The systematics of Australian Agathidinae (Hymenoptera: Braconidae), including the evolution of *Therophilus* and its colour mimicry pattern

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Title page image: *Therophilus unimaculatus*

This thesis is dedicated to my loving parents

Arthur and Mary Stevens

Thank you Mum and Dad for the broad open approach to life.

Table of Contents

Abstract

This study investigated the diversity and evolution of the Agathidinae in Australia. The Agathidinae are a large subfamily of braconid wasps with nearly 1,200 described species in over 50 genera worldwide. The subfamily has been relatively well-studied in the northern hemisphere but the Australian fauna is poorly known. This study presents a synopsis of the genera and species in Australia, including information on distributions, apparent species richness, species list, and keys to all genera present and to *Camptothlipsis* Enderlein, *Lytopylus* Foerster, and *Therophilus* Wesmael species. The phylogeny of the Agathidinae is also analysed using morphological and molecular data, with particular focus on the dominant genus in Australia, *Therophilus*, and its associated colour mimicry pattern.

The Australian Agathidinae has received little taxonomic attention since the last of the 36 recognised species were described nearly 100 years ago. Not surprisingly, this earlier work is insufficient for reliable identification of the genera and species present. This study, employing modern taxonomic concepts, found more than 200 undescribed species representing 10 genera occurring in Australia. The fauna is dominated by tropical genera with the northern tropical to sub-tropical regions of the continent hosting the greatest generic diversity. Only one genus, *Therophilus*, is widespread throughout Australia.

The cosmopolitan *Therophilus* is the most speciose agathidine genus in Australia with approximately 150 species recognised, 20 of which are described. The present study updates the taxonomy of the previously described *Therophilus* species, providing a more thorough assessment of intra-specific variation, and a key to species. In addition, four new species are described that support the morphological and molecular phylogenetic studies undertaken.

A conspicuous component of Australian *Therophilus* are the members associated with a putative mimicry complex of braconid wasps and other insects comprising species that display a distinctive black, red-orange and white colour pattern (referred to in this study as the BROW colour pattern). Previous phylogenetic analysis using both 28S and morphological data from mostly non-Australian taxa revealed *Therophilus* to be polyphyletic. There are currently no distinguishing morphological attributes to enable each of the divergent *Therophilus* lineages to be reliably identified, thereby making it

difficult taxonomically to designate each linage as a separate genus. Only one Australian *Therophilus* species was represented in the previous phylogenetic studies so the evolutionary affinities of the genus in Australia, including members that display the BROW colour pattern, remained unknown.

To investigate the evolution of Australian *Therophilus* and its putative mimicry colour pattern, previously published agathidine phylogenetic studies were expanded with the addition of predominantly Australian *Therophilus* species, many having the BROW colour pattern. The phylogenetic results further demonstrated the polyphyly of *Therophilus* and that the Australian fauna and the BROW mimicry pattern are not monophyletic.

This study represents an important contribution to the systematics of the Australian Agathidinae and provides a firm basis for identifying and describing the many undescribed Australian *Therophilus* species. The phylogenetic analyses further highlighted the importance of using multiple genetic markers, in conjunction with a broader taxonomic and geographical representation, to more robustly define the evolutionary relationships present.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of The University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Acknowledgments

