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Phylogenetics of Australian *Acacia* thrips: the evolution of behaviour and ecology

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Abstract

The species of thrips found on *Acacia* constitute a major component of the Australian thrips fauna, with at least 235 species in more than 30 genera, many of these being in the process of description as new. These thrips exhibit social behaviours, ranging from solitary and colonial species to a variety of more complex social organisations. Furthermore, the domiciliary habits of these species include domicile construction, gall induction, and opportunistic use of abandoned galls and domiciles. This suite of thrips also includes a variety of inquiline and kleptoparasitic taxa. To understand how these various traits have evolved and interact in this diverse group, we have reconstructed a phylogeny for 42 species of thrips associated with *Acacia* around Australia. We obtained DNA sequence data from two nuclear genes (*Elongation Factor-1 α* and *wingless*) and one mitochondrial gene (*cytochrome oxidase I*) and analysed these using maximum parsimony and maximum likelihood methods. A phylogeny resulting from such analysis allows inference of evolutionary transitions in domiciliary habits, social organisations, and parasitic behaviours. Gall induction and parasitic behaviour are postulated to each have a single origin, with no losses of either trait. Once parasitism evolved a remarkable radiation followed that allowed exploitation of very diverse hosts. Our data do not allow hypotheses of single versus multiple origins of domicile building to be resolved while opportunistic gall use appears to have arisen several times.

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1. Introduction

With almost the same land area but three times the number of vascular plant species as the United States of America, Australia is one of the most biologically diverse countries on this planet. Since much of the land is without roads or habitations, and thus logistically difficult to work in, any investigation of this biological diversity, both describing the taxa and examining their ecological and evolutionary relationships, involves considerable problems. This paper concerns one group of insects associated with one genus of plants: a particular suite of taxa of the Thysanoptera subfamily Phlaeothripinae found only on *Acacia* species. *Acacia* is the most species-rich plant genus in Australia and is distributed

throughout the continent, with approximately 1000 endemic species (Maslin, 2001). The phlaeothripine thrips associated with the non-floral tissues of these plants comprise more than 230 species, with this total comprising about one-quarter of the Thysanoptera species currently known (although not yet formally described) from Australia. Comprehensive biodiversity studies of such scope clearly require many years of observation by a range of different biologists with considerable funding. The results given here, based on studies over the past 5 years, are clearly preliminary, but the remarkable interplay that they indicate between insect behaviour, insect/plant co-speciation, insect host-plant shifts, as well as other biotic factors in this harshly arid environment, suggests that further studies on this system will produce much information of general biological interest.

The vast majority of Australian *Acacia* species do not have the bipinnate leaves that are typical of leguminous plants, but instead bear phyllodes, leaf-like expansions of the petioles. Three major series of these phyllodinous

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Acacia are recognised, Section Phyllodineae with about 390 species, Section Juliflorae with about 235, and Section Plurinerves with about 215 (Maslin, 2001). However, the thrips association with *Acacia* is strongly asymmetric, being almost exclusively with the members of the Juliflorae and Plurinerves. Similarly, the thrips found on *Acacia* do not constitute a random sample from the available fauna. Although the 230 or more species of phlaeothripine species involved are all phytophagous, they apparently form a single lineage that is distinct from the many phytophagous Phlaeothripinae found on other plants in this country.

Despite constituting a single lineage, the Phlaeothripinae on Australian *Acacia* are remarkably diverse, both in body structure and biology. Thrips in one group induce various *Acacia* species to produce galls on phyllodes, whereas those in a second more diverse group construct domiciles by glueing or sewing together two or more phyllodes. Protected spaces such as galls or domiciles are a valuable resource in areas of extreme aridity and insolation hence, a third group of thrips species includes kleptoparasites or inquiline that usurp this resource. However, by far the largest group is a diverse series of opportunist species that invade abandoned domiciles, including many produced by larval Lepidoptera and Coleoptera through mining or sewing together phyllodes. These thrips show a high level of host-specificity, regardless of their biology. There is weak evidence, both molecular and morphotaxonomic, that at least some of the species we currently consider to be polyphagous actually comprise series of host-specific siblings.

Descriptive taxonomy is only a first step in biodiversity studies. Without knowledge of the phylogenetic relationships between taxa, it is not possible to assess hypotheses about the evolution of behavioural and life history traits that they exhibit nor consider how labile or plastic such traits might be. A phylogeny makes it possible to determine the origins and losses of biological traits and to develop ideas about selective factors that may have influenced their evolution. Our objective here was to produce a phylogeny, parallel to our morphotaxonomic studies, based on a molecular data set derived from one mitochondrial and two nuclear gene fragments, and to map onto this phylogeny the various life history and behavioural traits. Many of the morphotaxa that are under description are known from few specimens, but molecular data were collected from 42 of the 235 species we recognise at present, including almost all of the major sub-lineages.

Various recent studies have examined social behaviour in Thysanoptera (Crespi et al., 1998; Crespi and Mound, 1997; Tsuchida and Ohguchi, 1998). Crespi (1992b) suggested that the evolution of eusociality in thrips may have been associated with haplodiploidy, but phylogenetic studies show that high levels of inbreeding

were present at the origin of eusociality in thrips, and the effect of inbreeding is likely to overwhelm the effect of haplodiploidy in lowering thresholds for altruistic behaviour (Chapman and Crespi, 1998; Chapman et al., 2000). Apart from eusociality, thrips have importance for evolutionary studies because of a number of other traits that are relevant to understanding the evolution of life history strategies (Kranz, 2000).

The 235 species of phlaeothripine thrips that we currently recognise from *Acacia* in Australia can be assigned to the following series of ecological or behavioural suites (Mound and Moritz, 2000). These suites are defined by the behavioural or ecological traits that are observed when collecting the insects in the field, and therefore provided a classification system by which the thrips fauna could be divided into manageable groups, given the lack of existing behavioural or taxonomic information. These suites are not intended to imply monophyly or represent any natural grouping of these taxa, but provide convenient classes to which questions of monophyly or evolution of traits may be applied. The reason that these suites are useful is because it appears that most of the Australian *Acacia* species that are hosts to thrips will have at least one representative from each of these suites present. That is to say, each host *Acacia* will have one or two species of gall-inducers, one or more domicile-building species, several parasitic species, and several opportunistic species, many of which appear to be specific to that host. Very few of the *Acacia* species that have gall-inducing thrips do not have a domicile-building thrips species and there are only two cases of hosts that have a domicile-building thrips but no gall-inducing species.

- (i) The gall inducers comprise 21 described species among three genera, *Kladothrips*, *Oncothrips*, and *Onychothrips*, and all species induce fully enclosed galls on the phyllodes of various *Acacia* species (Mound et al., 1996). Of the 21 species, six are known to have a gall-bound 'soldier' caste that defends the gall from kleptoparasites, and this, coupled with differential reduced fecundity in the gall-bound morph, suggests that these species are eusocial (Crespi, 1992b; Mound and Crespi, 1995). However, one species has similar 'soldier' morphs that lack lowered fecundity relative to the foundress (Kranz et al., 2001) and a further species produces very large numbers of a wingless morph that apparently does not function as soldiers.
- (ii) The domicile builders comprise many species that glue or tie phyllodes (leaf-like modified petioles) of the host *Acacia* species with an anal secretion to create an enclosed domicile. They include genera, such as *Carcinothrips*, *Dunatothrips*, *Lichanothrips*, and *Panoplothrips*, in which species employ a range of methods for constructing domiciles. This domicile-building behaviour only occurs within the

thrips associated with *Acacia* and has not been observed in any other thrips lineage. The diversity of methods for constructing domiciles may be the result of multiple origins of domicile building or diversification and speciation within a single lineage. All domicile builders produce their brood within the confines of the domicile and the founders often survive to produce more offspring once the first cohort has become adult. Other behavioural traits found within this suite are coordinated group foraging and co-founding of colonies (Crespi, 1992a; Crespi and Mound, 1997; Morris et al., 2002).

