2012-03-09

Phylogenetics of Thysanoptera (Insecta: Paraneoptera)

Rebecca S. Buckman
Brigham Young University - Provo

Phylogenetics of Thysanoptera (Insecta: Paraneoptera)

Rebecca S. Buckman

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

Michael F. Whiting, Chair
Byron J. Adams
Keith A. Crandall

Department of Biology

Brigham Young University

April 2012

ABSTRACT

Phylogeny of Thysanoptera (Insecta: Paraneoptera)

Rebecca S. Buckman
Department of Biology, BYU
Master of Science

The order Thysanoptera (Insecta: Paraneoptera), commonly known as thrips, includes organisms that exhibit a wide range of social and feeding behaviors that are of particular interest in evolutionary studies. These studies within thrips have been inhibited by the lack of knowledge of thrips relationships. The recognized classification scheme strives to reflect evolutionary relationships and is based upon morphology. Molecular data is next as morphology alone is not enough to resolve relationships. Few molecular studies have been conducted and all were limited in their taxon sampling and genetic sampling. To provide a foundation of future evolutionary studies, the objectives of this study are to (1) test the monophyly of the suborders Terebrantia and Tubulifera, (2) test the monophyly of the families and decipher their relationships, and (3) test the monophyly of the recognized subfamilies. Phylogenies were reconstructed based upon 5299 bp, from five genetic loci: 18S ribosomal DNA, 28S ribosomal DNA, *Histone 3* (*H3*), *Tubulin-alpha* (*TubA*) and *cytochrome oxidase c subunit* I (*COI*). 99 thrips species from seven of the nine families, all six subfamilies and 70 genera were sequenced. Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian analysis all strongly support a monophyletic Tubulifera and Terebrantia. Phlaeothripidae, Aeolothripidae, Melanthripidae and Thripidae are all monophyletic families. The relationship between Aeolothripidae and Merothripidae to the rest of Terebrantia is equivocal. Morphological and molecular data suggest Aeolothripidae or Merothripidae could be the basal lineage of Terebrantia. Four of the six subfamilies are recovered as monophyletic. The two largest subfamilies, Phlaeothripinae and Thripinae, are paraphyletic and require further study to understand relationships within them.

Keywords: Thysanoptera, phylogeny, Tubulin-alpha, Thripinae, Phlaeothripidae, thrips

ACKNOWLEDGEMENTS

Firstly, my profound appreciation goes to Dr. Laurence Mound (CSIRO Black Mountain, Canberra, Australia) for his substantial help with specimen collection and identification, and his morphological expertise. Without his help this project would not have been possible. Secondly, my gratitude goes to my advisor, Dr. Michael Whiting for his guidance and knowledge. He has provided me with the opportunity to pursue research and find something that I am passionate about. Appreciation goes to my committee members as they have given me support and were always willing to help. I would also like to thank my fellow students for all the time spent in discussions and aid of analysis implementation. Lastly, I would like give thanks to my family for all the love and support they give me in my studies and their encouragement to pursue my goals.

TABLE OF CONTENTS

Introduction 1

Materials and methods 5

Taxon sampling 5

Molecular Methods 5

Alignment 7

Phylogenetic reconstruction 8

Results 9

Alignment 9

Phylogenetic reconstruction 10

Testing classifications 11

Discussion 12

Tubulifera 13

Terebrantia 14

Conclusion 16

References 17

Figures and Tables 22

Introduction

The order Thysanoptera is a member of the Paraneoptera group of insects, which includes the orders Hemiptera, Psocoptera, and Phthiraptera. Commonly known as thrips, the ~6,000 described extant species range in size from 0.5-13mm and are distinguished from other insects by the presence of a single mandible and an eversible pretarsal blatter (arolium) and the presence of fringed wings. While fringe wings are also found on insects with reduced size including featherwing beetles (Ptiliidae), fairyfly wasps (Mymaridae), and some Psocoptera (*Nepticulomima*), Thysanoptera is the only order in which these wings are the basal condition for the order.

Species of thrips have a wide range of morphological features with some species very aberrant and unique. The Peanut-winged thrips (*Arachisothrips millsi*) has wings with heavy reticulation and the leading edge of the forewing ballooned, hallow and peanut-shaped (Stannard, 1952); much like the wings of the hemipteran family, Tingidae. Thysanoptera exhibit a wide range of social behavior including species that are solitary, subsocial, communal and eusocial (Crespi & Mound, 1997). On a molecular level, thrips are unique from other insects in the evolution of the mitochondrial genome (mtgenome). Thrips and other paraneopteran insects have an increased rate of nucleotide substitution in their mtgenomes and it has been suggested that this correlates with an increased rate of gene rearrangement (Shao *et al.* , 2001; Shao *et al.* , 2003). To date there is only one known mtgenome from thrips, *Thrips imaginis* (Shao & Barker, 2003), that shows a high degree of gene rearrangement. Of the 37 genes in the mtgenome it was inferred that 27 in *T. imaginis* have been translocated and/or inverted from the ancestral condition. We

have found that other thrips species also show highly rearranged mtgenomes, and that gene order is a phylogenetically conserved character suite within Thysanoptera (*unpublished data*).

The majority of research on thrips focuses on the handful of species with the largest economic impact. For example, *Frankliniella occidentalis* (Western Flower Thrips) was originally endemic to western North American but now has a world wide distribution (Kirk & Terry, 2003). Reitz (2009), showed that studies on *F. occidentalis* have accounted for one-third of the total Thysanoptera publications in the past 30 years. Along with *F. occidentalis* there are other thrips species that are vectors of Tospoviruses. With only ~10 of the 6,000 species vectors of Tospoviruses (Mound, 2001a), the majority of thrips diversity is largely ignored except in descriptive taxonomic studies. The focus on economic impact has resulted in a poor understanding of the phylogenetic relationships and evolutionary history across the order. With a clear understanding of thrips relationships, evolutionary studies of their behavior and molecular evolution will provide basic information that may be useful for the biological control of pest species.

The placement of Thysanoptera within Paraneoptera is still unclear (Kristensen, 1991). Paraneoptera is often considered to be the sister group to the Holometabolous insects (Kjer, 2004; Wheeler *et al.* , 2001) but it is still unclear how the constituent orders are related. There is evidence to support two hypotheses of relationships: a (Psocodea (Thysanoptera + Hemiptera)) relationship or a (Hemiptera (Thysanoptera + Psocodea)) relationship. The Thysanoptera +Hemiptera relationship is supported by mouthparts (Grimaldi & Engel, 2005) and fore wing structures (Yoshizawa & Saigusa, 2001). The Thysanoptera + Psocodea sister relationship is

supported by molecular data (Shao *et al.* , 2001; Wheeler *et al.* , 2001) and fossil evidence (Sharov, 1972; Zherikhin, 2002).

Taxonomy within thrips has been based entirely on morphology. There are multiple classification schemes in use (Bhatti, 1988; Bhatti, 1992; Bhatti, 2006; Zherikhin, 2002) with no consensus of intraordinal relationships (Mound *et al.* , 1980). Bhatti (1988; 1992; 2006) takes a phenetic approach and raised Thysanoptera to the superordinal level, creating Tubulifera and Terebrantia into orders that greatly increased the number of families without taking into consideration evolutionary relationships. Working with fossil specimens, Zherikhin (2002) proposes the order Thripida that contains two suborders: Thripina (traditional Thysanoptera) and Lophinoneurina (early Permian-late Cretaceous fossils). For a detailed comparison and review of classification schemes see Mound & Morris (2004).