Chapter 1: General Introduction

With more than 153,000 extant species, the Hymenoptera is one of the largest terrestrial invertebrate orders and can be found in most habitats including intertidal and freshwater environments (Aguiar *et al.* 2013). They perform important functional roles in natural and agricultural communities as herbivores, pollinators, and as predators and parasitoids. The evolution of parasitism of terrestrial insect and spider hosts has resulted in a massive radiation of species that has led to the parasitoid families being among the most speciose groups of the Insecta (LaSalle and Gauld 1993; Quicke 1997). However, they remain the least studied compared with the aculeate wasps, bees and ants, with only an estimated 10-20% of species having been described for Australia (Austin 1999). One of the largest parasitoid families is the Braconidae with nearly 20,000 valid species world-wide, and possibly three to four times this many species waiting description (Jones *et al.* 2009; Yu *et al.* 2012; Aguiar *et al.* 2013; Quicke 2015;). The Braconidae have been a rich source of biological control agents because their main host groups, Coleoptera, Diptera, and Lepidoptera, represent the most important orders for pest species (Shaw and Huddleston 1991). Braconidae are well represented in Australia with 36 of the 46 recognised subfamilies occurring on the continent (Stevens *et al.* 2000; Yu *et al.* 2012). However, the Australian fauna has received little attention relative to other regions, in particular the Holarctic, having only 738 described species (including 53 introduced) representing an estimated 20% of the actual fauna present (Stevens *et al.* 2000).

1.1 Subfamily Agathidinae

The cosmopolitan braconid subfamily Agathidinae is a relatively speciose group with about 1,200 described species in 51 genera (Yu *et al.* 2012; Sharkey and Chapman 2015). Agathidine wasps are virtually all solitary endoparasitoids of lepidopteran larvae, most commonly of concealed larvae, except for members of the tribe Disophrini which parasitise exposed larvae (Sharkey 1992). The Agathidinae play an important role as natural enemies of lepidopteran populations with numerous species having been deployed in biological control programs. Agathidines are generally more diverse in tropical and subtropical regions, although some genera display greater species richness in more temperate and arid environments (Sharkey 1997; van Achterberg and Long 2010; Sharkey and Chapman 2015). Little was known of the Australian fauna prior to this study with only 36 recognised species in eight genera, most having been described in the early part of the 20th century (Turner (1918a, b).

1.2 Taxonomic history of the Agathidinae

Higher level nomenclature

Blanchard (1845) was thought to be the first to recognise the agathidines as a higher level group of braconids when he erected Agathites with *Agathides* Nees as the type genus. However, this was brought into dispute when Simbolotti and van Achterberg (1992) indicated that the subfamily name Bassinae has formal priority over Agathidinae as it is based on *Bassi* Nees (1812) which was described two years earlier than *Agathides* Nees (1814). Papp (1998), who wrongly attributed van Achterberg and Polaszek (1996) with first recognising that Bassinae has priority, stated that it is a *nomen obliteratum* because the name had not been used for almost two centuries. Consequently, Papp (1998) disregarded the priority of Bassinae over Agathidinae. Wharton and van Achterberg (2000) proposed that *Bassi* Nees, 1812 (not completely described until Nees, 1814) was based on a junior objective synonym of *Alysia* Latreille, 1804, and therefore a junior homonym of *Therophilus* Fabricius, 1804; hence Bassinae is invalid and has not been adopted in any studies since. In addition, Wharton and van Achterberg (2000) showed that the subfamily name Agathidinae should be attributed to Haliday (1833) (as Agathenses).

Forster (1862) treated the group as two families, Agathoidae and the Eumacrodoidae, based on the shape of the head. Marshall (1885) did not follow this arrangement, instead treating them within the one group, Agathidides, which Cresson (1887) renamed as Agathidinae. Ashmead (1900) reintroduced Forster's concept but as two tribes within the Agathidinae: Agathidini (head rostriform, 'beak-like', with long malar space); and Eumicrodini (head not rostriform with short malar space). Szépligeti (1904) did not recognise these tribes, combining them once again under the subfamily Agathinae. Viereck (1914) showed that *Cremnops* Forster (1862) shared the same type species (*Ichneumon desertor* L.) as *Bracon* F. (1804). Consequently, Gahan (1917) replaced Agathidinae with the subfamily name Braconinae. This led to a period between 1917 and 1948 of nomenclatural confusion (e.g. Muesebeck 1927; Simmonds 1947) in which the subfamily was often referred to as Braconinae, until the reinterpretation of the name *Bracon* by the ICZN (1945) allowed the Agathidinae to be recognised as a separate group from the Braconinae. Shenefelt (1970b) provided the first worldwide taxonomic catalogue of the then 44 recognised genera and all species of Agathidinae, but no tribal classification or genus groups were recognised.