- (iii) The parasitic thrips include two broad categories of host utilisation. Some species can live as inquilines or commensals in the domiciles of their hosts with little or no apparent harm to the hosts (Morris et al., 2000; Mound and Morris, 2000), whereas others invade domiciles or galls and either eject or kill the original occupants (Crespi, 1992a). Unfortunately, the species in this suite are infrequently collected and thus there is very little behavioural information to reliably distinguish between the two groups. While some species can be readily recognised as kleptoparasites, and others as inquilines, there remain a number of genera and taxa for which such designations are unclear (Mound and Morris, 2000). The morphological variation in this suite is remarkable, ranging from small species that are relatively innocuous in structure, such as *Viciniothrips bullatus* Mound and Morris (2000), to large species with remarkable body armature, such as *Xaniothrips leukandrus* Mound and Morris (1999a,b).
- (iv) The fourth suite of *Acacia* thrips includes a diverse range of at least 100 opportunistic species that utilise abandoned domiciles such as galls or phyllode glues, empty lepidopteran leaf-ties and leaf mines, and other similar niches. The species in this last suite are usually solitary, but if a niche persists long enough individuals of a range of ages may be produced. This suite includes *Dactylothrips*, *Grypothrips*, *Rhopalothripoides*, and *Warithrips*, that appear in this study, as well as genera such as *Akainothrips*, *Csirothrips*, *Katothrips*, and *Kellyia* for which no data were available for this study.

Previous phylogenies of these thrips taxa using morphological data were not robust (Morris et al., 1999), due largely to the paucity of reliable synapomorphic morphological characters. Two trends in the evolution of thrips, intra-specific variation associated with sex and body size, and loss of character states due to wing loss and reduction in body size, make detailed morphological comparisons difficult. Taxonomists sometimes interpret the absence of a particular structure as an apomorphy, but such 'loss apomorphies' can be homoplasious (Mound et al., 1980). One result of the lack of reliable characters is that there is no satisfactory tribal-

level taxonomic structure for the sub-family Phlaeothripinae and little evidence to indicate relationships between many genera within the sub-family.

2. Materials and methods

2.1. Taxon sampling

In this study, we were able to obtain adequate sequence material for representatives of 14 of the 17 described Australian thrips genera recorded from *Acacia*. However, many species are newly revealed and currently undescribed; others could not be confidently assigned to current genera and some are tentatively assigned to new genera. These putative new genera have been assigned temporary codes for the purposes of this study (e.g., DOME1, GLARID1, TRIAD1, and PARACH); the intention is to describe these formally in later papers. The intention of this paper is not to address the taxonomy of the suite of species associated with *Acacia*, rather to provide a framework for future studies into the ecology, behaviour, evolution, and systematics of these species.

Sequences from at least two species from each genus were sought, although one or more representatives may be undescribed. Voucher specimens of all taxa have been deposited in the Australian National Insect Collection (ANIC) at CSIRO Entomology, Canberra.

Although there is no satisfactory tribal structure within the subfamily Phlaeothripinae, three lineages have been proposed (Mound, 1994). As far as it can be determined, all *Acacia* thrips fall into the *Liothrips* lineage and outgroup selection had to be from among one of the other genera in this lineage. The selected outgroup was from the genus *Gynaikothrips*, which has also been used for similar previous phylogenetic studies (Crespi et al., 1998; Morris et al., 1999). Morphological analysis by Morris et al. (1999) indicates that this genus is not a member of the ingroup.

2.2. DNA extraction

Specimens collected, host-plant affiliations, and behavioural suite associations are given in Table 1. DNA was extracted from fresh, frozen, and ethanol-preserved material. DNA was extracted from fresh material using a phenol/chloroform protocol as described in Crespi et al. (1998). For frozen and ethanol-preserved material, DNA was extracted using Chelex 100 resin (Walsh et al., 1991). Specimens for Chelex extraction were homogenised in 25 µl Tris buffer (pH 8.0) and then 100 µl of 5% Chelex resin was added, prior to a 5-h incubation period at 55 °C. After incubation, samples were vortexed and then centrifuged for approximately 5 s at 10,000g before 10 min incubation

Table 1

Thrips species used for phylogenetic analyses in this study, with details of host-plants and the behavioural suite to which each thrips species belongs

Species	Collection No.	Host <i>Acacia</i>	Behavioural suite
<i>Advenathrips inquilinus</i>	DM470	<i>A. aneura</i>	Parasitic
TRIAD1	DM338	<i>A. harpophylla</i>	Parasitic
<i>Carcinothrips leai</i>	DM352	<i>A. kempeana</i>	Domicile builder
<i>Dactylothrips</i> sp.	DM448	<i>A. stowardii</i>	Opportunistic
<i>Dactylothrips</i> sp.	DM496a	<i>A. cambagei</i>	Opportunistic
<i>Dunatothrips aneurae</i>	DM335	<i>A. aneura</i>	Domicile builder
<i>Dunatothrips armatus</i>	DM476	<i>A. aneura</i>	Domicile builder
<i>Dunatothrips skene</i>	DM434a	<i>A. catenulata</i>	Domicile builder
<i>Dunatothrips vestitor</i>	DM437a	<i>A. aneura</i>	Domicile builder
<i>Sartrithrips popinator</i>	DM423	<i>A. torulosa</i>	Domicile builder
<i>Sartrithrips luctator</i>	DM441	<i>A. stowardii</i>	Domicile builder
<i>Grypothrips</i> sp.	DM372	<i>A. cana</i>	Opportunistic
<i>Grypothrips</i> sp.	DM457	<i>A. oswaldii</i>	Opportunistic
<i>Gynaikothrips</i> sp.	DM278	<i>Ficus platypoda?</i>	Outgroup
<i>Kladothrips rugosus</i>	DM396	<i>A. maranoensis</i>	Gall-inducer
<i>Kladothrips hamiltoni</i>	DM475a	<i>A. harpophylla</i>	Gall-inducer
<i>Kladothrips maslini</i>	DM131	<i>A. orites</i>	Gall-inducer
GLARID1	DM404	<i>A. shirleyi</i>	Parasitic
<i>Koptothrips</i> sp.	DM467	<i>A. calcicola</i>	Parasitic
<i>Koptothrips flavicornis</i>	DM486	<i>A. melvillei</i>	Parasitic
<i>Koptothrips xenus</i>	DM473a	<i>A. cambagei</i>	Parasitic
PARACH1	DM424	<i>A. microcephala</i>	Domicile builder
PARACH2	DM426	<i>A. tephрина</i>	Domicile builder
PARACH3	DM467b	<i>A. calcicola</i>	Domicile builder
<i>Lichanothrips pulchra</i>	DM401	<i>A. harpophylla</i>	Domicile builder
<i>Lichanothrips magnificus</i>	DM385a	<i>A. harpophylla</i>	Domicile builder
<i>Oncothrips antennatus</i>	DM500	<i>A. aneura</i>	Gall-inducer
<i>Oncothrips habrus</i>	DM487	<i>A. melvillei</i>	Gall-inducer
<i>Oncothrips morrisoni</i>	DM467a	<i>A. calcicola</i>	Gall-inducer
<i>Oncothrips rodwayi</i>	DM498	<i>A. melanoxydon</i>	Gall-inducer
<i>Onychothrips pilbara</i>	B. Crespi 'SS'	<i>A. citrinoviridis</i>	Gall-inducer
<i>Onychothrips tepperi</i>	DM446	<i>A. aneura</i>	Gall-inducer
<i>Onychothrips zygus</i>	DM454a	<i>A. pickardii</i>	Gall-inducer
<i>Panoplothrips australiensis</i>	DM412	<i>A. shirleyi</i>	Domicile builder
<i>Rhopalothripoides froggatti</i>	DM491	<i>A. melanoxydon</i>	Opportunistic
<i>Viciniothrips bullatus</i>	DM434	<i>A. catenulata</i>	Parasitic
<i>Warithrips acaciae</i>	DM472	<i>A. kempeana</i>	Opportunistic
<i>Warithrips</i> sp.	DM277	<i>A. rhodophloia</i>	Opportunistic
DOME1	DM411	<i>A. catenulata</i>	Opportunistic
<i>Xaniothrips foederatus</i>	DM370	<i>A. aneura</i>	Parasitic
<i>Xaniothrips mulga</i>	DM481	<i>A. aneura</i>	Parasitic
<i>Xaniothrips xantes</i>	DM419	<i>A. argyrodendron</i>	Parasitic