The current classification uses phylogenetic principles to delimit classifications (Mound *et al.* , 1980). Two suborders are recognized, Tubulifera and Terebrantia. Tubulifera contains one family (Phlaeothripidae) and is characterized by a tubular tenth abdominal segment. This suborder of ~3500 species is further divided into a well-established subfamily Idolothripinae and an equivocal Phlaeothripinae. Some effort has been made to establish supra-generic classifications within Idolothripinae and Phlaeothripinae (Mound & Marullo, 1996; Mound & Palmer, 1983) and both groups are investigated here.

Terebrantia contains 13 families, five of which are known only from fossils. The extant species are dispersed among eight families. Three families, Merothripidae (18 spp.), Aeolothripidae (201 spp.) and Melanthripidae (76 spp.), retain the most plesiotypic character states of the order

(Pereyra & Mound, 2009). Other families include Heterothripidae (76 spp.), Stenurothripidae (24 spp.), Fauriellidae (5 spp.), and Uzelothripidae (1 sp). The largest family of Terebrantia, Thripidae (2066 sp.), comprises four subfamilies. Sericothripinae, Dendrothripinae and Panchaetothripinae are well-supported subfamilies, but the phylogenetic placement of the largest subfamily Thripinae relative to the other subfamilies is unclear.

Since 1980, there have been two hypotheses of intraordinal relationships (Figure 1). The utility of molecular data to investigate these relationships has been explored only superficially (Mound & Morris, 2004; Mound & Morris, 2007). The first molecular studies investigating relationships across the order were limited in their data sets and taxon sampling (Crespi *et al.* , 1996; Morris & Mound, 2003; Mound & Morris, 2007). The first ordinal molecular study (Crespi *et al.* ,1996) involved only eight ingroup taxa and was inferred from partial sequences of Cytochrome oxidase I (415 bp) and 18S ribosomal DNA (640 bp). The next effort in thrips phylogenetics was made by Morris and Mound (2003). They expanded the taxon sampling to include 52 thrips species (18 Tubulifera, 34 Terebrantia and 7 of 9 families) with a 600 bp sequence of 18S rDNA. Mound and Morris (2007) increased their character sampling to complete sequences of 18S rDNA (~1800 bp) for 38 species (8 of 9 families). All of these hypotheses, however, were limited by taxon sampling and number of molecular loci. Consequently, relationships are still dubious.

Here we infer a phylogeny for Thysanoptera with the largest molecular and taxon sampling to date. Our objectives are to: (1) test the monophyly of the suborders Terebrantia and Tubulifera,

(2) test the monophyly of the families and decipher their relationships, and (3) test the monophyly of the proposed subfamilies.

Materials and methods

Taxon sampling

Specimens were collected and stored in 95-100% ethanol and stored at -80°C (Table 1). We attempted to represent the taxonomic diversity of thrips by including representatives from all families, with the exception of two: the monotypic Uzelothripidae and Fauriellidae, which are only known from the type specimens. The 103 taxa in this study represent 101 thrips species, seven of the nine extant families, all six subfamilies and 70 genera. The greatest numbers of species were sampled from the two largest subfamilies, Phlaeothripinae and Thripinae that account for 76% (4500 species) of the total diversity. The 101 species represent 9% of the 767 genera and 1.7% of the 5864 species. In addition 15 species were included as outgroups representing Hemiptera, and Psocoptera (Table 1).

Molecular Methods

DNA was extracted by slicing the abdomen and thorax with a razor blade and the entire specimen was suspended in extraction buffer following manufacture protocols for the Qiagen DNEasy kit (Qiagen, Inc., Valencia, CA). In some cases, multiple individuals from the same population were extracted together to obtain a suitable DNA concentration. Vouchers were stored in 100% EtOH at -80°C until slide mounted and then deposited in the Insect Genomics

Collection, M. L. Bean Museum, Brigham Young University or the Australian National Insect Collection (CSIRO, Black Mountain, Canberra, Australia).

A total of five genetic loci (4 nuclear, 1 mitochondrial) were sequenced: 18S ribosomal DNA (~1,700 bp), 28S ribosomal DNA (~2,000 bp), Histone 3 (338 bp), Tubulin-alpha 1a (338 bp), and Cytochrome-oxidase I (~650 bp). All loci were amplified using Platinum taq DNA polymerase (Invitrogen, Carlsbad, CA) in 25 μl reactions. For ribosomal gene reactions (18S and 28S), 6.25 μl of water was replaced with 5 μl of betaine and 1.25 μl of DMSO. All genes were amplified under the following protocol*: 2 min. at 94°C and 35 cycles of 30 s. at 94°C, 30 s. at 46-55°C, and 45-85 s. at 72°C, with a final extension at 72°C for 7 min (*primer annealing temperatures and extensions times varied in accordance to primers and fragment length. See Table 2) on GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA).

Oligonucleotide primers were purchased from Integrated DNA Technologies (San Diego, CA). Primer sequences for 18S and 28S rDNA were obtained from Whiting (2002) with some primers optimized for thrips (Table 2). H3 primers are from Colgan *et al*. (1998) and primers used for COI amplification are the standard DNA barcoding primers (Folmer *et al.* , 1994). Tubulin-alpha1 (TubA) primers were developed through transcriptomics as general arthropod primers. For the amplification of TubA a nested PCR was used. Initial primers were DDVTubAF and DDVTubAR followed by thrips specific primers, TH_TubAF and TH_TubAR (see Table 2).

PCR products were visualized via 1-2% agarose gel electrophoresis with ethidium-bromide to confirm amplification and to test for product contamination. PCR products were cleaned with PrepEase® purification plates (USB Corporation, Cleveland, OH) according to manufacturer

instructions. PCR products were sequenced using BigDye chain termination chemistry and fractioned on an AB13730xl (Applied Biosystems Inc.) at the Brigham Young University DNA Sequencing Center (Provo, UT).

Alignment

Contigs were assembled and edited in SEQUENCHER® 5.0 (Genecodes, 2011). Consensus sequences were exported in FASTA format for alignment. Protein-coding sequences were imported into the program MEGA version 5 (Tamura *et al.* , 2011) for alignment by amino acid. Once the open reading frame was found, sequences were translated to amino acid, aligned by MUSCLE (Edgar, 2004) and then back translated.

Multiple sequence alignment of ribosomal DNA is difficult due to the multiple conserved and unconserved regions of the genes. In this paper multiple strategies of sequence alignment were tested with both 18S and 28S ribosomal genes. Both 18S and 28S rDNA were independently aligned four different ways with combinations of suborder alignments and Gblocks (Castresana, 2000) in MAFFT (Katoh & Toh, 2008) to test the sensitivity of the topology to the ribosomal gene alignments (see Figure 2). Reasoning behind independent alignment of suborders followed by profile alignment is due to the large distinction between the suborder taxa in the unconserved regions. Gblocks with the most relaxed parameters was utilized as a method to test the removal of the poorly aligned blocks on the tree topology. All combinations of the four 18S alignments and the four 28S alignments were concatenated with the three protein-coding genes using MacClade 4.08 (Maddison & Maddison, 2005) for a total of 16 complete datasets that were tested under parsimony and likelihood methods.