Tribal level classification

Bhat and Gupta (1977) undertook a detailed review of the Oriental agathidine fauna in which, based on the form of fore and mid-leg claws, they divided the regional fauna into two informal groups, the *Agathis* group (claws simple, with or without a basal lobe) and the *Cremnops* group (claws cleft). Nixon (1986) treated the European fauna in a similar manner, with the *Agathis-Microdus* and *Cremnops-Disophrys* genus groups recognised on the same criteria as Bhat and Gupta (1977). Van Achterberg (1990) proposed that the available tribal names for the *Agathis-Microdus* group were Agathidini Nees, and Vipioninae Gahan (later ruled as invalid (see Sharkey 1992)) for the *Cremnops-Disophrys* group. In his study van Achterberg (1990) also noted additional diagnostic characters for each group with pre-apical mid-tibial spines only present in the Agathidini Nees, and with the *Cremnops-Disophrys* group mostly having paired carina between the toruli.

Sharkey (1992) provided the first comprehensive tribal classification for the subfamily, further subdividing the Agathidini Nees (*sensu* van Achterberg (1990)) into three tribes, 1) Agathidini Blanchard, including *Agathis*, 2) Earinini Sharkey, and 3) Microdini Ashmead, including *Therophilus* (as *Bassus s.l.*), and the *Cremnops-Disophrys* group into two, Disophrini Sharkey and Cremnoptini Sharkey. Later, Simbolitti and van Achterberg (1999) did not consider the definition of the Agathidini and Microdini *sensu* Sharkey (1992) (named as Eumicrodini Foerster in Sharkey, 1996) to be valid because the defining characters could not reliably separate the two most speciose and taxonomically problematic genera, *Agathis* from *Therophilus* (as *Bassus s.l.*). Both genera were assigned by Sharkey (1992) to different tribes, *Agathis* to the Agathidini and *Therophilus* to Microdini. Therefore, Simbolitti and van Achterberg (1999) synonymised Microdini with Agathidini Nees, 1814, into a larger grouping defined on having simple non-cleft claws. The definition of the Agathidini *sensu* Simbolitti and van Achterberg (1999) is coincident with the genus groups *Agathis* and *Agathis-Microdus* of Bhat and Gupta (1977) and Nixon (1986), respectively.

Sharkey *et al.* (2006) provided the first implicit phylogenetic analysis of the subfamily using morphological and 28S sequence data for 62 ingroup taxa from 21 genera representing all five tribes of Sharkey (1992). This study supported the monophyly of Cremnoptini and Disophrini, and the recognition of Earinini, but with the inclusion of *Crassomicrodus* (ex Agathidini *sensu* Sharkey, 1992). Importantly, it also affirmed the synonymy of Microdini with Agathidini (*sensu* Sharkey, 1992) by Simbolitti and van Achterberg (1999), but also included the Earinini in this clade, referred to by Sharkey *et al.*

(2006) as Agathidini *s.l.*. Further, within this clade, the Agathidini was rendered polyphyletic, comprising two unrelated groups, Agathidini *s. str.* (equivalent to the Agathidini (*sensu* Sharkey *et al.* 2006)), and a small generic grouping, which he referred to as a New Tribe (abbreviated as 'n'tribe), which included the type species of *Therophilus*. However, because of uncertainties involving the relationships of the new tribe to the other clades, namely the lack of support for a monophyletic Agathidini *s. str.* + Earinini + new tribe, Sharkey *et al.* (2006) refrained from formally naming it.