The collection numbers given in this table correspond to the slide-mounted vouchers deposited in the Australian National Insect Collection.

at 95°C. Samples were then vortexed again and centrifuged for 30 s at 10,000g before use in PCR amplifications.

2.3. Polymerase chain reaction (PCR) amplification and sequencing

For PCR amplifications we used the following protocol: 94°C, 1 min denaturation, 48–52°C, 45 s annealing, 72°C, 1 min extension for 35 cycles, with a final cycle of 5 min extension at 72°C and 10 s cooling at 26°C. The polymerase enzyme used was Amplitaq Gold (Perkin–Elmer), which required a 9-min denaturation step for the first cycle only, as instructed by the manufacturer. PCR mixtures consisted of 50 µl of 1× reaction

buffer (Amplitaq Gold), containing 4 mM MgCl₂, 0.8 mM dNTPs, 10 pmol of each primer, 2 µl template DNA solution, and 1 unit Amplitaq Gold Polymerase. PCR products were purified, prior to sequencing using the BRESAspin PCR Purification Kit (Geneworks) and then labelled using the ABI Big-dye Ready-Reaction Kit (Perkin–Elmer). Sequencing of PCR products was performed on an ABI 373 automated sequencer through the Institute of Medical and Veterinary Science, Adelaide.

The gene elongation factor-1α was selected for use in this study, as it has been shown to be very useful in previous studies of other insects (Cho et al., 1995; Danforth and Ji, 1998; Danforth et al., 1999; Friedlander et al., 1998). The initial primers used to obtain

EF-1 α products were M51.9 (5'-CARGACGTATACA AAATCGG-3') and rcM4 (5'-ACAGCVACKGTYTG YCTCATRTC-3') (Cho et al., 1995). These primers yielded a product of approximately 500 bp. In some cases, however, these products could not be sequenced unambiguously, most likely due to the presence of two products of the same length but different sequences. The two products were identified by running PCR products on polyacrylamide gels to separate bands of slightly different lengths. These bands were then cut from the gels and then purified to sequence the fragments. As noted by Danforth and Ji (1998), EF-1 α occurs as two copies in all Hymenoptera and Diptera. The presence of two products in our amplifications suggests that this gene duplication of EF-1 α may not be confined to the holometabolous insect orders but could be more widespread than that previously believed. To ensure that our amplifications only generated a single product, a primer was designed to be specific for only one of the two copies of EF-1 α present in our taxa. This primer, G346 (5'-AG ACTCAACACACATAGGTTGGAC-3'), was then used as a reverse primer paired with M51.9 and two other forward primers designed from our initial sequences. The other two forward primers used were G304 (5'-GTATTGGCACCGTACCCGTTGG-3'), which lies 23 bp downstream of M51.9, and G333 (5'-CAGGATG TCTACAAGATCGGTGG-3'), which is a modification of M51.9. The sequences obtained using these primers include, when aligned, 422 bp of coding sequence and an intron of about 100 bp. The intron appears to occur at a highly conserved point in the sequence, as an intron is found in the same location in the F2 copy of both *Apis* and *Drosophila*, and also in *Artemia* (Danforth and Ji, 1998), but more comprehensive sequence and phylogenetic analyses are required to properly assess the evolutionary origins of the F1 and F2 copies from thrips.

The initial primers for amplifying a part of the *wingless* gene were LepWG1 (5'-GARTGYAARTGYC AYGGYATGTCTGG-3') and LepWG2 (5'-ACTICGC ARCACCARTGGAATGTRCA-3') (Brower and DeSalle, 1998). Further primers were designed from sequences obtained using LepWG1 and LepWG2 and then used in addition to LepWG1 to amplify a segment of *wingless* from the majority of taxa. The additional *wingless* primers used were G338 (5'-CATAACCTGCT GCATGCGTC-3') 38 bp downstream of LepWG1 and G348 (5'-GTTCGGTATCCGCGTCCACA-3') 52 bp upstream of LepWG2.

The COI products were obtained using one of the two forward primers, C1-J-2183 (5'-CAACATTTATTTTGG ATTTTTTGG-3') or C1-J-2195 (5'-TTGATTTTTTGG TCATCCAGAAGT-3') (Simon et al., 1994). These primers were paired with A2735 (5'-AAAAATGTTGA GGGAAAATGTTA-3'), designed by B. Crespi. The products thus obtained consisted of 545 bp of coding sequence.

2.4. Phylogenetic analyses

Sequences were aligned using Clustal X version 1.8 (Thompson et al., 1997) and then imported into PAUP* versions 4.0b4 and 4.0b8 (Swofford, 2000) for analysis. While the coding region of EF-1 α contained no indels, the intron required the insertion of a large number of gaps to facilitate alignment. Various alignments were tried, including reiterated alignment of the intron residues alone (using the 'align selected residues' command in Clustal X) and adjustment of the gap opening (GOP) and gap extension parameters (GEP). These different alignments did not vary significantly in the number of parsimony-informative characters and none of the different alignments altered the resulting tree topology (for parsimony analyses). The COI data had no indels but the *wingless* data contained a number of novel insertions of 3 or 6 bp in length. These insertions were, in most cases, only present in one taxon and thus have little impact on our results. The sequences used in our analyses can be found in GenBank under Accession Nos. AF448280–348, AF288993–995, AF288997–000, AF289003, 004, 006, 007, 009, AF289012–015, 017, 019, AF386676, 678, 679, 681, AF386683–686, 688, AF386690–693, 699, 701, 702, 704, AF386706–709, 712, AF386714–717, 722, 724, 725, AF386727–6731, and AF386733–737.

Data sets were analysed independently with maximum parsimony (MP) methods. MP analyses were conducted on unweighted data using the heuristic search option with tree bisection–reconstruction (TBR) branch swapping and random addition of taxa (100 replicates per search, with 20 trees held at each step). Following this, each individual data set was subjected to statistical testing to determine the most appropriate model of evolution for use in maximum likelihood (ML) analyses, using Modeltest 3.0 (Posada and Crandall, 1998). Modeltest calculates a neighbour-joining tree from the data and then calculates the likelihood scores for this tree for a series of 56 increasingly complex models of DNA sequence evolution. The likelihood scores for each model are then compared using hierarchical likelihood ratio tests to determine which model best fits the data. Each data set was then analysed using the ML optimality criterion employing the model proposed by Modeltest.

