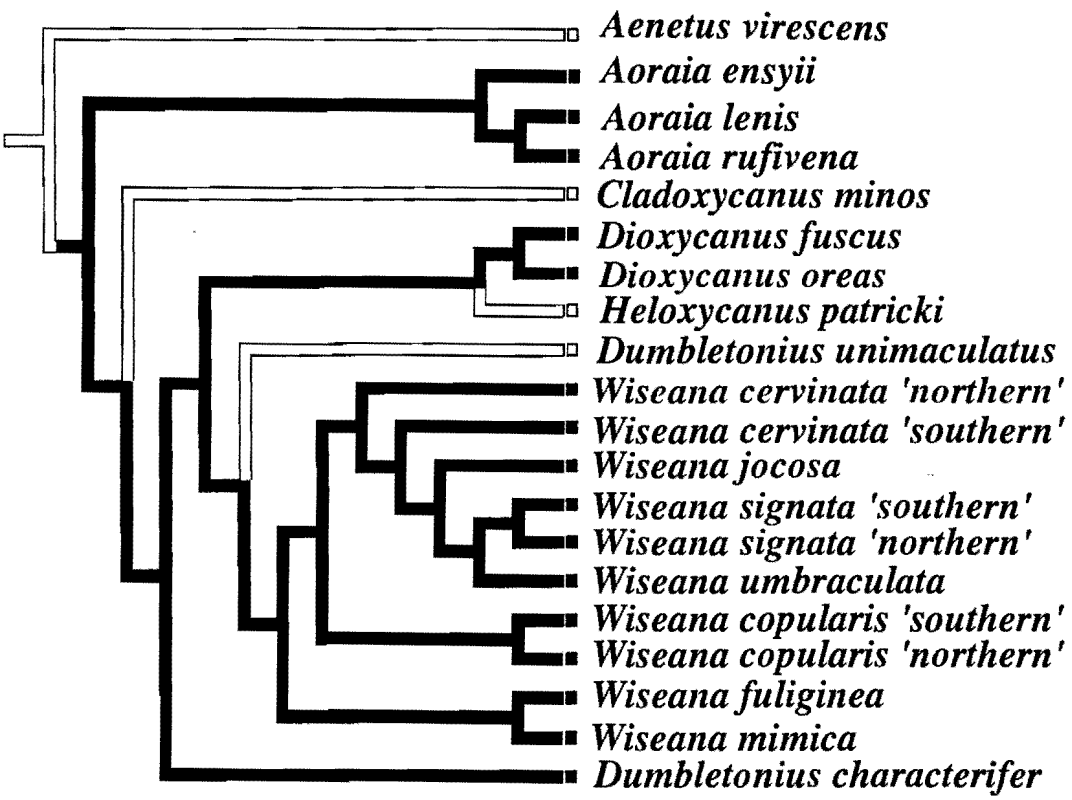

Figure 5: Hypothesised evolution of mid-posterior processes for New Zealand hepialid moths (Character 30) mapped on to the COI & II phylogeny. Shading indicates presence of mid-posterior processes.

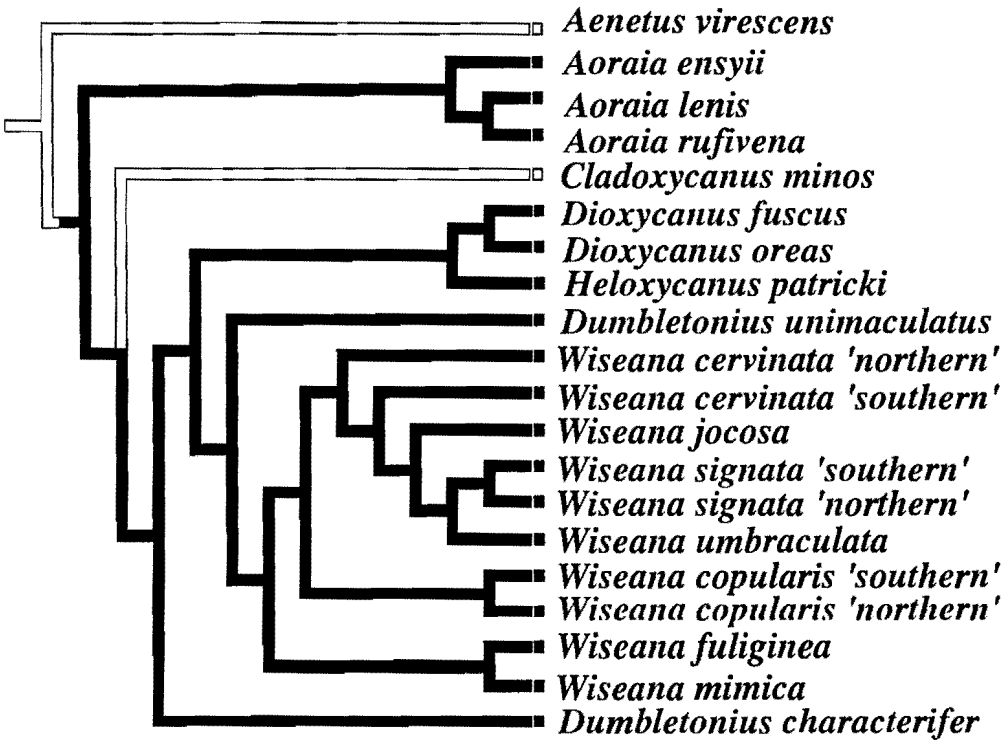

Figure 6: Hypothesised evolution of supraphallic papilla for New Zealand hepialid moths (Character 33) mapped on to the COI & II phylogeny. Shading indicates presence of the supraphallic papilla.

Morphological character partitions - Genitalic characters are useful for defining male specimens from the currently described genera and species (Dugdale, 1994). For example, males in the genus *Wiseana* have a bow-shaped and reduced pseudotegumen and *Wiseana cervinata* males have long twin processes while those in *W. fuliginea* are short. Male genitalic characters are also better for predicting of relationship, in comparison to female genitalia or other character partitions, as shown by the significantly lower levels of homoplasy for male genitalia when fitted to the COI & II tree.

Wiseana - Based on the COI & II sequence data, it was concluded that the genus *Wiseana* had evolved very recently and/or rapidly. The *Wiseana* taxa are very difficult to distinguish from external morphological characters and there are probably at least three cryptic species (Chapter 3). Fifty six of the 64 morphological characters examined here were found to be invariant within the genus. Harvey & Pagel (1991) proposed phylogenetic niche conservation as an explanation as to why phenotypically similar species are likely to be closely related. Ancestral *Wiseana* taxa probably existed in refugia during the last glacial period approximately 2.4 mya (Stevens *et al.*, 1988) and expanded into the new grassland niche created by the retreat of the glaciers approximately 850,000 ya. The high degree of phenotypic similarity in extant *Wiseana* taxa may be the result of the adaptive radiation that took place following the invasion of the new habitat.