Phylogenetic reconstruction

Multiple methods of tree construction were used to test the robustness of the topology under different optimality criteria. Parsimony analysis (MP) was implemented in TNT (Goloboff, 2008) using a heuristic search under 'new technology' search utilizing tree drifting, sectorial searches, tree fusing (Goloboff, 1999) and ratchet (Nixon, 1999) and TBR branch swapping and treating gaps as missing. Nodal support for the MP was calculated by 1000 bootstrap replications in TNT, along with partition Bremer analysis using TREEROT v.3 (Sorenson & Franzosa, 2007) implemented through PAUP v4.0b10 (Swofford, 2002).

For Likelihood (ML) and Bayesian analyses, jModelTest (Posada, 2008) was used to determine models of evolution for individual genes and the concatenated data set under the Akiake information criteria (AIC). ML analyses were implemented in RAxML (Stamatakis, 2006) partitioning by gene for 1000 replications. Nodal support for the ML analysis utilized the rapid bootstrap algorithm of RAxML with 1000 replications. The Bayesian analysis was run in MrBayes (Ronquist & Huelsenbeck, 2003) with no priors and a partitioned model according to gene and run with 20 chains for 20 million generations and a burn-in of 25%. Confirmation of convergence from Bayesian analysis used the program Tracer v1.5 (Rambaut & Drummond, 2007). Topologies between different alignments and phylogenetic methods were tested to see if they were statistically different from each other using a Shimodaira-Hasegawa (S-H) test implemented in RAxML.

Results

Alignment

In protein-coding genes no amino acid indels were found in Histone 3 and Tubulin-Alpha. COI contains five amino acid indels that appear to occur in approximately half of the taxa for a total of 1.3% gaps in the nucleotide alignment. In an initial alignment of 18S rDNA, putative autapomorphic insertions in expansion regions of the gene were found in three species: *Carientothrips* sp. (661 bp), *Hoplandrothrips* sp. nov. (180 bp), and *Merothrips floridensis* (83, 108 and 289 bp). The expansion regions were removed from the original sequences and new alignments were performed to remove the effect of these regions on the overall alignment. In 28S rDNA there were no expansion regions effecting the alignment.

Under parsimony and likelihood methods, the 16 different alignments produced different topologies. The MP analyses across the 16 alignments produced results that were highly incongruent with each other. The Likelihood topologies were robust across all alignments with the exception of five taxa (*Sophiothrips* sp., *Baenothrips* sp., *Heligmothrips* sp., *Merothrips floridensis*, and *Parabaliothrips* sp.) that regularly changed position. An S-H test of all ML topologies produced in comparison to the chosen topology was conducted and resulted with no significant difference (mean D[LH] = -15.2750, mean SD = 22.5992). Of the 16 alignments constructed, the 12 datasets that were aligned by suborder were rejected as a profile alignment assumes the two groups are phylogenetically distinct and would not allow a proper test of the hypotheses of Mound et al. (1980). From the remaining four alignments the first 18S alignment was used to retain the greatest amount of phylogenetic information of the unconserved regions.

In combination with the first 18S alignment the second 28S alignment (Gblocks) was used due to a high degree of poorly aligned areas. With the most relaxed parameters, Gblocks retained 48% of the original 3615 positions and reduced the percent gaps from 38.4% to 0.8%. The final concatenated dataset was 5299 bp in length: 18S rDNA=2165 bp (13.2% gaps), 28S rDNA=1771 bp, Histone 3= 338 bp, Tubulin-Alpha=338 bp, and COI=687 bp. Under parsimony and treating gaps as missing there are 2412 informative characters, 474 autapomorphic characters and 2413 invariable characters

Phylogenetic reconstruction

The parsimony analysis resulted with eight most parsimonious trees with a length of 23672 (CI = 0.233, RI = 0.626). A strict consensus resulted in two polytomies in the Thripinae, and one within the Phlaeothripinae (Figure 3). The SH test found the eight MP trees to be significantly different from the ML topology. From the eight most parsimonious trees there was a mean difference of -72.87363 in the likelihood scores (mean SD=29.30082). The large difference between the two analyses can be attributed to long branches as hypothesized in Mound & Morris (2007).

jModelTest recovered a six-parameter model with gamma distribution (G) and proportion of invariable sites (I) enabled for among-site rate variation for each loci and the concatenated data set. The GTRGAMMAI model was implemented in RAxML, partitioning model parameters by gene. The ML tree reconstructed has a score of -108770.0279 (Figure 4). The Bayesian analyses returned a tree with a mean likelihood score of -108251.58 and the topology closely resembles that of the ML analyses (Figure 5). To conduct an SH test between the ML

topology and the summary Bayesian tree, polytomies were randomly resolved with the smallest branch lengths possible using Mesquite (Maddison & Maddison, 2011). Ten trees with different randomizations of the polytomies were constructed and tested. The test of the ten trees resulted with a mean likelihood difference of -7.4292 (SD = 15.1424) and all trees not being significantly different from the ML topology.

Testing classifications

Tubulifera and Terebrantia were found to be monophyletic groups (100 bootstrap:151 bremer and 84:17, respectfully) across all tree reconstruction methods. The monophyletic Tubulifera contains just one family, Phlaeothripidae. Within Phlaeothripidae, the subfamily Idolothripinae is monophyletic (except in MP, *Phaulothrips* + *Compsothrips* separate) but poorly supported (50:n/a) and it renders Phlaeothripinae paraphyletic. Within Idolothripinae there are two recognized tribes: Pygothripini and Idolothripini. Neither tribe is recovered as monophyletic. Within Phlaeothripidae basal lineages are unclear but strongly supported relationships are found in the bulk of the derived taxa. *Baenothrips* is highly variable in its position in all alignments tested moving between a basal Phlaeothripidae position and a position among the Idolothripinae, sister to *Carientothrips*.

Of the three Terebrantia families that were tested for monophyly (Aeolothripidae, Melanthripidae and Thripidae), all were recovered as monophyletic with exception of Aeolothripidae in the MP analysis. In the ML analysis the backbone of the Terebrantia consists of three clades: Aeolothripidae, all other families and Thripidae. The Aeolothripidae takes a basal position as sister to the rest of Terebrantia but is not strongly supported (67:n/a). After the

branching of Aeolothripidae, the ML tree shows Terebrantia splitting into two clades, the Thripidae (100:19) and all the other families: Merothripidae, Melanthripidae, Stenurothripidae, and Heterothripidae (26:n/a). Stenurothripidae and Heterothripidae from a strong sister relationship (100:33) that is grouped in a clade with Melanthripidae (100:54). ML and Bayesian topologies differ in the placement of Merothripidae. Bayesian places Merothripidae as the basal lineage of Terebrantia, not in the clade with Melanthripidae.

Within Thripidae the ML analyses find Dendrothripinae, Sericothripinae and Panchaetothripinae as monophyletic groups. The Dendrothripinae is not recovered as monophyletic in the MP analysis and is hence not as strongly supported (67:n/a) as Sericothripinae (100:17) and Panchaetothripinae (100:113). Thripinae is the largest of the subfamilies and is paraphyletic in reference to all other subfamilies. In the Thripinae *Ayyaria chaetophora* has an inconsistent position between analyses and seem to be a source of confusion of Thripidae relationships. Across all three topologies *A. chaetophora* is recovered in three different poorly supported relationships with *Pseudanaphothrips* spp. (ML, <50 bootstrap), *Pseudodendrothrips mori* (MP, bremer of 8), or as part of an unresolved clade (Bayes).