Individual data sets were combined with each gene treated as a separate partition. This data set was tested for congruence of different partitions using the incongruence length difference (ILD) test (Cunningham, 1997; Farris et al., 1995) as implemented in PAUP* (500 reps). The combined data were subjected to MP and ML searches in the same manner as each individual data set. In addition to using an ML search using the model selected by Modeltest, a further search was performed to estimate site-specific rates for partitions within the data

set. The defined partitions were 1st, 2nd, and 3rd codon positions for each gene with the intron within EF-1 α also designated as a separate partition. The site-specific rates given for each partition allowed identification of those partitions with significantly higher rates of substitution and thus potentially be subject to saturation. Once saturated partitions were identified, we repeated parsimony and likelihood searches with these partitions excluded, to determine if the topology of the tree changed or if support for existing nodes was increased. When the data set had been modified in this manner, confidence measures for the nodes in the resulting trees were estimated using the bootstrap approach (Felsenstein, 1985), with values determined using 1000 heuristic search replicates for MP analyses and 30 replicates for ML analyses.

2.5. Hypothesis testing

To test hypotheses regarding monophyly of the behavioural suites represented in our analyses, we utilised the Shimodaira–Hasegawa (SH) test (Buckley et al., 2001; Goldman et al., 2000; Shimodaira and Hasegawa, 1999) as implemented in PAUP*. The SH test was used to compare the optimal tree resulting from ML analysis of our data set with trees that were obtained from ML analyses where the taxa from a given behavioural suite were constrained to be monophyletic. This then determines if the likelihood values for the two trees were significantly different.

3. Results

3.1. Separate analyses

DNA sequences for EF-1 α , *wingless*, and COI were obtained from 42 thrips species (see Table 1). The EF-1 α data set contains 544 characters of which 145 are parsimony-informative, the *wingless* data set contains 470 characters, with 155 being parsimony-informative, and the COI data set contains 545 characters, 225 of which are parsimony informative. The EF-1 α and *wingless* data sets resulted in 60 and 75 most parsimonious trees, respectively, and the COI data produced 22 most parsimonious trees. Analysis of these individual data sets using Modeltest 3.0 suggested that the most appropriate model of evolution for analysing the EF-1 α and *wingless* data sets was the TrN (Tamura and Nei, 1993) with a gamma-shape parameter (Γ) and proportion of invariable sites (I). The suggested model of evolution for the COI data set was the General Time Reversible (Rodríguez et al., 1990) with gamma-shape and invariable sites (GTR + Γ + I). The trees resulting from these ML analyses are shown in Fig. 1. For each of the separate data sets, the tree topology resulting from ML

analyses was very similar to that produced by parsimony analysis of the same data set. We used PAUP* to estimate likelihood scores ($-\ln$ likelihoods) for the MP trees and implemented the SH test to determine if the MP and ML trees were significantly different. In each case, the differences in likelihood scores between all trees (ML and MP) for any of the separate analyses were not significant. For this reason, the parsimony trees are not shown here. However, it is worth noting that one of these tests (comparison of trees from analysis of the *wingless* data set) found that one of the MP trees had the best likelihood score, implying that the ML analysis of this data may not have located the optimal tree. This indicates that ML analyses may also suffer from the “island effect” often found in MP analyses (Steel, 1994). There are substantial topological differences between trees resulting from each individual data set. The trees resulting from analysis of the EF-1 α data and the *wingless* data are broadly in agreement, with both data sets supporting four principal clades, each containing the same taxa. The primary differences between the EF-1 α and *wingless* topologies are in the placement of *Rhopalothripoides froggatti* and *Dactylothrips* (see Fig. 1). The trees resulting from analysis of the COI data are clearly different from those produced by analysis of the nuclear genes (EF-1 α and *wingless*). The disagreement between the COI data and the nuclear genes suggests that saturation may be causing information about the deeper nodes to be obscured by multiple substitutions at individual sites. Unlike the sequence data from the nuclear genes (EF-1 α and *wingless*) in which the base frequencies were approximately equal, the COI data showed a distinct bias towards purine bases (A and T). The observed proportion of A + T richness in the first and second codon positions was 65%, while third codon positions had 87% A + T. This type of base substitution bias is common for insect mitochondrial genes (Simon et al., 1994), can exacerbate the effects of multiple substitutions, and hasten saturation of the sequence data. One other potential reason for incongruence between the COI and nuclear gene trees is that a nuclear copy of the COI gene may have been amplified rather than the mitochondrial copy intended in some taxa. This would result in a tree that contains signal from sequences with different evolutionary histories, incongruent with trees based on homologous sequences. In our COI data, there is one sequence for which this may be the case (*Warithrips* DM277), as indicated by the significant difference in branch length for this taxon; however, we would not expect that a single, potentially erroneous, sequence would have an overwhelming effect on the results.

3.2. Combined analyses

Prior to combining the three data sets, we tested them for incongruence using the partition homogeneity or ILD