We hypothesised that the non-functional mouthparts of hepialid taxa might be free to vary and so reflect recent relationships. However, we found all mouthpart characters for *Wiseana* taxa to be invariant apart from the mandibles (Character 2) which were autapomorphic for *W. jocosa* and the maxillary palpi (Character 3) which supported relationship between the *W. signata* taxa and *W. umbraculata*.

The antennal pectinations wider than the flagellomere shaft (Character 12) support relationship between *W. signata* taxa and *W. umbraculata*. In all other *Wiseana* taxa the pectinations were narrower than the flagellomere shaft (Figure 7).

Scale shape and measurements have been used previously in attempts to distinguish *Wiseana* taxa, but have been found to be highly variable within and among taxa (MacArthur, 1986; Herbert, 1995). We found forewing discal cell, white scale shape (Character 20) to be stable but autapomorphic for each of the seven recognised *Wiseana* taxa (Figure 8). The *W. cervinata* 'southern' taxa had narrow, truncate scales while the 'northern' taxa had broad, truncate scales (Dugdale, 1994; Chapter 4). No scale shape differences were observed in the *W. copularis* or *W. signata* taxa.

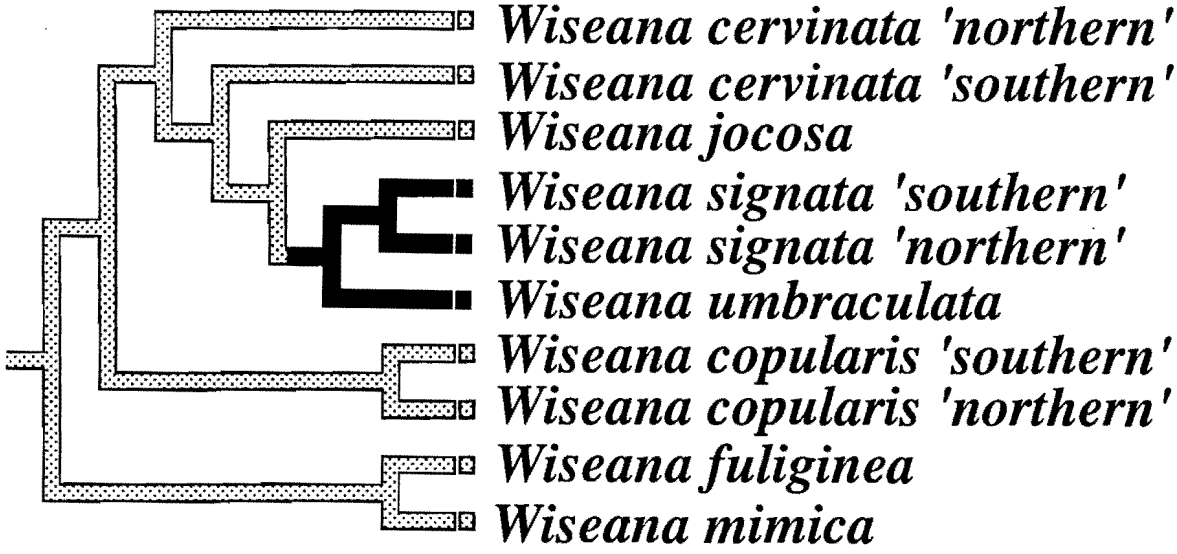

Figure 7: Shading indicates antennal pectinations wider than the flagellomere shaft in *Wiseana signata* taxa and *W. umbraculata* (Character 12).

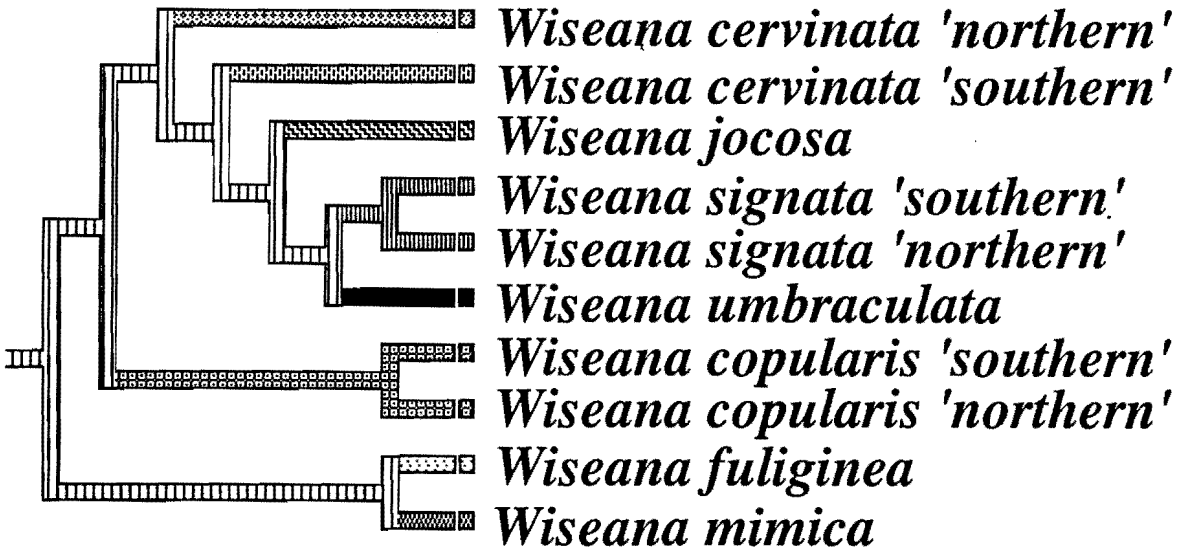

Figure 8: Male forewing discal cell white scale shape (Character 20) was autapomorphic within the genus *Wiseana* apart from the *W. copularis* and *W. signata* taxa. Autapomorphic character states are indicated by shading patterns.

Conclusions

An estimation of phylogeny provides a framework to test hypotheses regarding the evolution of morphological characters (Langtimm & Dewsbury, 1991; Harrison & Bogdanowicz, 1993). Mapping morphological characters derived from New Zealand's hepialid moths on a mtDNA COI and II phylogeny confirmed that characters hypothesised to be synapomorphies for the New Zealand *'Oxycanus'* lineages and the *'Oxycanus' s. str.* lineage in the cladistic analysis of a morphological data set (Chapter 2) covaried with phylogeny and were homologous. Further work is needed to assess whether characters such as 'oxycanus' wing venation have arisen independently in New Zealand and Australian hepialid taxa or indicate a relationship. Mapping morphological characters on an independently derived phylogeny allowed those that reflect phylogenetic pattern to be distinguished from those that were convergent. For example lack of scales on the dorsal surface of antennae did not indicate relationship between *Dioxycanus* and *Wiseana* taxa, but instead represented two independent losses. *Wiseana* taxa showed little morphological variation, but forewing discal cell white scale shape was useful in species identification.