Therophilus *relationships*

At a lower taxonomic level, one of the major findings of Sharkey *et al.* (2006) was that *Bassus* (as it was then circumscribed) was polyphyletic and, even though only 10 exemplar species were included, they fell out in four separate lineages; one lineage within the new tribe, and three separate lineages within the Agathidini *s. str.* Based on these results, Sharkey *et al.* (2009) began a process of redefining *Bassus* s.l. and dividing it into a number of smaller genera including the reinstatement of *Camptothlipis*, *Lytopylus* and *Therophilus*. The change made to the definition of *Bassus* by Sharkey *et al.* (2009) meant that no true *Bassus* species were represented in his 2006 phylogenetic analysis. Sharkey *et al.* (2009) conceded that *Therophilus* was still polyphyletic and that many species would end up being transferred from one polyphyletic genus (*Bassus*) to another (*Therophilus*), until further more detailed phylogenetic studies could better define the limits of natural generic grouping in this part of the agathidine tree.

In recent molecular phylogenetic studies of the Thailand and Neartic faunas Sharkey *et al.* have continued to investigate the polyphyly of *Therophilus* with a series of molecular phylogenetic studies which have led to the description of five new genera that represent relatively small monophyletic lineages with limited distributions. Sharkey *et al.* (2011) described the Nearctic genus *Neothlipsis* Sharkey that was shown to be closely related to *Camptothlipsis* within the Agathidini *s. str.* Sharkey and Stoelb (2012) refined *Therophilus s. str.* to represent a monophyletic taxon within his new unnamed tribe, with the remaining unrelated lineages within the Agathidini *s. str.* (referred to as *Therophilus s.l.*). Sharkey and Stoelb (2013) described a second genus, *Agathacrista* Sharkey, limited in distribution to the Oriental and eastern Palearctic regions, and resolved phylogenetically within the Agathidini *s. str.* as a sister group to *Bassus s. str.* and an undescribed new genus. Sharkey and Chapman (2015) described three Nearctic genera, *Aphelagathis* Sharkey, *Gelastagathis* Sharkey, and *Pneumagathis* Sharkey. *Aphelagathis* and *Pneumagathis* were placed on phylogenetic grounds within the Agathidini *s. str.* and closely related to

Neothlipsis. No sequence data could be obtained from *Gelastagathis* so it is not known where this small, distinctive genus fits phylogenetically, although it was hypothesised on morphological features to be closely related to both *Aphelagathis* and *Pneumagathis*. The recent revisions of the Thailand and Nearctic faunas have served to divide a small part of *Therophilus s.l.* into numerous small genera. However, the bulk of species from other regions inevitably reside in other lineages and represent unnamed genera. These lineages are difficult to clearly differentiate on any distinguishing morphological features making it problematic as they appear only diagnosable using molecular data.

1.3   The Australian fauna

The agathidine fauna of Australia has received little attention and is therefore largely unknown. No new species of Agathidinae had been described from Australia since Turner (1918a, b) treated 25 new species. Prior to Turner only eight species had been described (Parrot 1953; Stevens *et al.* 2000). Two endemic genera were previously recognised, the relatively speciose *Agathiella* Szépligeti and the monotypic *Platyagathis* Turner, but *Agathiella* has since been synonomised with *Therophilus* (Sharkey *et al.* 2009).

The current study has revealed a much larger agathidine fauna for the region than previously recognised, rendering the keys compiled by Turner (1918a, b) for the more speciose genera as grossly inadequate. A key to the Australian genera has never been developed so a combination of several overseas keys (e.g. Chou and Sharkey 1989; Sharkey 1996; 1997; Simbolotti and van Achterberg 1992; 1999) were used here to assist in the identification of the genera present on the continent. Nine genera (*Agathis* Latreille, *Biroia* Szépligeti, *Braunsia* Kriechbaumer, *Cremnops*, *Disophrys*, *Euagathis* Szépligeti, *Hypsostypos* Baltizar, *Platyagathis* and *Therophilus*) were formally recognised to be present in Australia. The occurrence of *Agathis*, however, is questionable. Turner (1918a) noted that typical *Agathis* had not been observed to occur in Australia and Parrot (1953) expressed doubt that Brulle's (1846) species belonged to *Agathis*. To date, using the more contemporary taxonomic concepts of Sharkey *et al.* (2006) and van Achterberg and Long (2010), no Australian species of *Agathis* have been found among material in Australian and overseas collections, with all specimens previously identified as *Agathis* in fact belonging to *Therophilus*.