Discussion

For the purpose of the discussion, only the Bayesian and ML analyses are referenced in this paper as they implement models that are able to adequately handle the accelerated evolution of this group and they are more congruent with the current morphological classification. The MP topology is taken into consideration when appropriate. The monophyly of Terebrantia and

Tubulifera support one of the hypotheses of Mound *et al.* (1980), but the familial relationships within Terebrantia are not congruent with those suggested in that same hypothesis (Figure 1:A).

Tubulifera

Within Phlaeothripidae, the monophyly of subfamily Idolothripinae based upon morphology is by no means clear despite the studies of Mound and Palmer (1983). Mound and Palmer never found a strong set of synapomorphies for Idolothripinae that did not include exceptions. Despite this, Idolothripinae is recovered as monophyletic under statistical models which are congruent the weak morphological synapomorphies of broad stylets, absence of sternal glandular areas in males (with some exceptions), and short stout sub-lateral setae (S2) on tergite IX of males (also with some exceptions).

Phlaeothripinae includes all other species that are not members of Idolothripinae, and work is needed to develop phylogenetically significant classifications for these species. Currently there are three 'lineages' recognized within Phlaeothripinae (Mound & Marullo, 1996; Stannard, 1957). These lineages are not strongly based on characters, but appear to reflect the biologies of the included species: '*Liothrips*'-leaf-feeding species, '*Phlaeothrips*'-hyphal-feeding species, and '*Haplothrips*'-flower-feeding species. Despite the known weakness of the morphological support, two of these 'lineages', *'Haplothrips'* and *'Liothrips'*, are congruent with groupings recovered in this study (see Figure 3). *'Haplothrips'* is strongly supported clade (98:28) that includes *Dyothrips pallescens* which is slightly aberrant and the *Leptothrips* spp. that apart from apomorphies, share most character states with *Haplothrips.* The inclusion of two oddities, *Lissothrips* and *Scopaeothrips bicolor* needs further study as both have morphological characters

that are very distinct from *Haplothrips* spp. The *'Liothrips'* lineage is the other strongly supported clade (99:32) within Phlaeothripinae that are all leaf-feeders and sometimes gall inducing (*Kladothrips, Leeuweenia* and *Teuchothrips*). The *'Phlaeothrips'* lineage is shown to be paraphyletic but includes the basal species of Phlaeothripidae. A more extensive phylogenetic study of this subfamily is needed to solidify or reject the validity of these lineages as recognized classifications.

Terebrantia

The monophyly of Aeolothripidae is recovered and its position at the base of the Terebrantia is congruent with fossil evidence. It had been thought that Aeolothripidae was the most primitive of the extant Thysanoptera when looking at fossil specimens (Sharov, 1972). In 1974, Mound & O'Neill revisited the taxonomy of Merothripidae and suggested the Merothripidae retained the most plesiomorphic thrips character states including a well-developed tentorium and paired trichobotheria on tergite IX. These plesiomorphic characters (Mound & Heming, 1991; Mound & O'Neill, 1974) of the Merothripidae support its placement at the base of the Terebrantia as suggested by the Bayesian topology. Along with the Merothripidae, the Aeolothripidae and Melanthripidae are the only other families that have retained plesiomorphies which have been lost in the other six families (Pereyra & Mound, 2009). The monophyly of Melanthripidae is recovered, and is distinct from the Aeolothripidae with which it has been associated traditionally based upon cross veins and width of the forewing. A suggested relationship of the Melanthripidae to the Heterothripidae is particularly intriguing. In body structure, Heterothripidae and Stenurothripidae are quite similar, although the sensoria on antennal segments three and four are different in appearance. Melanthripidae are very different from

members of these two families in body structure, but the antennal sensoria in some Australian Melanthripidae are similar to the sensoria of Heterothripidae species. This apparent relationship requires further study.

The family Thripidae is recovered as monophyletic and across all topologies, there is a consensus of a split of Thripidae into two major groups. One clade includes Dendrothripinae, Sericothripinae, *Frankliniella* and other related Thripinae while the other contains Panchaetothripinae, *Thrips* and close relatives. Dendrothripinae and Sericothripinae relationships are poorly supported and overall no strong hypotheses can be made about relationships within that clade of Thripinae. The subfamily Panchaetothripinae is recovered as monophyletic. In 2007, Mound & Morris were not able to comment on the placement of Panchaetothripinae as this changed from a basal lineage to well within Thysanoptera, depending upon the analysis (ML vs. MP). This variation was attributed to as possible long-branch attraction. Here Panchaetothripinae remains stable and nested well within Thripidae in all analyses, rejecting the hypothesized basal position of the subfamily. The sister clade to Panchaetothripinae is strongly supported but a further investigation needs to be done as no clear synapomorphies can be determined for the group.

Within Thripinae, the *Thrips*-group (Mound, 2001b) is retained as monophyletic as supported by ctenidiate characters, and is placed as only distantly related to the other major ctenidiate Thripinae – the *Frankliniella* group. It is interesting that *Thrips australis*, a species placed by European workers in a separate genus, is not only retained within the genus *Thrips*, but is placed close to another common Australian species, *T. imaginis*. The strongly supported *Anaphothrips* + *Thrips* group relationship is surprising as there is no strong structural evidence. *Anaphothrips*

spp. has the plesiotypic presence of a pair of setae in front of the fore ocellus (lost in *Thrips* and *Taeniothrips*) and a different arrangement of setae on the lobe of the forewing. Similarly, a suggested relationship between *Pseudanaphothrips* and *Frankliniella* is recovered, supported morphological data (Mound, 2001b) and the Bayesian and MP topologies.

The failure to demonstrate monophyly of both the Phlaeothripinae and Thripinae is no surprise. The classifications within these two groups are based on structural similarities and differences for which there is limited evidence of any phylogenetic significance. Such classifications are driven more by the need to provide identifications, or to sort museum collections, than by any interest in evolutionary relationships. Given the extent of homoplasy amongst many structural character states used in thrips taxonomy, and the frequent use of "loss apomorphies" in the available classifications, molecular data will be of particular importance for investigating the patterns of radiation and relationships within these two major groups.

Conclusion

The phylogeny produced in this study is once again, just another step in deciphering thrips relationships. The morphological classification of families and most subfamilies are validated by molecular data, but below the subfamily level much work is needed. Thripinae, Idolothripinae and Phlaeothripinae relationships are far from clear and a more extensive sampling of genera is needed to help stabilize those taxa that bounce around the tree. A wider geographical sampling is also needed as Australia and the United States account for over half of the sampled taxa.

References

Bhatti, J.S. (1988) The orders Terebrantia and Tubulifera of the superorder Thysanoptera (Insecta): A critical appraisal. *Zoology (Journal of pure and applied zoology)*, **1**, 167-240.

Bhatti, J.S. (1992) The order Tubulifera (Insecta): Its characters and classification into families. *Zoology (Journal of Pure and Applied Zoology)*, **3**, 127-162.

Bhatti, J.S. (2006) The classification of Terebrantia (Insecta) into families. *Oriental Insects*, **40**, 339-375.

Castresana, J. (2000) Selection of Conserved Blocks from Multiple Alignments for their use in Phylogenetic Analysis. *Molecular Biology and Evolution*, **17**, 540-552.

Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G. & Gray, M.R. (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, **46**, 419-437.

Crespi, B.J., Carmean, D.A., Vawter, L. & Dohlen, C. (1996) The molecular systematics of Thysanoptera. *Systematic Entomology*, **21**, 79-87.