Acknowledgements

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Appendix 1:

Character Descriptions and Character States for New Zealand hepialids.

Adult male - head

(1) Labrum/clypeus definition: (0) labrum not differentiated, (1) distinct labral sclerite present, mound-like and raised (2) distinct labral sclerite present, weakly raised.

(2) Mandibles: (0) Absent, (1) reduced to a plate on the epicranial wall, (2) present.

(3) Maxillary palpi: (0) sclerotized, (1) not sclerotized.

(4) Labial palpi segmentation: (0) 3 segmented, (1) 2 segmented.

(5) vom Rath's organ (0) present, (1) absent.

(6) Prelabium shape: (0) obscurely bilobed, (1) strongly bilobed, (2) simple.

(7) Prelabium palpal spacing: (0) contiguous, (1) separated.

(8) Prelabium palpal insertion: (0) raised, (1) not raised.

(9) Labial palpi basal segment: (0) rami absent, (1) rami present.

(10) Clypeus shape: (0) higher than wide, (1) quadrate, (2) strip-like.

(11) Antennal pectination: (0) pectinations absent, (1) tripectinate, pectinations positioned apically on flagellomere shaft, ovate apically, (2) tripectinate, pectinations positioned apically on flagellomere shaft, broad rounded apices, (3) bipectinate, posterior pectinations, ovate apically, (4) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, triangular in shape, rounded apices, (5) bipectinate, sub-pectinations apically positioned on flagellomere shaft, laterally flattened, broad, oval apices, (6) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, rectangular in shape, broad, truncate apices, (7) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, longer than deep triangles, narrow, rounded apices, (8) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, triangular, narrow rounded apices, (9) bipectinate, pectinations apically positioned on flagellomere shaft, laterally flattened, triangular, rounded apices, pallid.

(12) Antennal pectination width: (0) pectinations absent, (1) pectinations not as wide as flagellomere shaft, (2) pectinations as wide as flagellomere shaft, (3) pectinations wider than flagellomere shaft.

(13) Dorsum of 3-4 proximal antennal flagellomeres, (0) with linear, truncate scales, (1) linear, truncate scales absent.

(14) Antennae, microsensilla: (0) absent, (1) uniform, (2) proximal, on one central mound, (3) proximal, on two lateral mounds.

(15) Sensilla chaetica on dorsal surface of flagellar segments: (0) absent, (1) present centrally.

Adult Male - wings

(16) Wing venation: (0) Forewing veins R_4 and R_5 arise from common stem which splits off from R_{2+3} ,(1) forewing veins R_4 and R_5 arise separately from a combined R_{2+3} stem.

(17) Hindwing Veins Sc & R_1: (0) not fused apically, (1) fused apically.

(18) Hindwing vein curvature Sc & R_1: (0) Veins straight, (1) veins strongly curved.

(19) Forewing longitudinal scale pattern: (0) absent, (1) present.

(20) Discal cell white scale shape: (0) obovate, (1) long, linear, truncate apically, (2) long, tapering evenly to a truncate apex, (3) short and broadly ovate, (4) short, pear-shaped, evenly rounded apex, (5) long, linear, broad and truncate apically, (6) very long, narrow and tapering, narrow rounded apex, (7) tapering narrow-broad truncate, (8) oval in mid-section, narrow rounded apex, (9) long and narrow with long, sharp apex, (A) very short and blunt, (B) oval in mid-section, tapers abruptly to an acute apex, (C) oval in mid-section, tapering to a rounded apex, (D) oval in mid-section, apiculate.

(21) Hindwing colour: (0) green, (1) brown, (2) fawn/buff, (3) yellow/orange - red/pink.

Adult male - prothorax

(22) Episternal tooth: (0) triangular, not reaching the distal margin of laterocervicale, (1) slender, not reaching the distal margin of laterocervicale, (2) slender, reaching distal margin of laterocervicale, (3) strap-like, and reaching distal margin of laterocervicale.

Adult male - legs

(23) Arolium: (0) absent, (1) present.

Adult male - genitalia

(24) Sternum 8 shape: (0) apically smooth, (1) subapical teeth, (2) armed, broadly emarginate.

(25) T9: (0) strongly sclerotized region, (1) present as a faint sclerite, (2) absent.

(26) Extension of pseudotegumen into a dorsal hood: (0) absent, (1) present, below height of twin processes, (2) present, reaching above height of twin processes.

(27) Twin processes: (0) absent, (1) present, small (0.07-0.15 mm), (2) present, large (0.33-0.44 mm), (3) present, very large (0.74 mm).

(28) Pseudotegumen dorsal margin shape: (0) straight, parallel sided, (1) straight, decreasing separation dorsal to ventral, (2) splayed dorsally, parallel ventrally, (3) parallel dorsally, decreasing separation ventrally, (4) curved, not straight.

(29) Pseudotegumen dorsal margin: (0) knife-edged, bare, smooth flange, (1) knife-edged, small irregular teeth on flange, (2) knife-edged, bare, smooth, flange, lateral sharp teeth-like processes, (3) rounded, thickened margin, no flange.

(30) Mid posterior process shape: (0) absent, (1) present, apically acute, (2) present, apically truncate.

(31) Ventral processes: (0) absent, (1) present, moderately thick, short, apically acute, (2) present, moderately thick, short, apically acute, anterior margin extended to fuse with trulleum, (3) long, slender, subspinose-acuminate apically, (4) moderately thick, short, apically truncate.

(32) Lateral processes on pseudotegumen: (0) absent, (1) present, weak, (2) present, strong.

(33) Supraphallic papilla: (0) absent, (1) present, reduced, (2) present, short, thumb-like, (3) present, long, finger-like.

(34) Extension of posterior margin of saccus: (0) absent, (1) present, small flange, (2) present, moderately large flange, heavy sclerotization, concavity.

(35) Extension of lateral and distal margins of saccus: (0) absent, (1) present.

(36) Trulleum shape and sclerotization: (0) no strongly defined region, (1) broadly v-shaped, lightly sclerotized, mesal prominence, (2) broadly rectangular, strongly sclerotized, centrally widened or deeply concave.

(37) Valvae: (0) acuminate process absent, (1) acuminate process present.

Adult female - genitalia

(38) T7 cuticular processes: (0) absent, (1) present.

(39) T8 cuticular processes: (0) absent, (1) present.

(40) S7 & 8: (0) fused, (1) widely separated, (2) narrowly separated.

(41) Position of spiracle 8 on pleural area: (0) below anterolateral corner, (1) anteroventral to anterolateral corner, (2) anterior to extended posterolateral corner.

(42) S9: (0) reduced, (1) triangular, (2) large, long, broad.