In general, investigations have shown agathidines to be more diverse in tropical and subtropical regions, although some genera (e.g. *Agathis* and *Earinus*) display greater species richness in more temperate climatic zones. Several genera, *Agathirsia* Westwood,

Agathis, *Coccygidium* and *Crassomicrodus* Ashmead, have species groups that have radiated in arid environments (Wharton 1993; Sharkey 1997; Pucci and Sharkey 2004). The Australian fauna also displays a similar trend with the northern tropical and subtropical regions of the continent having a greater abundance and richness at both the generic and species levels (Stevens *et al.* 2010). This is in stark contrast to the southern temperate and arid environments that are dominated by *Therophilus s.l.*, which has undergone extensive radiation throughout Australia with species richness in the southern temperate and Mediterranean climatic regions being as great or greater than in the northern tropical and subtropical environments (Stevens *et al.* 2011). Turner (1918a) had also noted this trend for *Therophilus* (as *Agathiella*) species.

An interesting aspect of the Australian agathidine fauna is the presence of two major colour forms, each putatively believed to be aposematic and part of different mimicry complexes. One form is a contrasting yellow-brown and black colour pattern that exhibits a variety of arrangements on the body and wings. This colour pattern is confined to the tropical genera *Coccygidium*, *Cremnops* and *Disophrys* and the variety of pattern arrangements exhibited appear relatively low. Overseas tropical agathidine genera, *Alabagrus* Sharkey, and *Sesioctonus* Viereck, also display similar colour forms and are believed to be representatives of a large putative mimicry complex that contains over 2,000 ichneumonoid species, as well as an unknown number of species from numerous pterygote orders, including several hundred species of hemipteran reduvids (Briceńo 2003; Leathers and Sharkey 2003). Leathers and Sharkey (2003) proposed that species of *Alabagrus* suspected of being in this mimicry complex are Batesian mimics, on the basis that an offensive odour detectable to humans is not exuded nor is a painful sting known, unlike some other braconid and reduvid members.

The second major colour form is a distinctive contrasting <u>b</u>lack, <u>r</u>ed-<u>o</u>range, and <u>w</u>hite (BROW) pattern exhibited in various ways across the body segments but which does not extend over the wings (e.g., Chapter 3: Figure 17, Stevens et al. (2011)). Within the Agathidinae, the BROW pattern is confined to species of *Therophilus* from both northern and southern regions of Australia. Non-Australian *Therophilus* species are generally entirely (or metasoma only) pale red to orange (Sharkey 1985). However, two species from Malaysia exhibiting a colour pattern approaching the BROW condition have been identified. The occurrence of the BROW pattern is widespread across numerous Australian braconid subfamilies (e.g. Braconinae, Doryctinae and Helconinae,) and has also been observed in numerous lepidopteran, reduvid and mirid species (Quicke *et al.* 1992;

Belokobylskij *et al.* 2004). The depiction of members of this putative mimicry complex are in a colour plate (Plate 6) of the textbook *Insects of Australia* (Naumann 1991). Further taxa displaying similar BROW patterns are also illustrated on Plate 3 and 5.