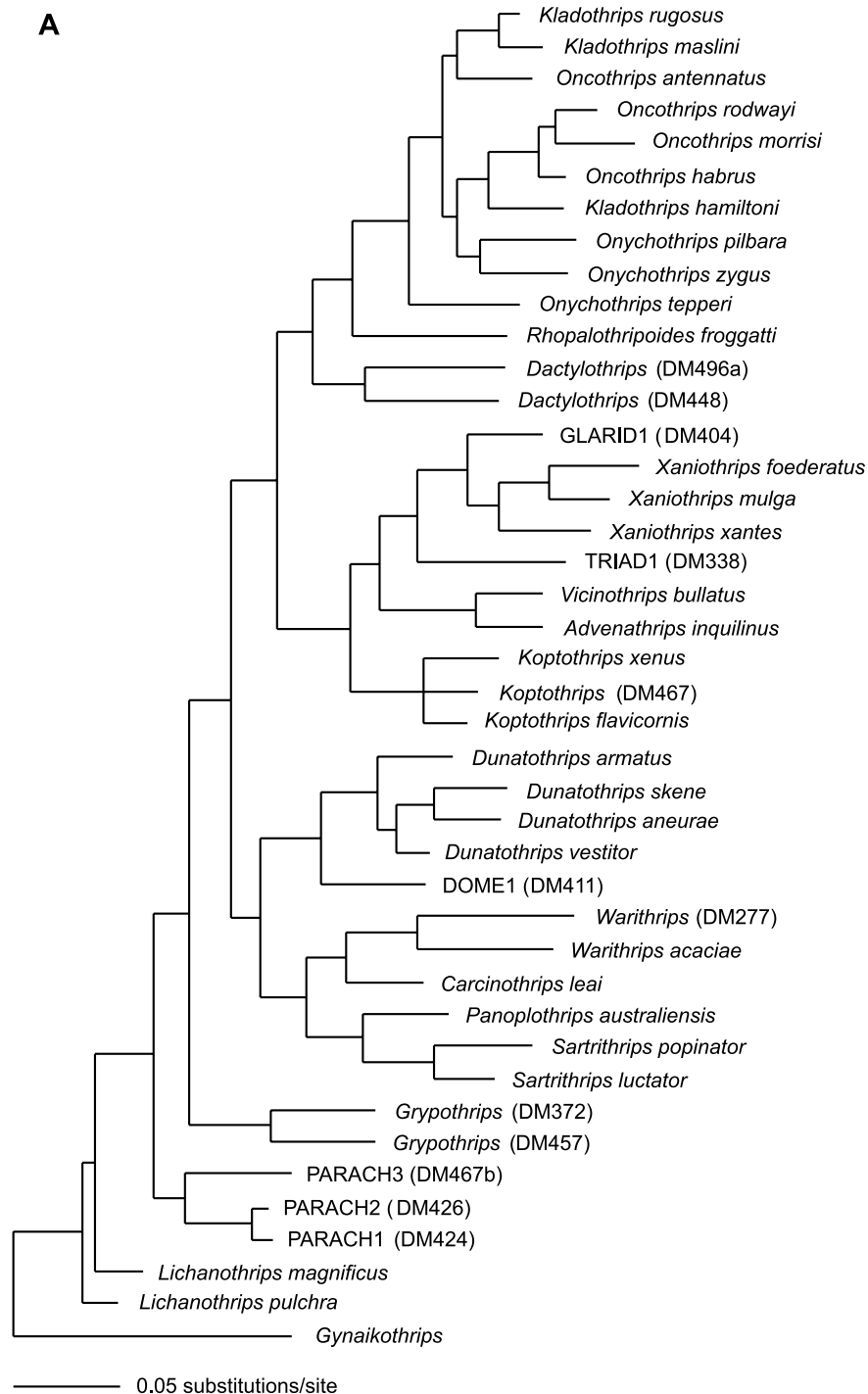


Fig. 1. Trees resulting from ML analyses of EF-1 α , *wingless*, and COI data sets: (A) tree resulting from TrN + I + G analysis of EF-1 α data, (B) tree resulting from TrN + I + G analysis of *wingless* data, and (C) tree resulting from GTR + I + G analysis of COI data.

test (Cunningham, 1997; Farris et al., 1995). The result of these tests on the three combined data sets suggested that they were significantly incongruent and thus should not be combined ($P = 0.002$). We then applied the ILD test to each combination of pairs for the individual data sets. These tests indicated that the EF-1 α and *wingless* data sets were sufficiently congruent with each other to combine

them ($P = 0.75$) but that neither was congruent with the COI data ($P = 0.002$ for both comparisons). Despite the shortcomings of the COI data, we elected to combine the data sets for two reasons. Our primary reason for combining the COI data with the EF-1 α and *wingless* was that even with saturation affecting the data, some signal remains and the 'noise' introduced by saturation could

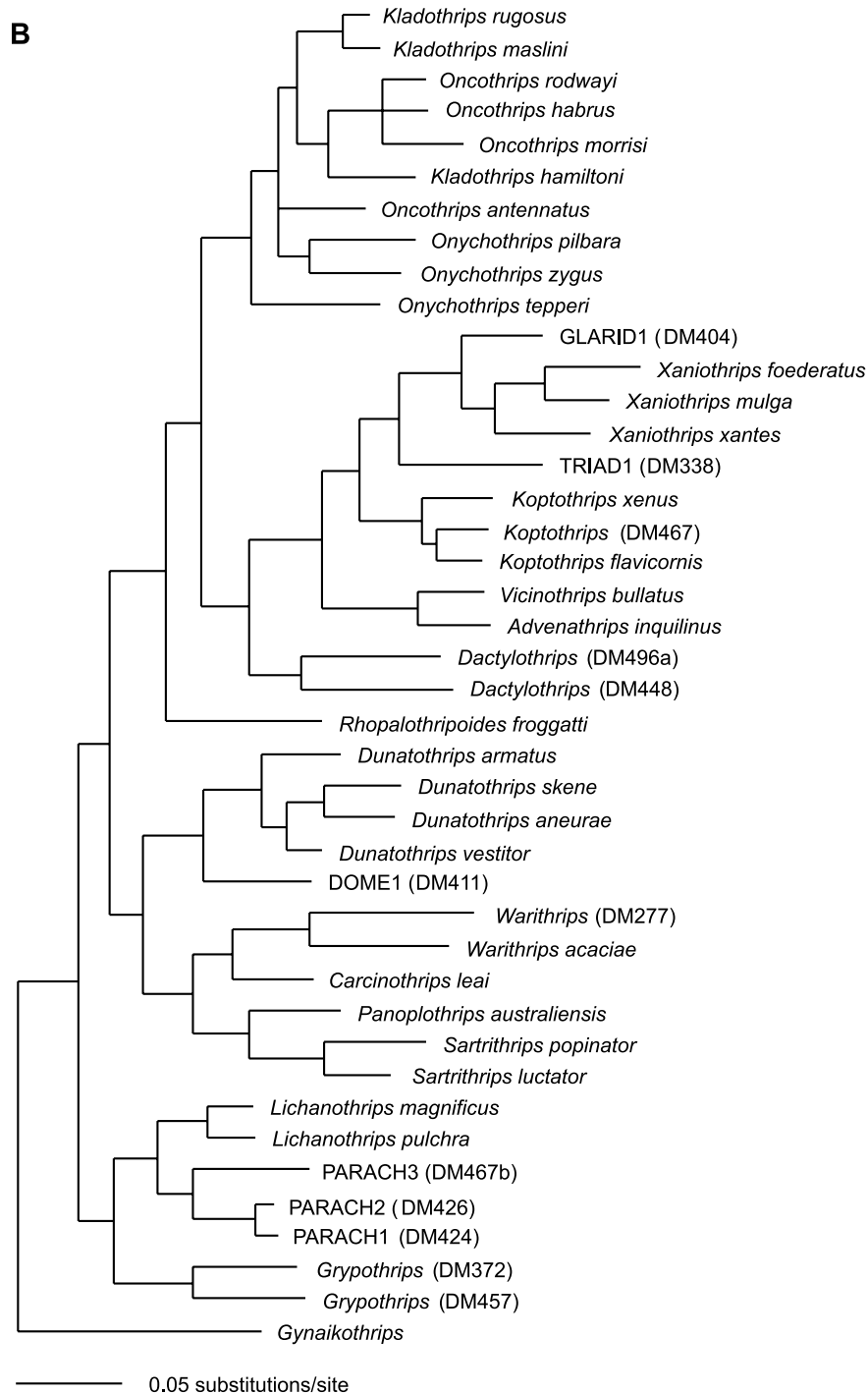


Fig. 1. (continued)

be overwhelmed by the signal in other data sets (Broughton et al., 2000; Wenzel and Siddall, 1999). The second reason for including our COI data in the combined analyses is that the ILD test has been shown to be very conservative and may be an unreliable guide to character incongruence (Davis et al., 1998; Messenger and Maguire, 1998; Yoder et al., 2001).

The combined data set contains 1565 characters of which 532 are parsimony-informative. Maximum par-

simony analysis of this combined data set yielded 14 equally most parsimonious trees. The evolutionary model selected as most appropriate for ML analysis of these data was the general time reversible with gamma-shape rate variation and invariant sites (GTR + Γ + I). The tree resulting from a GTR + Γ + I heuristic search is shown in Fig. 2.