(43) S9 side and median pieces: (0) junctions not discernible, (1) side and median pieces separated by clefts, (2) side and median pieces separated by weakly sclerotized zone.

(44) Sclerotization of dorsal plate midline: (0) absent, (1) weak sclerotization, (2) strong sclerotization.

(45) Paranal groups of setae on diaphrama: (0) absent, (1) present.

(46) Position of ovipore: (0) ovipore simple, (1) ovipore on a weakly bilobed, erect papilla, (2) ovipore on a strongly bilobed, erect papilla.

(47) Intergenital lobes: (0) open, (1) firmly apposed, (2) fused for all or part of length.

(48) Antrum floor: (0) membranous, (1) sclerotized, (2) thick and folded, (3) strongly sclerotized.

(49) Ductus bursae: (0) spines present, (1) spines absent.

(50) T8 apical tuft: (0) absent, (1) present.

Larvae

(51) Arrangement of stemmata on head capsule: (0) in two parallel rows, (1) in one straight and one curved row, (2) in two parallel arcs.

(52) Head capsule setae SO_3, G_2, G_1: (0) in a strong curve, (1) in a straight line.

(53) Mesothorax prosternum: (0) absent, (1) present.

(54) Metathorax prosternum: (0) absent, (1) present.

(55) Abdominal segments A3-6 prosternum: (0) fused with V_1 pinaculum, (1) small, separate, (2) large, separate.

(56) Metathoracic seta L_3: (0) on rhomboidal SD_1, SD_2 pinaculum, (1) not on rhomboidal SD_1, SD_2 pinaculum.

Pupae

(57) Antennal pedicel and scape: (0) antennae smooth, (1) broad, stout, outwardly decurved thorn-like process dorsally, (2) crenulate carina.

(58) Vertex: (0) sunken with mesal furrow, (1) produced into a cone, (2) produced into 2 divergent, strongly sclerotized cones.

(59) Frons: (0) plane, (1) convex, (2) convex with conical process, (3) convex with decurved bifurcate process.

(60) Gena: (0) planoconvex, (1) on a prominent mound.

(61) Length of labial and maxillary plates: (0) of similar length, (1) labial plate extends further than maxillary plate.

(62) Mesonotum: (0) low prominence absent, (1) low prominence present.

(63) Chaetotaxy of abdominal segment A1: (0) seta D_1 & D_2 present, (1) seta D_1 present.

(64) Carina sublaterally on A4-6, anterolateral of the spiracle: (0) absent, (1) present.

Chapter 7

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

B. Brown, R.M. Emberson and A.M. Paterson

Abstract

We describe a method for identifying the members of the endemic genus *Wiseana* Viette from New Zealand. Seven species have been described in the genus: *W. cervinata, W. copularis, W. fuliginea, W. jocosa, W. mimica, W. signata* and *W. umbraculata*. No morphological characters have yet been identified to distinguish between the larvae of different 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 genitalic characters. Molecular markers were generated via amplification of the cytochrome oxidase subunit I and II (COI & 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*.

Key words - hepialid, *Wiseana*, mtDNA COI & II, restriction fragment length polymorphism, diagnostic.

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Introduction

Wiseana Viette is one of five endemic genera in the *'Oxycanus'* lineage of hepialid moths (Dugdale, 1994) from New Zealand. Using a morphological species concept, Dugdale (1994) recognised 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) (Dugdale, 1994).

The larvae of *Wiseana* are commonly referred to as 'porina' and they 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 the South Island. Dugdale (1994) also lists *W. mimica* as a damaging species.

Wiseana taxa are difficult to distinguish using adult morphological characters, although males of all species can be identified by a combination of antennal, scale and genitalic characters (Dugdale, 1994; Chapter 2). Females of all species have been found to have continuous, overlapping external morphological characteristics (MacArthur, 1986; Herbert, 1995) and are reliably identified only using characters from the bursa copulatrix (Dugdale, 1994). External characters to distinguish between *W. cervinata* and *W. copularis* females are being tested (E.G. White, personal communication). Dugdale (1994) reported no authenicated field-collected larval specimens for *W. fuliginea, W. jocosa* or *W. mimica* and no morphological characteristics to distinguish between the larvae of *W. cervinata* and *W. copularis*. Herbert (1995) found the larvae of each species, reared in the laboratory from positively identified adults, to be morphologically very similar. Progress, however, is being made in the search for larval morphological characters to distinguish between *Wiseana* taxa (John 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 measures has previously treated 'porina' pests as a one-species problem, principally dealing with the *Wiseana 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 *et al.*, 1969; French, 1973; Barlow *et al.*, 1986).

Ten *Wiseana* haplotypes were identified from mitochondrial DNA COI & II sequences (Chapter 3). Haplotypes corresponding to the seven currently recognised species were recovered, plus an additional haplotype for each of *Wiseana cervinata, W. copularis* and *W. signata.* Phylogenetic relationships for the genus *Wiseana* were inferred from the mitochondrial DNA sequences. Lack of synapomorphies deeper in the clade, a predominance of transitional nucleotide substitutions and the presence of many autapomorphic characters for *Wiseana* haplotypes, indicated 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 recognised *Wiseana* species using 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 also less sensitive to degradation 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 and Powers, 1996; Armstrong *et al.*, 1997). The technique is also relatively inexpensive compared with 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. PCR and RFLPs of mtDNA have produced diagnostic tests for *Aedes* mosquitoes (Kambhampati & Rai, 1991), *Anopheles* mosquitoes (Mitchell *et al.*, 1992), gypsy moths *Lymantria dispar* (Bogdanowicz *et al.*, 1993), root knot nematodes *Meloidogyne* spp. (Hugall *et al.*, 1994), ermine moths of the *Yponomeuta padella* complex (Sperling *et al.*, 1995) and tobacco budworm *Heliothis virescens* and corn earworm *Helicoverpa zea* (Roehrdanz, 1997).

In this paper we report a method for distinguishing between *Wiseana* taxa using PCR RFLP on the mitochondrial DNA cytochrome oxidase I and II (COI & II) regions.

Material and Methods

Collections - *Wiseana* adults were collected by light trapping, placed directly into 96% ethanol and stored at 4°C before DNA extraction. Each *Wiseana* taxon has a unique distribution 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 *Wiseana cervinata*. Consequently, where possible for each species, several populations were sampled. *Wiseana umbraculata* specimens were collected from eight populations. *Wiseana cervinata* 'southern' and *W. cervinata* 'northern' specimens were collected from four populations each. Eight populations of *W. copularis* 'southern' and five of *W. copularis* 'northern' were sampled. *Wiseana fuliginea* and *W. mimica* were sampled from two populations each and *W. jocosa* from six populations. *Wiseana signata* 'southern', and 'northern' were sampled from two and four populations respectively. The locations of collecting sites are shown in Figure 1 and site details and numbers of individuals screened are shown in Appendix 1.