Quicke *et al.* (1992) observed that numerous braconines can give a painful sting and many give off a pungent odour when handled that would be likely to act as deterrents to potential predators. On this basis they hypothesised that the BROW pattern observed in braconine taxa acts as an aposematic signal to would be predators (Quicke *et al.* 1992). Hence, the BROW pattern amongst braconines is hypothesised to predominantly represent Müllerian mimicry. It is not known if *Therophilus* species possess a painful sting or can produce a pungent odour when disturbed. However, *Therophilus* species do possess long ovipositors that can be over 1.5 times their own body length which could be capable of delivering a painful rebuke to a potential predator. If this is the case then it would stand that the *Therophilus* species displaying the BROW pattern would be Müllerian co-models. The selective pressures for these putative mimicry complexes are not known although they would likely be predator, host and/or habitat related (Quicke *et al.* 1992).

1.4 Aims

In this thesis, I present research that investigates the Australian Agathidinae using morphological and molecular data, with particular emphasis on the most species rich and widespread genus on the continent, *Therophilus s.l.* Each of the results chapters are formatted as journal papers. **Chapters 2 and 3** have already been published and it is intended that **Chapter 4** will also be submitted for publication in the near future. **Chapter 5** presents a general discussion regarding the broader research implications and limitations of the study, as well as future research directions to extend the current findings. This thesis has been arranged in a logical progression of research ideas and findings as indicated in the aims of the research project outlined below:

Chapter 2 (published as Stevens *et al.* (2010), Zootaxa 2480: 1–26) ― Provide a synopsis of the Australian Agathidinae at the generic level. The specific aims of this study were to:

a) develop a dichotomous key to genera of the region using contemporary generic concepts;

b) provide a corrected taxonomic list of species based on examination of primary types, including synonyms and holotype information;

c) provide information on the species richness and distribution of genera, their biology, and occurrence of likely mimicry colour patterns

Chapter 3 (Published as Stevens *et al.* (2011), Zootaxa 2887: 1–49) ― Treats *Camptothlipsis*, *Lytopylus* and *Therophilus s.l.* species that were previously considered to belong to *Bassus* with an emphasis on *Therophilus* as the most diverse Australian genus. The aims of this study were to:

a) redescribe the existing *Therophilus* species and developes a dichotomous key to their identification;

b) record species' distributions and host records, particularly those associated with native Australian lepidopteran pests *Etiella behrii* Zeller (Pyralidae) and *Epiphyas postvittana* (Walker) (Tortricidae);

c) document the presence and variation in the BROW colour pattern across species;

d) describe four new species to support morphological and molecular phylogenetic studies on the Australian fauna, including a new species of *Camptothlipsis*; and

e) redescribe the introduced *Lytopylus rufipes* (Nees von Esenbeck) to facilitate its identification.

Chapter 4 ― Presents a phylogenetic study to determine relationships among Australian *Therophilus*, using morphological and DNA sequence data, and uses the resultant trees to investigate the pattern of evolution of the BROW mimicry complex. Its specific aims were to:

a) produce a revised and expanded agathidine phylogeny using morphological and 28S rRNA data to investigate the evolution of Australian *Therophilus* in relation to the world fauna;

b) determine if the BROW pattern displayed by Australian *Therophilus* forms a monophyletic, readily recognisable taxonomic unit within the genus; and

c) investigate more closely the evolutionary relationships among Australian *Therophilus* species using an expanded dataset comprising morphology in conjunction with four genetic markers (16S rRNA, 28S, cytochrome oxidase I (CO1), and long wavelength rhodopsin (LW rh)).

14

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Chapter 2: Synopsis of Australian agathidine wasps (Hymenoptera: Braconidae: Agathidinae)

Statement of Authorship

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Contribution to the Paper	All morphological work including acquiring specimens, examination of genera and spp., creating keys, writing ms		
Overall percentage (%)	90		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	3/12/2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i.   the candidate's stated contribution to the publication is accurate (as detailed above);

ii.   permission is granted for the candidate in include the publication in the thesis; and

iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Prof. Andrew Austin		
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Chapter 3: Diversity, distribution and taxonomy of the Australian agathidine genera *Camptothlipsis* Enderlein, *Lytopylus* Foerster and *Therophilus* Wesmael (Hymenoptera: Braconidae: Agathidinae)