To determine if portions of the data might be saturated, particularly the COI third codon positions, we

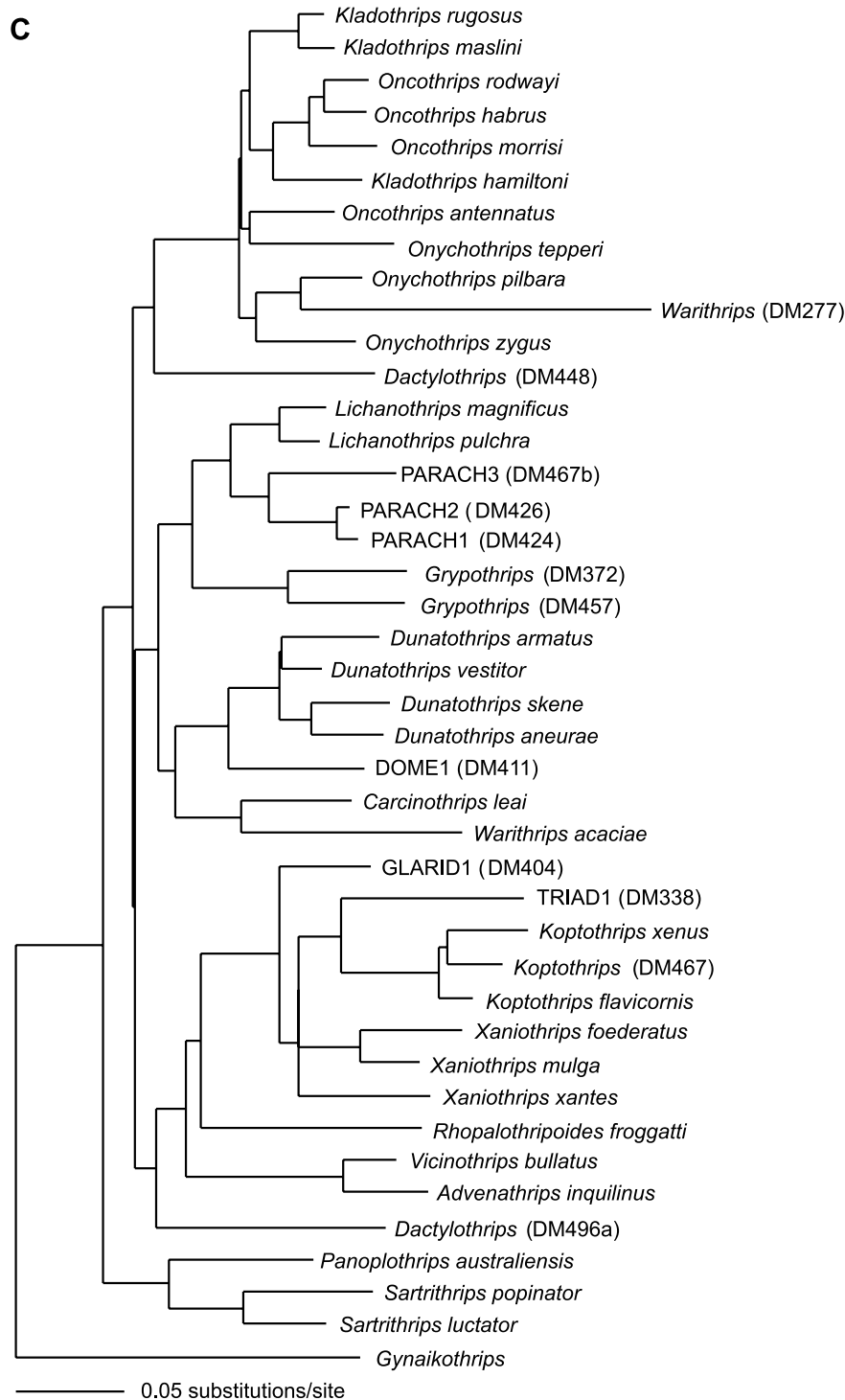


Fig. 1. (continued)

repeated the GTR analysis of the combined data using site-specific rates rather than a gamma-shape parameter to estimate the relative rate of substitutions in the different data partitions. The site-specific rate analysis indicated that the rate of substitutions in COI third codon positions (4.26 substitutions per site) was more than twice as great as any of the other coding regions

(0.00–1.52 subs./site) or the non-coding intron region of EF-1 α (1.81 subs./site). Thus, we repeated the GTR + Γ + I analysis with the third codon positions of COI excluded from the analysis and compared the result with the tree from our initial combined ML analysis. The two trees have identical topologies, with minor variation in branch lengths. Repeat of the MP analyses

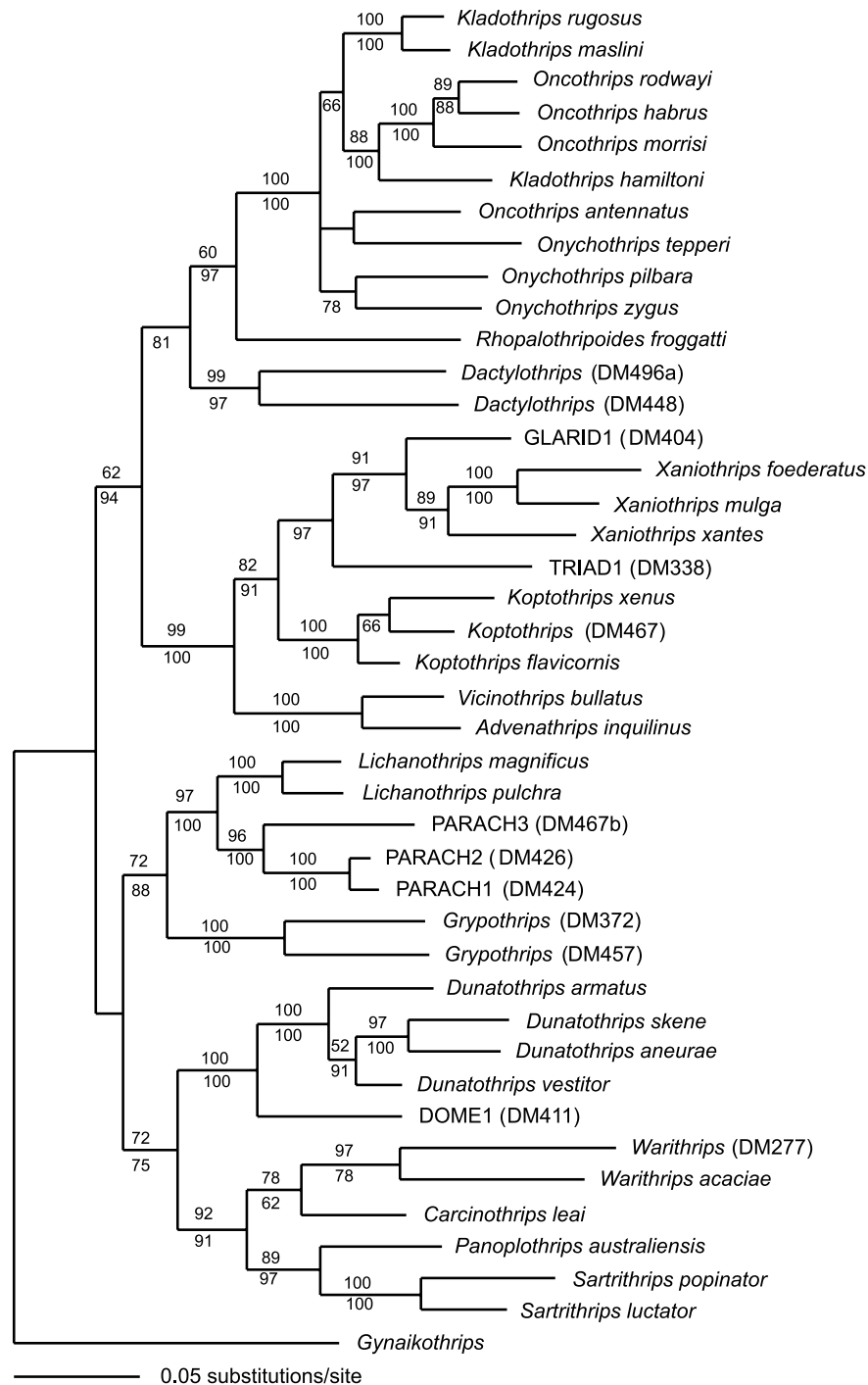


Fig. 2. Trees resulting from ML analyses of combined data (EF1- α + *wingless* + COI), with parsimony bootstrap values (1000 reps) shown above the branches and likelihood bootstrap values (30 reps) shown below the branches.

with COI third codon positions excluded produced a single topology not significantly different from the ML analysis ($P = 0.322$ for ML tree with COI third position excluded). Thus, we conclude that excluding the COI third codon positions from our ML analyses does not unduly affect the resulting topology. Saturation of the COI third codon positions does significantly affect the results of the MP analyses as excluding these sites causes

the result to converge on the topology given by the ML analyses.