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

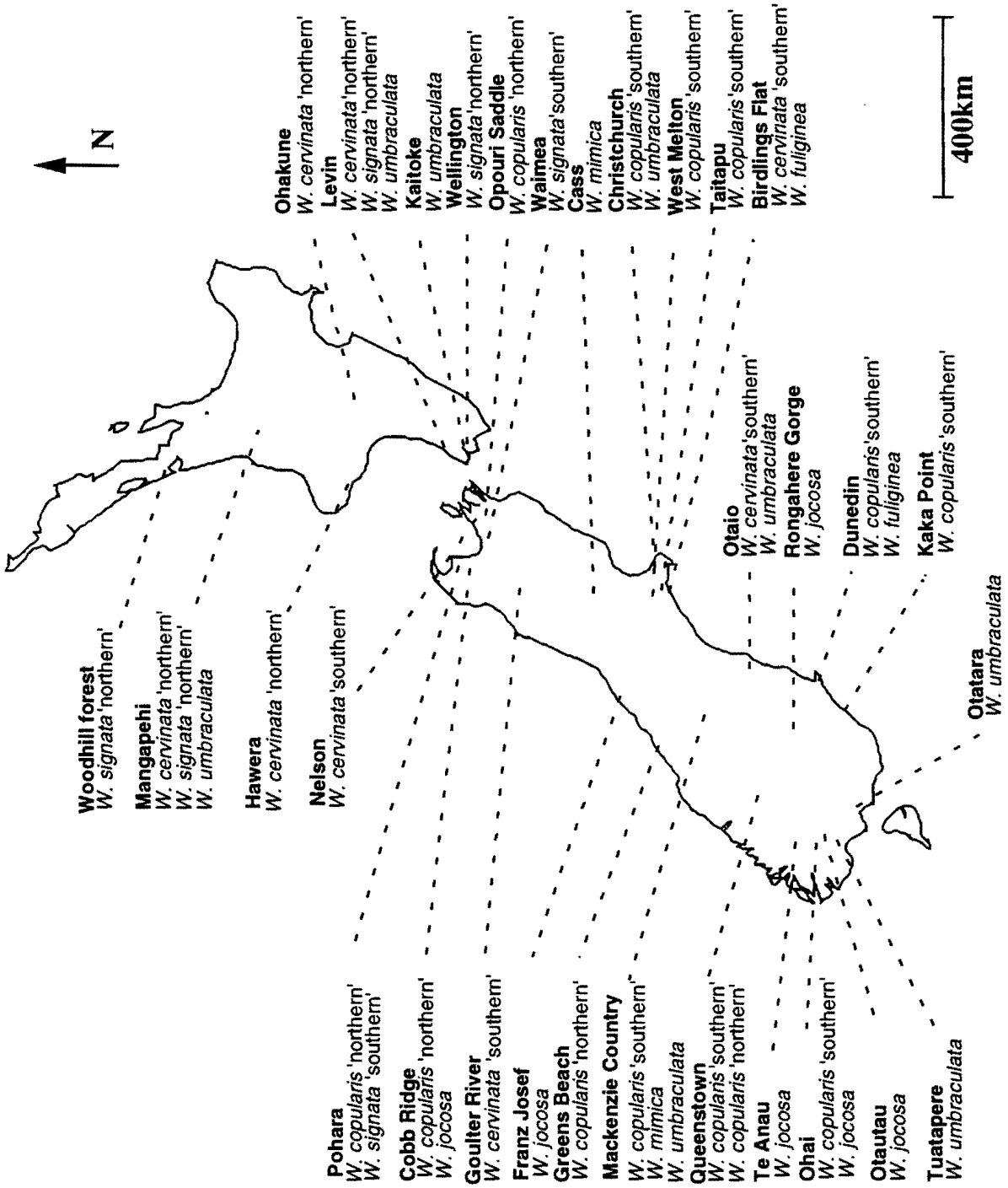

Figure 1: Map of New Zealand showing the location of collection sites and *Wiseana* taxa sampled at each, for the RFLP study.

Preliminary identification of restriction sites - A 527 base pair (bp) region of the COI & II region had been sequenced previously for phylogenetic analysis (Chapter 3). Restriction sites in this region were searched for using the range of enzymes listed in DNAMAN (Lynnon Biosoft, 1994-95), before the amplification of the entire COI & II region.

DNA extraction, PCR and restriction digests - Muscular tissue from the thorax of specimens was homogenised and total DNA extracted using a proteinase-K digestion and high salt precipitation (White *et al.*, 1990). The COI & II gene regions were amplified via the polymerase chain reaction (PCR) (Saiki *et al.*, 1988) using the primers mtDNA 4 (5´TACAATTTATCGCCTAAACTTCAGCC) (Sperling and Hickey, 1994) and mtDNA 18 (5´CCACAAATTTCTGAACATTGACCA) (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 10x *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 *Taq* DNA polymerase (Boehringer Mannheim) and 0.9 µl of 20 ng/µl DNA. A Perkin- Elmer 2400 thermal cycler was used with a cycling profile of 94°C for 2 minutes 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 minute 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 one hour, with a 100 bp ladder (Gibco BRL), before being visualised under UV.

Subsequently, the PCR product from the entire COI & II region was digested in a 10 µl reaction with restriction enzymes in a water bath for 2 hours at temperatures according to the manufacturer's (Boehringer Mannheim) recommendations. Reactions with 10 u/µl enzymes comprised: 1 µl of 10x manufacturer's buffer, 0.35 µl of 10 u/µl enzyme, 3 µl PCR product and 5.65 µl deionized water. Reactions with enzymes at concentrations other than 10 u/µl comprised: 1 µl of 10x buffer, 3.5 units of enzyme, 0.1 µl 100x 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 hours at 135 v, with a 100 bp ladder (Gibco BRL) and visualised under UV.

To improve resolution of the fragments, the AsnI and TaqI products were run on 3% metaphor gels at 100 v for 5 hours and 4 hours 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. There were no restriction sites for Hind III. The enzymes PstI, HinfI, BglII, and Tru91 cleaved the product but no polymorphisms were evident. SauIIIa, AluI, DraI, RsaI and MaeIII cleaved the product and polymorphisms were evident in a small number of taxa. Four enzymes were useful in distinguishing *Wiseana* taxa (Appendix 2), including the additional haplotypes of *W. cervinata*, *W. copularis* and *W. signata* previously identified from mtDNA sequence data. The enzymes, AsnI, TaqI, HindII and HaeIII cleaved the product and exhibited polymorphisms that were consistent for individuals within and between populations of *Wiseana* taxa. The enzyme AsnI produced a diagnostic pattern for all *Wiseana* taxa apart from *W. fuliginea* and *W. mimica* which had an identical pattern, as did *W. signata* 'southern' and *W. signata* 'northern' (fig. 2). The enzyme TaqI produced 4 patterns (fig. 3). *Wiseana cervinata* 'southern' and *W. copularis* 'southern' exhibited unique patterns. A further pattern was shared by *W. signata* 'southern' and 'northern' and *W. umbraculata* and the final pattern was shared by the remaining taxa. Hind II produced unique patterns for *W. jocosa* and *W. copularis* 'northern', while the enzyme did not cleave the remaining taxa (fig. 4). Hae III cleaved *Wiseana signata* 'northern' only (fig. 5).