Statement of Authorship

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Chapter 4: Phylogenetic affinities of Australian *Therophilus* Wesmael (Hymenoptera: Braconidae: Agathidinae) and evolution of the BROW colour pattern

Abstract

The Australian agathidine fauna is dominated by the cosmopolitan *Therophilus* Wesmael. A conspicuous component of Australian *Therophilus* are species associated with a putative mimicry complex of braconid wasps and other insects that display a distinctive <u>b</u>lack, <u>r</u>ed-<u>o</u>range and <u>w</u>hite (BROW) colour pattern. Previous phylogenetic analyses using both 28S and morphological data from mostly non-Australian taxa revealed *Therophilus* to be polyphyletic. However, there are currently no distinguishing morphological attributes to distinguish the divergent *Therophilus* lineages, making it difficult to designate each linage as a separate genus. Only one Australian *Therophilus* species was represented in this previous study so evolutionary affinities of the genus for this region, including members of the BROW mimicry complex, remained unknown. To investigate the relationships among Australian *Therophilus* and the evolution of this distinctive colour pattern, the current study provides a revised and expanded phylogeny using predominantly Australian *Therophilus* species, morphology and several molecular markers in various combinations. The resultant trees resolved at least three divergent clades, supporting the hypothesis that *Therophilus* is polyphyletic and that within the Australian fauna the BROW colour pattern has evolved multiple times.

Introduction

The braconid subfamily Agathidinae is a relatively speciose group of endoparasitoids of lepidopteran larvae with nearly 1,200 described species in over 50 genera worldwide (Sharkey 1997; Sharkey *et al.* 2006, 2009; Yu *et al.* 2012). The Agathidinae are well-represented in Australia with 40 described and more than 200 undescribed species in 10 genera (Stevens *et al.* 2010, 2011). The Australian fauna is dominated by the large cosmopolitan genus *Therophilus* Wesmael that occurs throughout the continent, including Tasmania, and comprises about two-thirds of Australian agathidine diversity (Stevens *et al.* 2010).

A conspicuous component of the Australian agathidine fauna is a distinctly contrasting <u>b</u>lack, <u>r</u>ed-<u>o</u>range and <u>w</u>hite colour pattern (referred to as the BROW pattern by Stevens *et al.* 2010, 2011) displayed by many taxa. The pattern consists of black and red-orange in varying amounts on the anterior portion of the body (head, mesosoma and legs) with black and white in varying amounts on the posterior body (metasoma and hind legs) (see title page image, Chapter 3: Fig. 12). The BROW mimicry colour pattern has evolved independently in numerous dipteran, lepidopteran, mirid and reduviid species (Naumann 1991, depicted in Plates 3, 5, and 6), and several braconid subfamilies including

Helconinae, Braconinae and Doryctinae (Quicke *et al.* 1992; Belokobylskij *et al.* 2004; Iqbal *et al.* 2006). Within the Australian Agathidinae, the BROW pattern appeared to be confined mostly to *Therophilus* species, although it is also known in two Australian *Disophrys* Foerster species (Stevens *et al.* 2010). More recently, revisions of the Oriental fauna have indicated the presence of the BROW colour pattern to be more widespread within the Agathidinae with two *Bassus* F. species from Vietnam (van Achterberg and Long 2010) and three *Agathacrista* Sharkey species from Thailand (Sharkey and Stoelb 2013) also displaying this pattern. Although it has evolved independently many times across multiple insect taxa, it is not known whether the same has occurred within Australian *Therophilus* or if the colour pattern is linked to a monophyletic lineage.