Crespi, B.J. & Mound, L.A. (1997) Ecology and evolution of social behaviour among Australian gall thrips and their allies. *Evolution of Social Behaviour in Insects and Arachnids*, (ed. by J. Choe and B.J. Crespi), pp. 166-180. Cambridge University Press, Cambridge.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrihenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294-297.

Genecodes (2011) *Sequencher 5.0*. Genecodes Co., Ann Arbor, Michaigan.

Goloboff, P.A. (1999) Analyzing large data sets in reasonable times: solutions for compsite optima. *Cladistics*, **15**, 415-428.

Goloboff, P.A., Farris, J. S. (2008) TNT, a free program for phylogenetic analysis. *Cladistics*, **24**, 774-786.

Grimaldi, D. & Engel, M.S. (2005) *Evolution of Insects*. Cambridge University Press, New York.

Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics*, **9**, 286-298.

Kirk, W.D.J. & Terry, L.I. (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology*, **5**, 301-310.

Kjer, K.M. (2004) Aligned 18S and insect phylogeny. *Systematic Biology*, **53**, 506-514.

Kristensen, N.P. (1991) Phylogeny of Extant Hexapods. *Insects of Australia*, Vol. 1, (ed. by I.D. Naumann, P.B. Carne, J.F. Lawrence, E.S. Nielsen, J.P. Spradbery, R.W. Taylor, M.J. Whitten and M.J. Littlejohn), pp. 125-140. Melbourne University Press, Carlton, Victoria.

Maddison, D.R. & Maddison, W.P. (2005) *MacClade 4: analysis of phylogeny and character evolution*. Sinauer Associations, Sunderland, MA.

Maddison, W.P. & Maddison, D.R. (2011) *Mesquite: a modular system for evolutionary analysis*. Version 2.75, http://mesquiteproject.org.

Morris, D.C. & Mound, L.A. (2003) Thysanoptera phylogeny - the molecular future. *Entomologische Abhandlungen*, **61**, 153-155.

Mound, L.A. (2001a) So many thrips-so few tospoviruses? *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*, 15-18.

Mound, L.A. (2001b) The *Thrips* and *Frankliniella* genus-groups: the phylogenetic significance of ctenidia. *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*, 379-386.

Mound, L.A. & Heming, B.S. (1991) Thysanoptera. *Insects of Australia*, Vol. 1, (ed. by I.D. Naumann, P.B. Carne, J.F. Lawrence, E.S. Nielsen, J.P. Spradbery, R.W. Taylor, M.J. Whitten and M.J. Littlejohn), pp. 510-515. Melbourne Univeristy Press, Carlton, Victoria.

Mound, L.A., Heming, B.S. & Palmer, J.M. (1980) Phylogenetic relationships between the families of recent Thysanoptera. *Zoological Journal of the Linnean Society of London*, **69**, 111-141.

Mound, L.A. & Marullo, R. (1996) The Thrips of Central and South America: an Introduction. *International Memoirs on Entomology*, **6**, 1-488.

Mound, L.A. & Morris, D.C. (2004) Thysanoptera Phylogeny-The morphological background. *Acta Phytopathologica et Entomologica Hungarica*, **39**, 101-113.

Mound, L.A. & Morris, D.C. (2007) The insect Order Thysanoptera: Classification versus Systematics. *Zootaxa*, **1668**, 395-411.

Mound, L.A. & O'Neill, K. (1974) Taxonomy of the Merothripidae, with ecological and phylogenetic considerations (Thysanoptera). *Journal of Natural History*, **8**, 481-509.

Mound, L.A. & Palmer, J.M. (1983) The generic and tribal classification of spore-feeding Thysanoptera (Phlaeothripidae: Idolothripinae). *Bulletin of The British Museum (Natural History) Entomology*, **46**, 1-174.

Nixon, K.C. (1999) The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics*, **15**, 407-414.

Pereyra, V. & Mound, L.A. (2009) Phylogenetic relationships within the genus *Cranothrips* (Thysanoptera, Melanthripidae) with consideration of host associations and disjunct distributions within the family. *Systematic Entomology*, **34**, 151-161.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253-1256.

Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.5*.

Reitz, S.R. (2009) Biology and ecology of the Western Flower Thrips (Thysanoptera: Thripidae): The making of a pest. *Florida Entomologist*, **92**, 7-13.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **29**, 359-370.

Shao, R. & Barker, S.C. (2003) The highly rearranged Mitochondrial Genome of the Plague Thrips, *Thrips imaginis* (Insecta: Thysanoptera): Convergence of two novel gene boundaries and an extraordinary arrangement of rRNA genes. . *Molecular Biology and Evolution*, **20**, 362-370.

Shao, R., Campbell, N.J.H., Schmidt, E.R. & Barker, S.C. (2001) Increased rate of gene rearrangement in the Mitochondrial genomes of three Orders of Hemipteroid Insects. *Molecular Biology and Evolution*, **18**, 1828-1832.

Shao, R., Dowton, M., Murrell, A. & Barker, S.C. (2003) Rates of gene rearrangement and nucleotide substitution are correlated in the Mitochondrial genomes of Insects. *Molecular Biology and Evolution*, **20**, 1612-1619.

Sharov, A.G. (1972) The Phylogenetic relations of the Order Thysanoptera. *Entomological Review of Washington*, **51**, 506-508.

Sorenson, M.D. & Franzosa, E.A. (2007) *TreeRot, Version 3*. Boston University, Boston, Massachusetts.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688-2690.

Stannard, L.J. (1952) Peanut-winged thrips. *Annals of the Entomological Society of America*, **45**, 327-330.

Stannard, L.J. (1957) *The Phylogeny and Classification of the North American Genera of the Suborder Tubulifera (Thysanoptera)*. Vol 25. University of Illinois Press, Urbana.

Swofford, D.L. (2002) *PAUP* Phylogenetic Analysis Using Parsimony (and other methods)*. Sinauer Associations, Sunderland, Boston, Massachusetts.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731-2739.

Wheeler, W.C., Whiting, M., Wheeler, Q.D. & Carpenter, J.M. (2001) The phylogeny of the extant hexapod orders. *Cladistics*, **17**, 113-169.

Whiting, M.F. (2002) Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta*, **31**, 93-104.

Yoshizawa, K. & Saigusa, T. (2001) Phylogenetic analysis of paraneopteran orders (Insecta : Neoptera) based on forewing base structure, with comments on monophyly of Auchenorrhyncha (Hemiptera). *Systematic Entomology*, **26**, 1-13.