3.3. Hypothesis testing

The SH tests were performed using the trees resulting from ML searches, the tree from the unconstrained analysis versus the trees obtained from a constrained

analysis. The gall-inducing taxa form a monophyletic group in the tree resulting from our ML analyses, as do the parasitic species, and as such the constraint tree is the same as the unconstrained tree and thus there is no significant difference between them. However, it is possible to search for the optimal tree in which those taxa are not monophyletic and then use an SH test to see if the difference between these constrained trees and the tree resulting from the unconstrained analysis is significant. The results of such tests show that the optimal tree in which the gall-inducing thrips are constrained to be paraphyletic is significantly different (less likely topology) from the optimal unconstrained tree in which these species are monophyletic ($P = 0.003$). A similar test of the optimal tree in which the parasitic species are constrained to be paraphyletic shows that such a tree is significantly different from the unconstrained tree ($P = 0.002$). When the data are analysed with the domicile-building species constrained to be monophyletic, the SH test indicates that the resulting tree is significantly different from the most parsimonious unconstrained trees ($P < 0.0001$). If the opportunistic species are constrained to be monophyletic, the resulting tree is also significantly different from unconstrained trees ($P < 0.0001$).

4. Discussion

4.1. Monophyly of the behavioural/ecological suites

In the following sections, we will discuss the monophyly of the four behavioural/ecological suites. To illustrate the relationships between taxa within each suite, the behavioural suites are mapped onto the phylogeny resulting from ML analysis of our combined data and this is shown in Fig. 3.

4.1.1. The gall-inducing suite

Despite being the most studied group of thrips from *Acacia*, monophyly of the gall-inducing species has been assumed in the past but has not been rigorously tested. Although we were unable to test all of the gall-inducing species in our phylogenetic analysis, we included multiple species representatives from each genus known to induce galls. The 10 species that we did include formed a strongly supported monophyletic group (bootstrap value of 100%). Our analyses indicate that the most closely related genera to the gall-inducing taxa are *Dactylothrips* and *Rhopalothripoides*. Pairwise divergences (based on the combined data set with COI third positions excluded) indicate that divergence among the gall-inducing species varies from 0.8% to 5.1%, whereas divergences between the gall-inducers and *Dactylothrips* or *Rhopalothripoides* range from 7.3% to 9.3%. This suggests that the gall-inducing species are

a monophyletic group distinct from the most closely related taxa (based on these analyses). Monophyly of gall induction implies that the ability to induce galls has arisen only once amongst the thrips on *Acacia*. Although there are a number of other origins of galling on non-*Acacia* species in other Australian thrips, there is no evidence to suggest that gall-induction amongst thrips might be monophyletic (Morris et al., 1999; Mound, 1994). Furthermore, there appear to be no losses of the gall-inducing trait amongst the thrips species found on *Acacia*. The advantages of living in a sealed structure, such as a gall, that provides both food and shelter, apparently limit the possibilities for re-evolving free-living strategies.

4.1.2. The domicile-building suite

Although all of the domicile-building species in our analysis are contained within one clade, that clade also includes a number of thrips from the opportunistic suite. The topology of our unconstrained trees suggests that there may have been a single origin of domicile-building behaviour with several subsequent losses. The alternative is that there have been multiple origins of domicile building. The single origin hypothesis would require a minimum of three losses, in DOME1 (DM411), in the branch leading to the *Warithrips* species, and in the *Grypothrips*. A multiple-origin hypothesis with no losses would require four origins of domicile building. These two hypotheses represent two extremes, with a number of intermediate hypotheses involving a combination of multiple origins and losses, being possible (e.g., two origins and two losses or three origins and one loss). As each of these hypotheses would entail the same number of evolutionary steps, there is no direct evidence to favour one hypothesis over another. However, there is some evidence for multiple origins of domicile-building behaviour from field observations. Each of the domicile-building clades (*Lichanothrips*/PARACH, *Dunatothrips*, and *Sartrithrips*/*Panoplothrips*/*Carcinothrips*) has characteristic forms of architecture and adhesive (Mound and Morris, 1999b, 2001) supporting their independent evolution. If there were only a single origin of domicile building, then the question of why this behaviour might have been lost multiple times in favour of an opportunistic strategy arises. One possible advantage for evolving an opportunistic strategy might be to reduce or escape kleptoparasitism by other thrips by not being sufficiently reliable or predictable as a host to attract parasites. However, some species in both *Warithrips* and *Grypothrips* inhabit both abandoned galls and abandoned glued phyllode domiciles and as such would still have to defend their resource niches against the kleptoparasites that would normally invade such habitats. More likely reasons for evolving opportunistic habits would be advantages in avoiding the energetic costs of