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

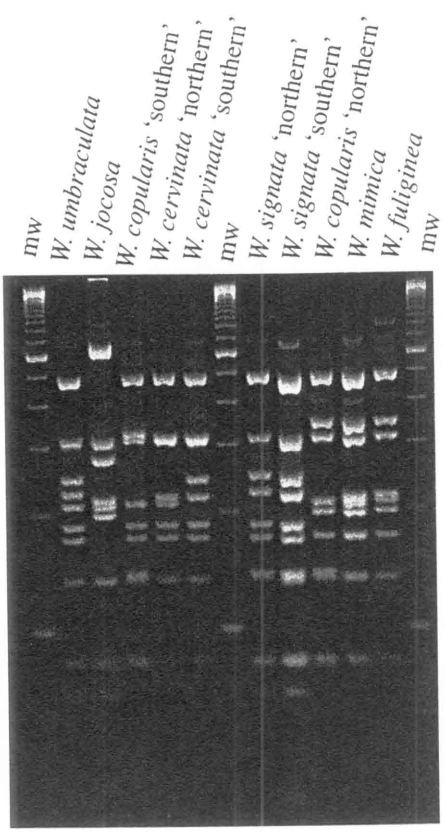

Figure 2: AsnI restriction patterns for *Wiseana* taxa, from 2200 bp amplified mtDNA COI and II.

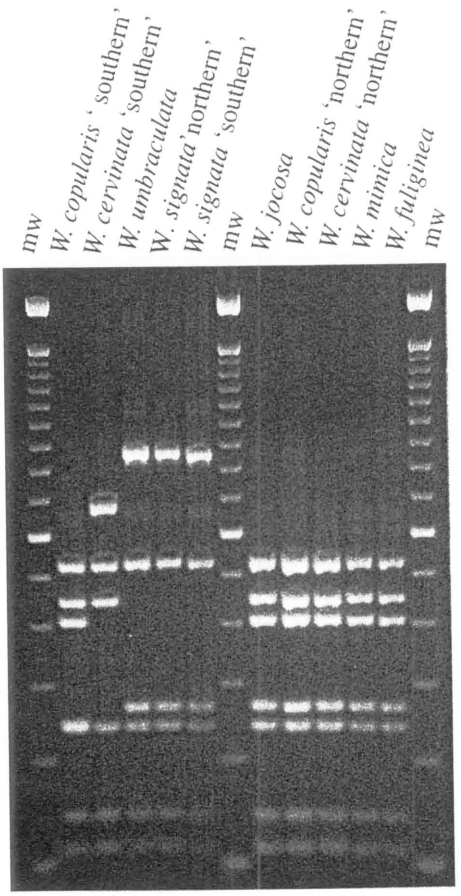

Figure 3: TaqI restriction patterns for *Wiseana* taxa, from 2200 bp amplified mtDNA COI and II.

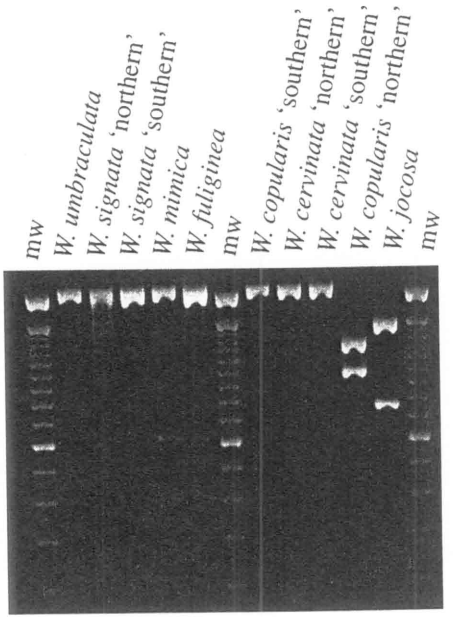

Figure 4: HindII restriction patterns for *Wiseana* taxa, from 2200 bp amplified mtDNA COI and II.

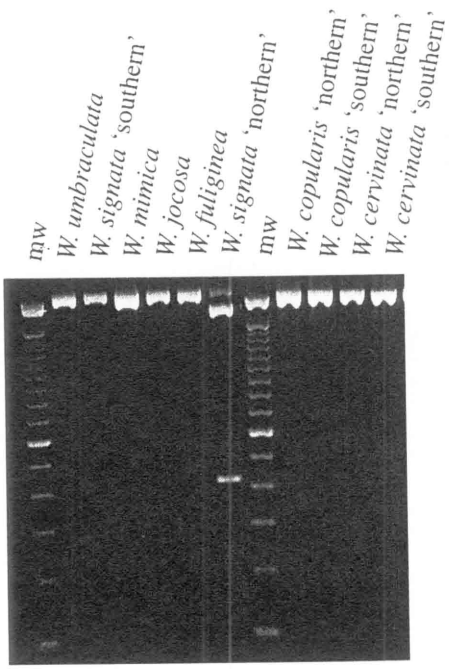

Figure 5: HaeIII restriction patterns for *Wiseana* taxa, from 2200 bp amplified mtDNA COI and II.

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).

DNA methods have not previously been used to identify *Wiseana* taxa. This technique produced diagnostic markers for *W. cervinata* 'southern', and 'northern', *W. copularis* 'southern' and 'northern', *W. jocosa*, *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 & II sequence differed by only one nucleotide and corrected divergences show that these taxa differ by only 0.19% (Chapter 3). The sequence from the ITS2 region for these taxa was identical (Chapter 4). The variable region from the mitochondrial 16s rRNA gene, which is thought to be evolving as rapidly as the control region, (Parker and Kornfield, 1996) was sequenced for *W. fuliginea* and *W. mimica,* but again both produced an identical sequence (B. Brown, unpublished data).

Mitochondrial DNA COI & II sequences identified additional haplotypes of *W. cervinata*, *W. copularis* and *W. signata* (Chapter 3). Cleavage of the entire COI & 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 adds to the accumulating evidence that *W. cervinata* as currently described may be two species. *Wiseana cervinata* populations south of latitude 40°S have an adult emergence time from September until December, while populations north of this latitude emerge from September until March (Dugdale, 1994). Herbert (1995) reported allozymes 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' haplotype RFLP pattern was recovered from populations at Mangapehi (WO), Ohakune (RI), Hawera (TK) and Levin (WN). 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 this 'northern' haplotype. The phylogeny of *Wiseana* taxa recovered from mtDNA COI and II sequence data (Chapter 3) indicated that *W. cervinata* 'southern' and 'northern' are not closely related sister taxa.