Zherikhin, V.V. (2002) Order Thripida. *History of Insects*, (ed. by A.P. Rasnitsyn and D.L.J. Quicke), pp. 133-143. Kluwer Academic Publishers, Norwell, Massachusetts

Figures and Tables

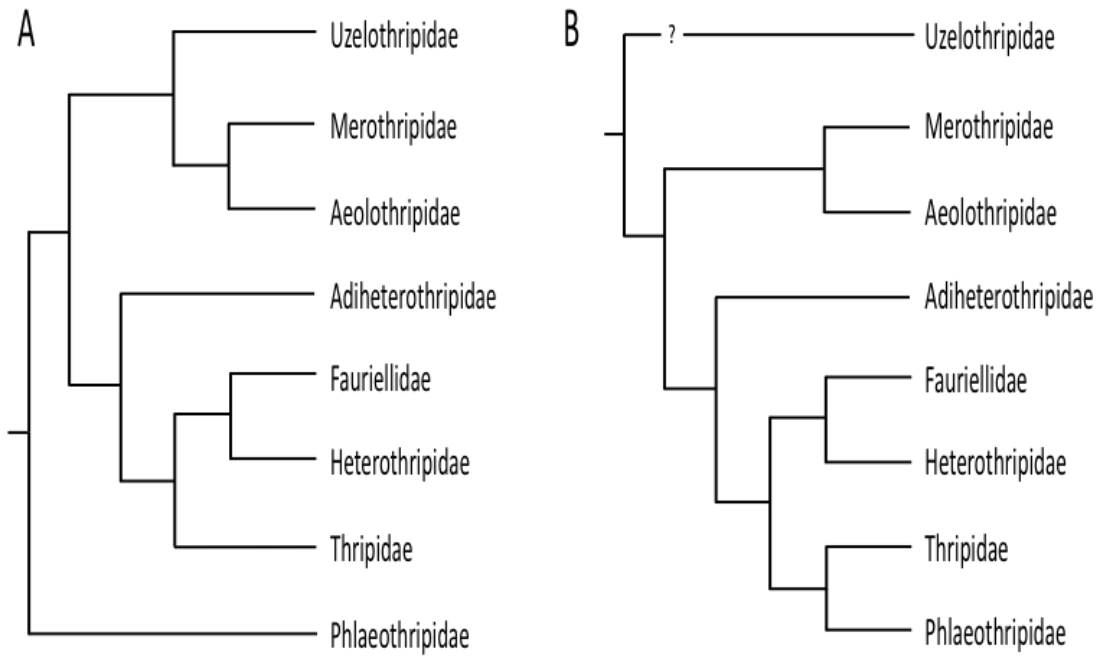

Figure 1: Proposed hypothesis of Mound et al. (1980). In this figure the Melanthripidae were still included in the Aeolothripidae and the Stenurothripidae is referred to as the Adiheterothripidae. (B) Phlaeothripidae is actually nested within Thripidae predicted to be sister to Panchaetothripinae.

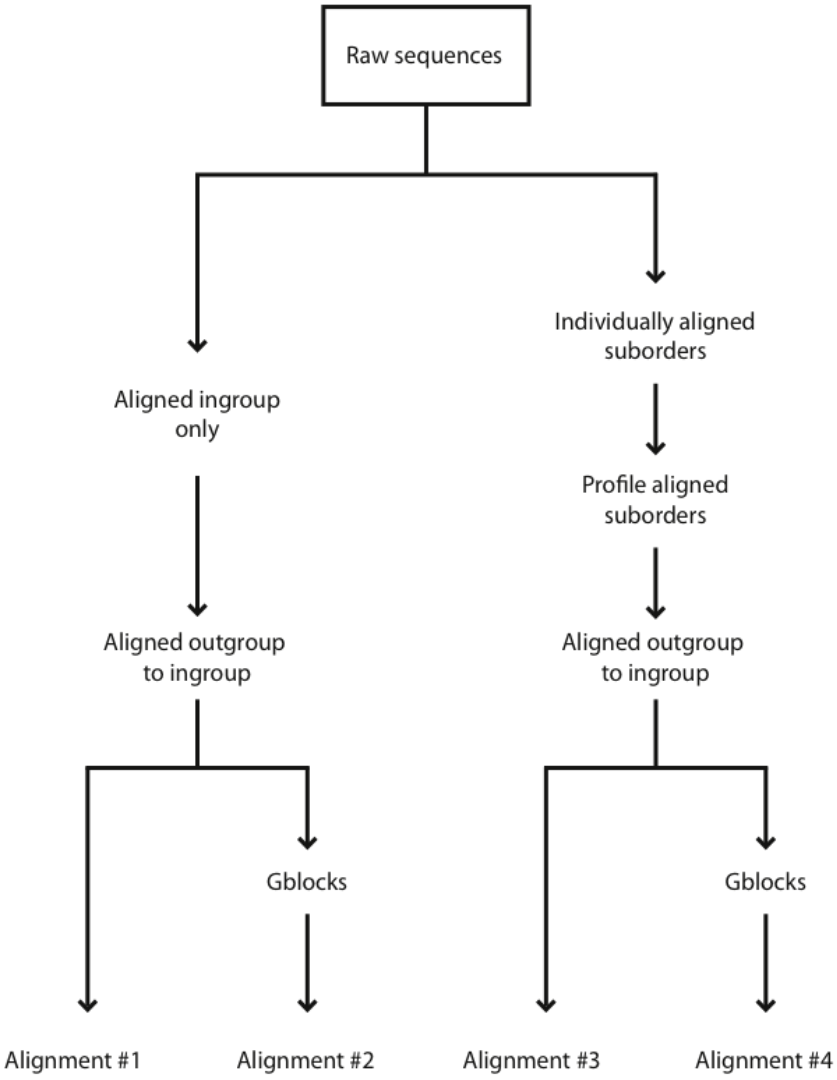

Figure 2: A flow chart showing the production of the four alignments made for both 18S and 28S rDNA.