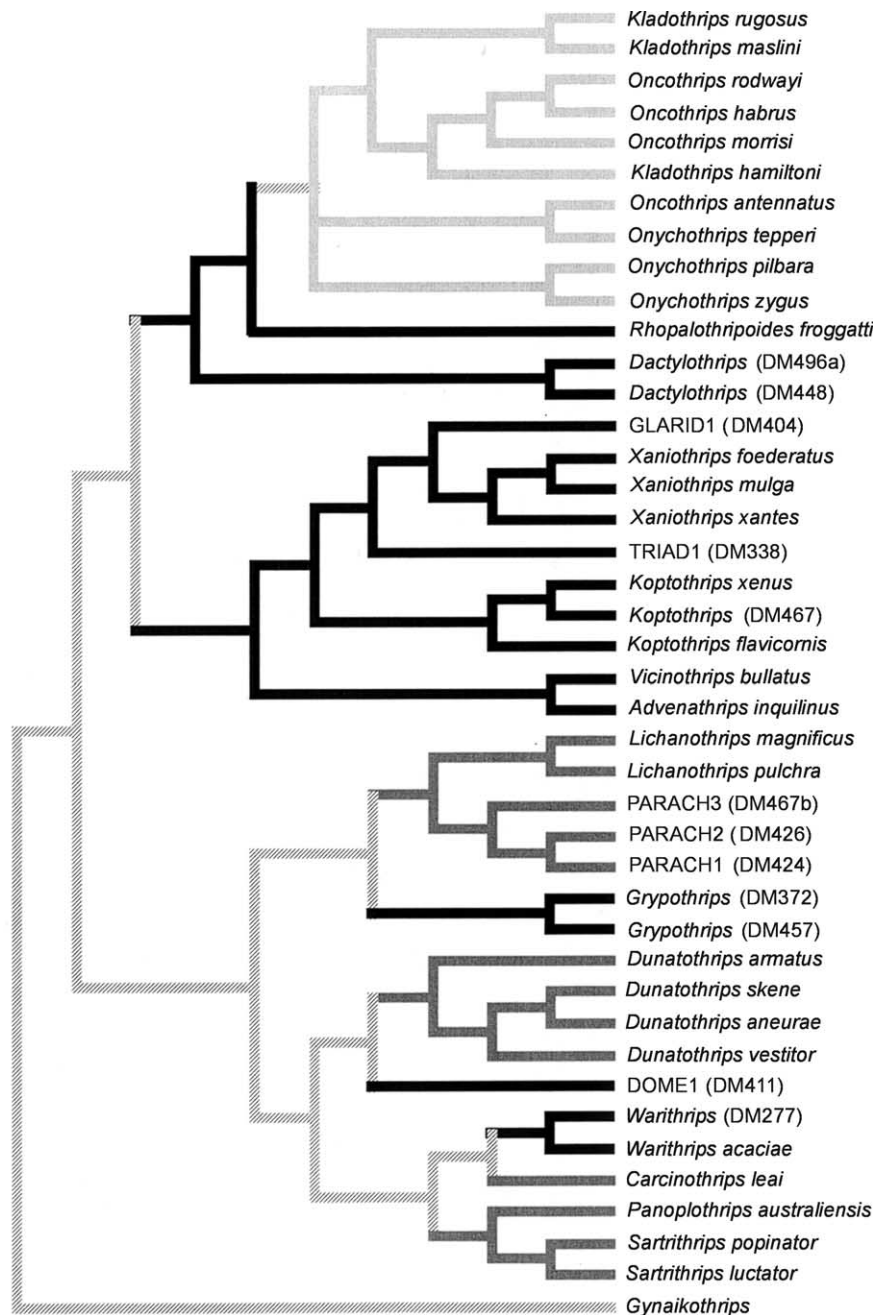


Fig. 3. Tree resulting from ML analysis of combined data with the four behavioural/ecological suites mapped. Grey bars indicate the gall-inducing suite, black bars indicate the opportunistic suite, black stippled bars denote members of the parasitic suite, and grey stippled bars indicate members of the domicile-building suite.

constructing a domicile, or shifts to host species with greater abundances of abandoned galls or domiciles. As the trait of domicile building is apparently unique among Thysanoptera, it is tempting to assume that there has been a single origin and the behaviour has been subsequently modified over evolutionary time. However, the answers to these questions require more detailed comparative ecological data to determine whether the three forms of domicile building are, in fact, homologous.

4.1.3. The parasitic suite

The most unanticipated result of our analyses is the monophyly of the parasitic species of thrips, with a bootstrap value of 99%. Furthermore, topologies in which the parasitic species are constrained to be paraphyletic have significantly lower (higher $-\ln$ likelihood) likelihood values, as indicated by our SH tests above. There are several a priori reasons why this result is unexpected. First, previous morphological analysis of *Acacia* thrips including *Koptothrips* (kleptoparasites of

gall-inducers) and *Xaniothrips* (kleptoparasites of domicile-builders) indicated that these genera might not even be in the same lineage within the Phlaeothripinae (Morris et al., 1999). One taxonomist has even suggested that *Xaniothrips* should be placed in its own family, the Xaniothripidae (Bhatti, 1992), although recent work has refuted this (Mound and Morris, 1999a). Second, diverse studies on Hymenoptera have indicated that social parasitism has evolved frequently, but parasitic clades are often, or even predominantly, closely related to the host species (Wilson, 1971). Although this clearly does not hold true for all Hymenoptera, it is often the case that social parasites have a sister-group relationship with their hosts (e.g., ants, Wilson, 1971; Sanetra and Buschinger, 2000; Ward, 1996; bees, Lowe and Crozier, 1997; Michener, 1974; Wilson, 1971). Our results strongly indicate that in the *Acacia* thrips there has been only a single origin of kleptoparasitism and inquilinism and, as far as our taxonomic representation goes, without reversion to non-parasitic habits. The kleptoparasitic/inquiline clade includes *Koptothrips*, the species of which specialise in invading thrips galls (Crespi and Abbot, 1999), *Xaniothrips*, which specialises in invading glued phyllode domiciles (Mound and Morris, 1999a), and genera such as *Advenathrips* (Morris et al., 2000), *Vicinohrips* (Mound and Morris, 2000), and the undescribed TRIAD (DM338), which live as inquilines or commensals in glued-phyllode domiciles. Because the two most basal genera in this clade are inquilines, it seems possible that kleptoparasitism evolved after ‘softer’ forms of exploiting host domiciles had arisen.

4.1.4. The opportunistic suite

In contrast to the monophyly of the gall-inducing and the parasitic suites, the opportunistic thrips species are polyphyletic within the phylogeny of *Acacia* thrips (see Fig. 3). One possible explanation is that the opportunistic state is plesiomorphic and some or all of the other behavioural/ecological suites are apomorphies. Support for this notion comes from the monophyletic group containing two lineages of opportunistic genera *Rhopalothripoides* and *Dactylothrips* basal to all of the gall-inducing thrips. However, to determine whether the opportunistic state is plesiomorphic requires testing with broader taxon sampling.

4.2. Evolution of behavioural and ecological traits

It is expected that host-plant relationships might affect speciation patterns in some of the behavioural/ecological suites but not others. This is true for the gall-inducing species, where the clade containing the *Kladothrips* species and *Oncothrips morrisoni*, *O. habrus*, and *O. rodwayi* utilise host-plants from *Acacia* Section Plurinerves (Morris et al., 2001). The remaining species are found primarily on hosts from *Acacia* Section

Juliflorae. This close host relationship might be expected if the ability to induce galls is closely tied to host physiology, so that gall induction in widely divergent hosts requires major adaptive shifts. The domicile-building species also follow a similar pattern of host-plant relationships, despite our expectations to the contrary based on the assumption that domicile building should require a less specific chemical interaction with the host. *Dunatothrips*, *Carcinothrips*, and *Panoplothrips* are all found exclusively on Juliflorae whereas the *Lichanothrips* and PARACH genera are found on Plurinerves. That host-plant relationships are conserved in thrips that manipulate the plant in some way is not unexpected. In contrast to the close host-plant relationships seen in the gall-inducing and domicile-building suites, the parasitic suite might not be expected to have close ties to their host-plants but instead would be more linked to the thrips species that they parasitise. The *Koptothrips* that invade thrips galls are found primarily on plants of the Plurinerves group. This does not necessarily indicate a link with the host-plants, rather it may be a result of an inability to readily parasitise those gall-inducing thrips found on Juliflorae; thus, *Koptothrips* are found infrequently on this group of *Acacia* species. The *Xaniothrips* that usurp thrips phyllode domiciles have apparently had no difficulty in adapting to different host-plants. *Xaniothrips* species invade domiciles constructed by *Dunatothrips*, *Sartrithrips*, and *Carcinothrips* on Juliflorae as well as *Lichanothrips* domiciles on Plurinerves, but are not found in domiciles of PARACH species or *Panoplothrips*. This indicates that factors relating to the gall-inducing or domicile-building host thrips play a larger role in determining kleptoparasite distribution than the host-plant.

The different groups of thrips on *Acacia* present an interesting range of behavioural complexities that are well suited to examining this interplay between evolutionary flexibility and constraints imposed by specialisation. This study suggests that the degree to which behaviour and ecology are constrained or labile will play a significant role in the evolution of an organism. Thus, an important area for future work will be to compare the trends seen here with evolutionary plasticity of life history modes in other organisms. It is expected that the results presented here will facilitate ongoing research into the systematics and evolution of this group of insects by providing a framework of relationships and a brief introduction to the behavioural diversity among the major groups within ‘the *Acacia* thrips.’

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