The biological significance and the geographical boundaries of the *W. copularis* and *W. signata* mtDNA haplotypes have not yet been established, but each can be distinguished by this technique. *Wiseana copularis* 'southern' produces a unique pattern with AsnI and TaqI, while *W. copularis* 'northern' can be distinguished using HindII. *Wiseana signata* 'northern' can be distinguished using HaeIII. *Wiseana signata* 'southern' produces a pattern identical to those of *W. signata* 'northern' (Asn1) or *W. signata* 'northern' and *W. umbraculata* (TaqI). However, both *W. signata* 'northern' and *W. umbraculata* can be identified by unique patterns produced by HaeIII and AsnI respectively.

This technique offers economical, rapid and accurate identification of *Wiseana* taxa. In conjunction with morphological characters, 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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Appendix 1: Locations of populations sampled for each *Wiseana* taxon. ([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.

Population	Code[a]	Grid Reference[b]	Number of individuals screened
W. fuliginea			
Birdlings Flat	MC	M37 860-090	2
Dunedin	DN	I44 133-783	1
W. mimica			
Cass	NC	K34 963-090	2
Mackenzie Country	MK	H38 802-615	1
***W. copularis* '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
***W. signata* 'southern'**			
Waimea	NN	N27 182-844	1
Pohara	NN	N25 012-421	2
***W. signata* 'northern'**			
Mangapehi	WO	S17 116-960	2
Woodhill Forest	AK	Q10 367-934	2
Levin	WN	S25 997-496	2
Wellington	WN	R27 703-918	2
***W. cervinata* ' 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

Appendix 1 continued.

Population	Code[a]	Grid Reference[b]	Number of individuals screened
***W. cervinata* '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
***W. copularis* '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
W. jocosa			
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
W. umbraculata			
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

Appendix 2: Expected fragment sizes following the amplification of *Wiseana* COI & II mtDNA with primers mtD4 and mtD18 and digestion with AsnI, TaqI, HindII or HaeIII.

Restriction Enzyme	Taxa	Predicted size of resultant fragment (bp)
AsnI	*W. fuliginea* & *W. mimica*	50, 90, 140, 185, 200, 210, 220, 300, 310, 500
	W. copularis 'northern'	50, 90, 140, 150, 180, 195, 210, 300, 350, 500
	W. signata 'southern' & 'northern'	50, 80, 90, 140, 150, 180, 190, 220, 260, 310, 500
	W. cervinata 'southern'	50, 90, 140, 180, 190, 220, 230, 260, 315, 500
	W. cervinata 'northern'	50, 90, 140, 180, 190, 210, 220, 230, 315, 500
	W. copularis 'southern'	50, 90, 140, 150, 180, 190, 210, 300, 320, 500
	W. jocosa	50, 80, 90, 135, 195, 200, 230, 280, 310, 680
	W. umbraculata	50, 80, 90, 135, 180, 190, 210, 230, 250, 310, 500
TaqI	*W. fuliginea, W. mimica, W. cervinata* 'northern', *W. copularis* 'northern', *W. jocosa*	50, 125, 150, 250, 270, 400, 460, 540
	W. signata 'southern' & 'northern', *W. umbraculata*	50, 125, 150, 240, 270, 530, 890
	W. cervinata 'southern'	50, 125, 150, 250, 450, 530, 690
	W. copularis 'southern'	50, 125, 150, 250, 260, 400, 450, 530

Appendix 2 continued.

Restriction Enzyme	Taxa	Predicted size of resultant fragment (bp)
HindII	*W. jocosa*	790, 1400
	W. copularis 'northern'	1000, 1200
	W. cervinata 'southern' & 'northern', *W. copularis* 'southern', *W. fuliginea, W. mimica, W. signata* 'southern' & 'northern', *W. umbraculata*	2200
HaeIII	*W. signata* 'northern'	420, 1770
	W. cervinata 'southern' & 'northern', *W. copularis* 'southern' & 'northern', *W. fuliginea, W. jocosa, W. mimica, W. signata* 'southern', *W. umbraculata*	2200

Chapter 8
General Conclusions

"The fundamental task of systematists is to infer the pattern of hierarchical relationships among taxa." (DeSalle and Brower, 1997).

This study has contributed to this ideal by applying phylogenetic methodology to recover hierarchical ancestor-descendent relationships in the *'Oxycanus'* lineages (Dugdale, 1994) of hepialid moths from New Zealand. Phylogenies were recovered using morphological characters in Chapter 2, molecular characters from the mtDNA COI & II gene regions in Chapter 3 and from the nrDNA ITS2 region in Chapter 4. The summarising of information from different phylogenies is an unresolved issue for systematists and two methods, total evidence and taxonomic congruence, were explored in Chapter 5. In Chapter 6, the COI & II phylogeny was used to test whether apparent synapomorphies supporting clades in the morphological phylogeny were homologous and to trace the evolution of morphological character states. In Chapter 7, RFLP patterns, produced by cleaving the entire mtDNA COI & II gene regions with restriction enzymes, were used to develop a diagnostic test for *Wiseana* taxa.

The New Zealand *'Oxycanus'* lineage *s. str.*, comprising *Dioxycanus, Dumbletonius, Heloxycanus* and *Wiseana* taxa, was confirmed as a monophyletic grouping as morphological synapomorphies mapped on to the independently derived COI & II phylogeny were homologous. Australian taxa, added to both the morphological and COI & II analyses, fell outside the New *Zealand 'Oxycanus'* lineage *s. str.*

Morphological and COI & II phylogenies were mostly congruent, but conflicted in their placement of *Dumbletonius* taxa and arrangements within the genus *Wiseana*. *Dumbletonius characterifer* and *D. unimaculatus* were recovered in the same clade in the morphological phylogeny and separately in the COI & II phylogeny. In the COI & II phylogeny, *D. characterifer* was recovered as the basal taxon for the *'Oxycanus'* lineage *s. str.* or in a clade with *Cladoxycanus*, but never in a clade with *D. unimaculatus*. *Dumbletonius unimaculatus* was recovered in a clade with *Wiseana* taxa when the COI & II data set was analysed using both maximum parsimony and maximum likelihood methods and from the ITS2 data set (*'Oxycanus'* lineages only) under both maximum parsimony and maximum likelihood. There was high spectral support for this clade compared with the (*Dumbletonius characterifer, D. unimaculatus*) clade.

Re-examination of the apparent morphological synapomorphies for the (*D. characterifer, D. unimaculatus*) clade indicated all may be plesiomorphic rather than homologous characters, but have been retained in the same state in these taxa. This suggests that the genus *Dumbletonius* is not a monophyletic group.