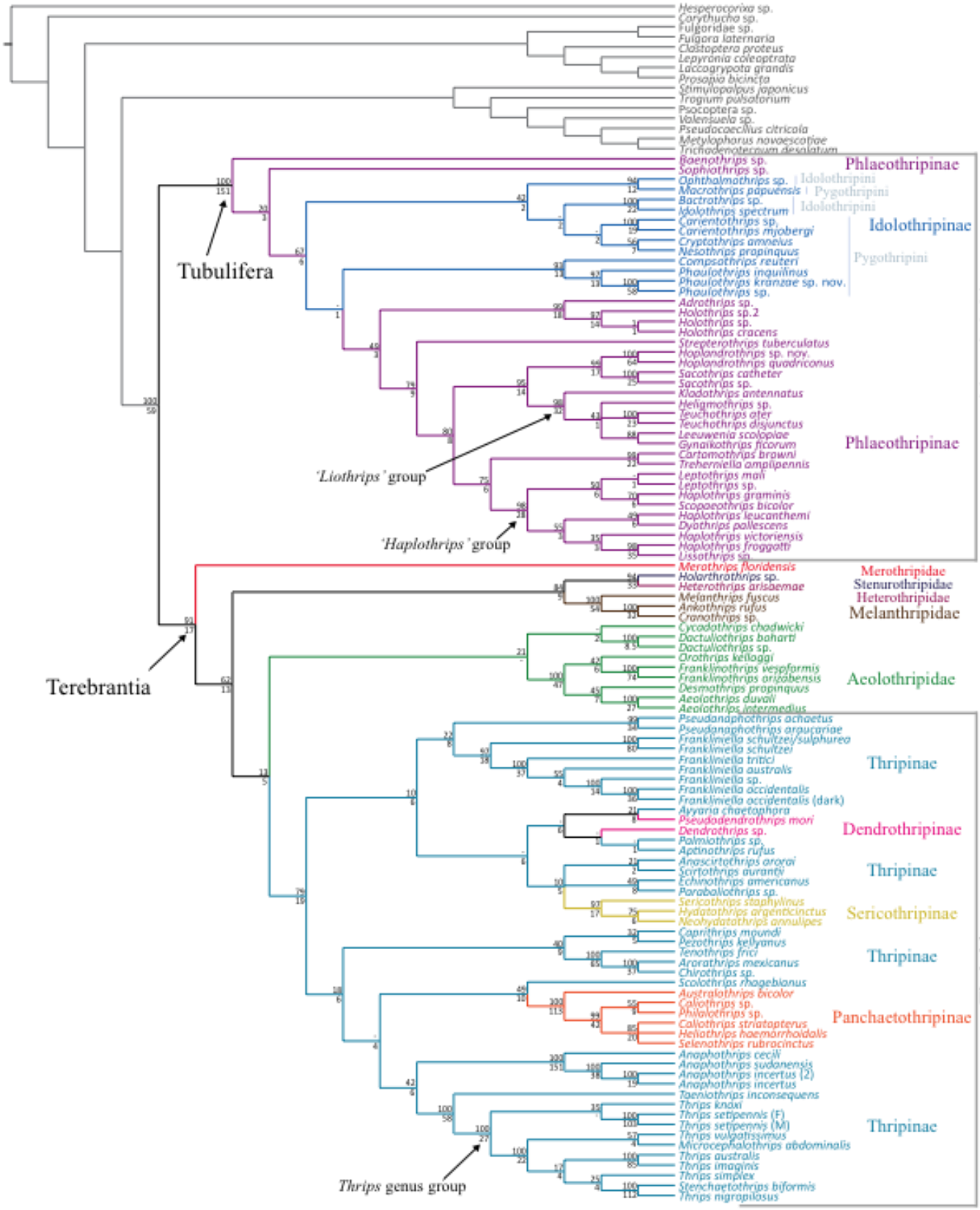

Figure 3: Strict consensus of eight most parsimonious trees found in TNT. At the nodes, the number above is the bootstrap value and the combined partitioned Bremer support is below.

Figure 4: Tree from the Maximum Likelihood analysis (RAxML) with bootstrap values.

Figure 5: Tree constructed from the Bayesian analysis. Node numbers are the posterior probabilities.

Table 1. Taxa sampled.

Taxa included in 18S, 28S, H3, TubA, and COI nucleotide sequence data sets. Classification follows that of Mound (2011).

Taxon	Specimen Code[a]	Geographical region	18S	28S	H3	TubA	COI
Terebrantia							
Aeolothripidae							
Aeolothrips duvali	TH96	USA: NM	X	X	X	-	X
Aeolothrips intermedius	TH124	England	X	X	X	X	X
Cycadothrips chadwicki	TH11	AUS: NSW	X	X	X	X	X
Dactuliothrips boharti	TH73	USA: CA	X	X	X	-	X
Dactuliothrips sp.	TH15	USA: AZ	X	X	X	X	X
Desmothrips propinquus	TH12	AUS: ACT	X	X	X	X	X
Franklinothrips orizabensis	TH13	USA: CA	X	X	X	X	X
Franklinothrips vespformis	TH106	Netherlands[1]	X	X	X	-	X
Orothrips kelloggi	TH14	USA: AZ	X	X	X	-	X
Heterothripidae							
Heterothrips arisaemae	TH46	USA: MD	X	X	X	X	X
Melanthripidae							
Ankothrips rufus	TH70	USA: UT	X	X	X	X	X
Cranothrips sp.	TH20	AUS: NT	X	X	X	X	X
Melanthrips fuscus	TH49	Crete	X	X	X	X	X
Merothripidae							
Merothrips floridensis	TH47	AUS: ACT	X	X	X	X	X
Stenurothripidae							
Holarthrothrips sp.	TH48	Israel	X	X	X	X	X
Thripidae							
Dendrothripinae							
Dendrothrips sp.	TH63	France	X	X	X	X	-
Pseudodendrothrips mori	TH16	Chile	X	X	X	X	X
Panchaetothripinae							
Australothrips bicolor	TH60	-	X	X	X	-	X
Caliothrips sp.	TH62	AUS: QLD	X	X	X	X	X
Caliothrips striatopterus	TH128	AUS: WA	X	-	X	X	X
Heliothrips haemorrhoidalis	TH21	AUS: ACT	X	-	-	X	X
Philalothrips sp.	TH61	AUS: WA	X	X	X	X	X
Selenothrips rubrocinctus	TH22	AUS: WA	X	X	X	X	X
Sericothripinae							
Hydatothrips argenticinctus	TH28	AUS: SA	X	X	X	X	-

Table 1. Continued

Taxon	Specimen Code[a]	Geographical region	18S	28S	H3	TubA	COI
Neohydatothrips annulipes	TH29	USA: IL	X	X	X	X	X
Sericothrips staphylinus	TH30	AUS: TAS	X	X	X	X	X
Thripinae							
Anaphothrips cecili	TH31	AUS: SA	X	X	X	X	-
Anaphothrips incertus (1)	TH66	AUS: QLD	X	X	X	X	-
Anaphothrips incertus (2)	TH116	AUS: ACT	X	X	X	X	-
Anaphothrips sudanensis	TH121	AUS: WA	X	X	X	X	X
Anascirtothrips arorai	TH129	AUS: WA	X	X	X	X	-
Aptinothrips rufus	TH69	AUS: ACT	X	X	X	X	X
Arorathrips mexicanus	TH32	AUS: NSW	X	X	X	-	X
Ayyaria chaetophora	TH33	Vietnam	X	X	X	X	-
Caprithrips moundi	TH67	AUS: ACT	X	X	X	-	-
Chirothrips sp.	TH82	USA: UT	X	X	X	X	X
Echinothrips americanus	TH64	-	X	X	X	X	EF467234
Frankliniella australis	TH34	Chile	X	X	X	X	X
Frankliniella occidentalis	TH35	Chile	X	X	X	X	X
Frankliniella occidentalis (dark)	TH71	USA: UT	X	X	X	X	X
Frankliniella schultzei	TH36	AUS: ACT	X	X	X	X	X
Frankliniella schultzei/sulphurea	TH130	AUS WA	X	X	X	X	X
Frankliniella sp.	TH103	USA: UT	X	X	X	X	-
Frankliniella tritici	TH97	USA: NC	X	X	X	X	X
Microcephalothrips abdominalis	TH38	AUS: ACT	X	X	X	X	X
Palmiothrips sp.	TH65	Israel	X	X	-	X	X
Parabaliothrips sp.	TH68	AUS: NSW	X	X	-	X	-
Pezothrips kellyanus	TH37	Sicily	X	X	X	X	X
Pseudanaphothrips achaetus	TH111	AUS: TAS	X	X	X	-	X
Pseudanaphothrips araucariae	TH39	AUS: QLD	X	X	X	X	-
Scirtothrips aurantii	TH40	South Africa	X	X	X	X	EU100995
Scolothrips rhagebianus	TH113	AUS: WA	X	X	X	X	X
Stenchaetothrips biformis	TH41	Taiwan	X	X	X	X	
Taeniothrips inconsequens	TH72	USA: UT	X	X	X	X	X
Tenothrips frici	TH42	AUS: ACT	X	X	X	X	X
Thrips australis	TH131	AUS: ACT	X	X	X	X	X
Thrips imaginis	TH43	AUS: ACT	X	X	X	-	AF335993
Thrips knoxi	TH110	AUS: NSW	X	X	-	X	X
Thrips nigropilosus	TH44	USA: SC	X	X	X	X	AM932052
Thrips setipennis (M)	TH105	AUS: NSW	X	X	X	X	X

Table 1. Continued

Taxon	Specimen Code[a]	Geographical region	18S	28S	H3	TubA	COI
Thrips setipennis (F)	TH107	AUS: NSW	X	X	X	X	X
Thrips simplex	TH45	AUS: ACT	X	X	X	X	X
Thrips vulgatissimus	TH104	England	X	X	X	X	X
Tubulifera							
Phlaeothripidae							
Idolothipinae							
Bactrothrips sp.	TH90	AUS: VIC	X	X	X	X	X
Carientothrips mjobergi	TH117	AUS: ACT	X		X	X	-
Carientothrips sp.	TH91	AUS: VIC	X	X	X	X	-
Compsothrips reuteri	TH88	India	X	X	X	X	X
Cryptothrips amneius	TH114	AUS: ACT	X	X	X	X	X
Idolothrips spectrum	TH18	AUS: SA	X	X	X	X	-
Macrothrips papuensis	TH86	PNG[2]	X	-	X	X	-
Nesothrips propinquus	TH52	AUS: ACT	X	X	X	X	-
Ophthalmothrips sp.	TH79	South Africa	X	X	X	X	X
Phaulothrips inquilinus	TH19	AUS: SA	X	-	X	X	X
Phaulothrips kranzae sp. nov.	TH126	AUS: SA	X	X	X	X	-
Phaulothrips sp.	TH94	AUS: VIC	X	X	X	X	-
Phlaeothripinae							
Adrothrips sp.	TH125	AUS: NSW	X	X	X	X	X
Baenothrips sp.	TH58	AUS: ACT	X	X	X	X	-
Cartomothrips browni	TH115	New Zealand	X	X	X	X	X
Dyothrips pallescens	TH24	Thailand	X	X	X	X	X
Gynaikothrips ficorum	TH23	AUS: QLD	X	X	-	X	X
Haplothrips froggatti	TH55	-	X	X	X	X	X
Haplothrips graminis	TH84	USA: FL	X	X	X	-	X
Haplothrips leucanthemi	TH83	USA: MT	X	X	X	X	X
Haplothrips victoriensis	TH109	AUS: TAS	X	X	X	X	X
Heligmothrips sp.	TH123	AUS: NSW	X	X	-	X	X
Holothrips cracens	TH51	India	X	X	X	X	X
Holothrips sp.1	TH50	AUS: QLD	X	X	X	X	X
Holothrips sp.2	TH93	AUS: VIC	X	X	-	X	X
Hoplandrothrips sp. nov.	TH59	-	X	X	X	X	X
Hoplandrothrips quadriconus	TH119	AUS: ACT	X	X	X	X	X
Kladothrips antennatus	TH57	AUS: WA	X	X	X	X	X
Leewenia scolopiae	TH25	AUS: QLS	X	X	X	X	X

Table 1. Continued

Taxon	Specimen Code[a]	Geographical region	18S	28S	H3	TubA	COI
Leptothrips mali	TH81	USA: UT	X	X	X	X	X
Leptothrips sp.	TH99	USA: VA	X	X	X	X	X
Lissothrips sp.	TH56	-	X	X	X	X	-
Sacothrips catheter	TH112	AUS: QLD	X	X	X	X	-
Sacothrips sp.	TH26	AUS: NSW	X	X	X	X	X
Scopaeothrips bicolor	TH120	Mexico[3]	X	X	X	X	X
Sophiothrips sp.	TH54	India	X	X	X	X	-
Strepterothrips tuberculatus	TH122	AUS: WA	X	X	X	X	X
Teuchothrips disjunctus	TH27	AUS: ACT	X	X	X	X	X
Teuchothrips ater	TH92	AUS: VIC	X	X	X	X	X
Treherniella amplipennis	TH98	USA: FL	X	X	X	X	X
Outgroups							
Hemiptera							
Cercopidea							
Aphrophoridae							
Lepyronia coleopterata	n/a		AY744782	AY744816	AY744854	-	GU446982
Cercopidae							
Laccogrypota grandis	n/a		GU446823	GU446913	GU447146	-	GU447001
Prosapia bicincta	n/a		AY744789	AY744823	AY744861	-	GU446987
Clastopteridae							
Clastoptera proteus	n/a		AY744781	AY744815	AY744853	-	GU446981
Fulgoroidea							
Fulgoridae							
Fulgoridae sp.	HP26	USA: LA	X	X	X	-	X
Fulgora laternaria	n/a	French Guiana	EU645792	EU645859	EU645915	-	-
Heteroptera							
Corixidae							
Hesperocorixa sp.	HP27	USA: UT	DQ133581	DQ133586	X	X	X
Tingidae							
Corythucha sp.	HP28	USA: CO	DQ133582	DQ133587	X	X	X
Psocoptera							
Psocoptera sp.	PS02		X				
Caeciliusoidea							
Caeciliusidae							
Valenzuela sp.	PS01		AF423793	-	X	X	-

Table 1. Continued

Taxon	Specimen Code[a]	Geographical region	18S	28S	H3	TubA	COI
Psocomorpha							
Pseudocaeciliidae							
Pseudocaeilius citricola	n/a		AY630527	-	GU569321	-	-
Psocidae							
Metylophorus novaescotiae	n/a		AY630558	-	EF662154	-	-
Trichadenotecnum desolatum	n/a		EF662297	-	EF662182	-	-
Troctomorpha							
Amphientomidae							
Stimulopalpus japonicus	n/a		AY630459	-	GU569345	-	-
Trogiomorpha							
Trogiidae							
Trogium pulsatorium	n/a		AY630453	-	DQ104786	-	-

[a]Brigham Young University Insect Genomic Collection
[1]From a reared colony. ENTOCARE CV Wageningen, Netherlands.
[2]PNG = Papua New Guinea
[3]Found on plants in Australian quarantine imported from Mexico.

Table 2. Primers used organized by gene and then primer pairs used for amplification

Primers	Direction	Sequence (5' ⇒ 3')	Annealing Temp. (C)	Elongation Time
18S rDNA				
18S 1.2F	Forward	Whiting (2002)	49°	1:15
18S THb2.9	Reverse	TAT CTG ATC GCC TTC RAA CCT C		
18S THa0.7	Forward	GCT CGT AGT TGG ATC TGT GY	54°	1:15
18S THbi	Reverse	GTT AGY AGG YTA GAG TCT CGT TCG		
18S a2.0	Forward	Whiting (2002)	50°	1:15
18S 9R	Reverse	Whiting (2002)		
28S rDNA				
28S THrd1a	Forward	AAG GAT TCC STY AGT AGC GG	52°	1:15
28S B	Reverse	Whiting (2002)		
28S THrd3a*	Forward	CAC AAG TAC CGT GAG GGA AA		
28S THrd3b*	Reverse	TTT CCC TCA CGG TAC TTG TG		
28S A	Forward	Whiting (2002)	46°	1:15
28S rD7b1	Reverse	Whiting (2002)		
28S Rd4.8a*	Forward	Whiting (2002)		
28S rD5b*	Reverse	Whiting (2002)		
Histone 3				
H3 AF	Forward	Colgan *et al.* (1998)	52°	0:45
H3 AR	Reverse	Colgan *et al*. (1998)		
Tubulin Alpha[1]				
DDVTubAF	Forward	GAR CCC TAC AAY TCY ATT CT	50°	0.50
DDVTubAR	Reverse	GAA ACC RGT KGG RCA CCA GTC		
TH_TubAF	Forward	ACA YTC VGA YTG YGC CTT CAT GG	58°	0:45
TH_TubAR	Reverse	CGG TAC ARG AKR CAG CAV GCC AT		
Cox I				
LCO1490	Forward	Folmer *et al.* (1994)	49°	1:00
HCO2198	Reverse	Folmer *et al.* (1994)		

*Internal primers used only during sequencing.
[1]Nested PCR. Only the nested primers were used for sequencing.