Evidence suggested that *D. characterifer* was basal taxon of the *'Oxycanus'* lineage *s. str.* and recovery of *D. characterifer* in a clade with *Cladoxycanus* in some of the COI & II maximum parsimony trees was a case of long branch attraction, a well known failing of the parsimony method. This highlights the advantages of having several phylogenies, produced from independent data sets and different methods available for comparison, as congruence between these data sets can be taken as evidence that the phylogeny is accurate.

The identification of additional haplotypes for *Dumbletonius* taxa or *Cladoxycanus minos*, to break up long branches and improve the accuracy of the phylogeny would be useful and longer sections of DNA may provide more synapomorphies to support and clarify relationships, especially the placement of *Dumbletonius characterifer*. However, the underlying shape of the tree and the branch lengths will affect the degree of resolution possible.

The morphological distinctiveness of *Cladoxycanus minos* resulted in its placement in a separate lineage by Dugdale (1994). The recovery of *Cladoxycanus minos* as a separate branch in both morphological and COI & II phylogenies and identification of 12 autapomorphic morphological characters, supports its distinctiveness and placement in a separate lineage. *Cladoxycanus minos* was confirmed as sister taxon to the New Zealand *'Oxycanus'* lineage *s. str.* since the eight apparent morphological synapomorphies these taxa shared were found to be homologous when mapped on the COI & II phylogeny.

The COI & II phylogeny placed the Australian *Oxycanus* and *Jeana* taxa as sister group to the two New Zealand *'Oxycanus'* lineages. However, the morphological phylogeny suggested that the Australian taxa were sister groups to the New Zealand *'Oxycanus'* lineage *s. str.* If the COI & II hypothesis is correct, the two morphological characters shared by the Australian taxa and the New Zealand *'Oxycanus'* lineage *s. str.*, (the dorsal hood and the flange on the posterior margin of the vinculum/saccus complex of the male pseudotegumen), would have arisen in the ancestor at the base of the (*Oxycanus, Jeana, Cladoxycanus, 'Oxycanus'* lineage *s. str.*) clade and have been lost in

Cladoxycanus. Examination of more Australian taxa in the genus *Oxycanus,* and a molecular phylogeny with better support than was available from the COI & II phylogeny, would indicate if interpreting these characters as homologous was correct.

In all analyses, *Aenetus* and *Aoraia* taxa were recovered as separate branches basal to the *'Oxycanus'* lineages. This supports Dugdale's (1994) placement of *Aenetus* and *Aoraia* taxa in separate lineages. The Australian genera *Fraus* and *Trictena* also fell outside the New Zealand *'Oxycanus'* lineages. The Australian genus *Oxycanus* was shown to be paraphyletic with *Oxycanus sphragidias* being recovered in a clade with *Trictena* taxa. The relationship of *O. sphragidias* to the remaining Australian *Oxycanus* taxa is unknown. All *Oxycanus* taxa including *O. sphragidias* have the 'oxycanus' wing venation pattern, whereas *Trictena* taxa do not. A comprehensive re-examination of Australian hepialid taxa is needed to establish whether 'oxycanus' wing venation is a synapomorphy for an Australasian hepialid clade or whether it has arisen several times independently.

The interspersing of New Zealand and Australian taxa in the COI & II phylogeny and the estimations of times of divergence suggest that the New Zealand hepialid fauna results from several post-Gondwanan break up dispersal events. The estimated time of divergence for *Aenetus* from other New Zealand hepialids of 3-4 my is considered to be an underestimate. *Aenetus* taxa are not known to disperse over water, but yet are found on islands estimated to have been separate for at least 25 my. The relationship between New Zealand *Aenetus* and *Aenetus* taxa from Australia, New Caledonia, New Guinea and the islands of the Banda Arc requires clarification. The estimated time of divergence and radiation within the genus *Wiseana*, approximately 1-1.5 mya, corresponded with known geological events. Recent and rapid radiation within *Wiseana* was supported by lack of variable morphological characters and short internodes between the branches in the COI & II phylogeny, but in all analyses the monophyly of the genus was supported.

Combining data sets did not resolve problem areas within the *'Oxycanus'* lineages, such as the placement of *Dumbletonius characterifer*. The advantages of having several estimates of phylogeny from independent data sets were again highlighted when the combined data set produced a topology different to those produced from the morphological and COI & II data sets. It was hypothesised that unequal rates of evolution between the ITS2 and other data sets caused the parsimony method to become inconsistent when the data sets were combined and so produce an inaccurate phylogeny.

Accurate methods of identification and a stable classification have been lacking for *Wiseana* taxa. Morphology and COI & II data sets produced many alternative but equally likely, arrangements of taxa within this genus. Only relationships between *W. signata* and *W. umbraculata* received strong support. New haplotypes were recovered for *W. cervinata*, *W. copularis* and *W. signata* taxa from the COI & II and ITS2 nucleotide sequences. Amplification of the entire COI & II gene regions via the PCR reaction and subsequent cleaving with restriction enzymes produced unique RFLP patterns for all *Wiseana* taxa except *W. fuliginea* and *W. mimica*. This diagnostic test will assist in the positive identification of damage-causing larvae, enable a better understanding of their ecology and behaviour and consequently more effective control measures. Further work should include assessing the genetic variation in *W. fuliginea* and *W. mimica* populations and developing a diagnostic test for these taxa.

The most resolved phylogeny for *Wiseana* was from the combined morphology, COI & II, ITS2 and allozyme data set, using *Dumbletonius unimaculatus* as an outgroup. Two clades were recovered, but the exact placement of *W. jocosa* and *W. copularis* taxa in the (*W. cervinata* 'southern', *W. cervinata* 'northern', *W. copularis* 'southern', *W. copularis* 'northern', *W. fuliginea, W. jocosa, W. mimica*) clade was uncertain. Spectral analysis indicated approximately equal support values for a (*W. jocosa, W. signata, W. umbraculata*) clade, as seen in the COI & II phylogeny and for a (*W. jocosa, W. fuliginea, W. mimica*) clade, as seen in the morphological phylogeny. The remaining clade comprised (*W. signata* 'southern', *W. signata* 'northern', *W. umbraculata*). The recovery of two clades within the genus *Wiseana* is in agreement with previous hypotheses. It is worth noting that all the known pest species of *Wiseana* occur in one clade. Accurate identification of the damage-causing larval stage, using RFLPs, may help establish whether *W. jocosa* is also a pest species.

This study has contributed to a fundamental goal of systematics, that is the recovery of phylogenetic relationships among taxa. The utility of independent data sets for character mapping, assessing congruence and the accuracy of combined analyses, and the utility of spectral analysis in measuring support for alternative hypotheses, were also demonstrated by this study.

References

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