In the first phylogenetic analysis of the Agathidinae using both molecular and morphological data, *Therophilus* (as *Bassus*) was demonstrated to be polyphyletic (Sharkey *et al.* 2006). Sharkey and Stoelb (2012) defined *Therophilus* as comprising two main lineages: *Therophilus s. str.* falling within a 'New Tribe'; and *Therophilus s.l.* falling within the Agathidini *s. str.* (as Microdini). To date, no single character, or suite of distinguishing morphological features, are known that enable the reliable identification of each of these *Therophilus* lineages, thus making it difficult to define them as separate genera (Sharkey *et al.* 2009; Stevens *et al.* 2010; Stevens *et al.* 2011; Sharkey and Stoelb 2012; Sharkey and Chapman 2015).

Only one Australian *Therophilus* species (as *Bassus*) was represented in the analysis of Sharkey *et al.* (2006) and was placed within *Therophilus s.l.* Therefore, despite the dominance of *Therophilus* in Australia, its evolutionary affinities in the region are largely unknown, as is the relationship amongst members of the BROW complex. Thus, it is unclear whether the polyphyly of *Therophilus,* first demonstrated by Sharkey *et al.* (2006), occurs in Australia or whether the continent's fauna is monophyletic.

The main aims of this study were to: 1) produce a revised and expanded phylogeny of Agathidinae using morphological and 28S sequence data to investigate the evolution of Australian *Therophilus* in relation to the world fauna; 2) determine if the conspicuous BROW component of Australian *Therophilus* forms a monophyletic group, or if the colour pattern has evolved multiple times within divergent lineages; and 3) investigate more closely the evolutionary relationships among Australian *Therophilus* species using a revised morphological character matrix in conjunction with four genetic markers (16S rRNA, 28S rRNA, cytochrome oxidase I (CO1), and long wavelength rhodopsin (LW rh)).

Materials and Methods

Taxon sampling

The morphological and 28S molecular databases of Sharkey *et al.* (2006) were expanded with the addition of 25 Australian taxa, including representatives displaying the BROW colour pattern. Specimens were predominately obtained from Malaise traps set up at various sites around Australia, mostly in South Australia and New South Wales (Table 1). Material was also obtained from Malaise trap samples stored in ethanol at the Australian National Insect Collection (ANIC) and the Queensland Museum (QMBA). The examination of pinned material from Australian and international collections revealed that the Malaise trap samples did not cover the full morphological diversity present in Australian agathidines. Therefore, nine pinned specimens representing two *Braunsia*, one *Camptothlipsis* and six *Therophilus* species were also sequenced for the 28S gene.

Morphology

Two morphological data-sets were used in this study. The first data-set (Data-set 1) used the 40 characters and associated states of Sharkey *et al.* (2006) (Appendix 1), whose original purpose was targeted more at investigating the broader tribal level relationships of the subfamily. This data matrix (Appendix 2), with numerous modifications (Appendix 3), was expanded from 62 to 89 ingroup taxa with the addition of mostly Australian species. The second morphological data-set (Data-set 2) represents a re-interpreted character matrix incorporating additional characters to provide potentially more phylogenetic information at a lower taxonomic level, particularly for *Therophilus*. This data-set consists of 44 characters (Appendix 4) scored for 35 species (Appendix 5).

Table 1. List of taxa included in each of the four analyses including locality details, accession codes and comments on previous taxonomic classification used and if BROW colour pattern exhibited or if unknown.

Taxon list	Locality	Accession codes				Comments
		16S	28S	CO1	LW rh	
Out-group						
Ascogaster sp. M161		AF029114	AF029121	AF379988		
Ascogaster sp. 5					EU107011 / EU107037	
Cardiochiles sp. 3		EU107065		EU106958	EU107017 / EU107043	
Cardiochiles sp. 5			EU106922			
Diospilus fomitis Mason	Canada					
Malagsigalphus sp. 1	Madagascar		DQ201888			
Sigalphus gyrodontus He & Chen	Vietnam		AJ416966			
S. irrorator (Fabricius)	France		Z97942			
S. sp. DLJQ-AC		AF003509	AF029137	AF379995		
Agathidinae						
Agathidini *s.l.*						
Agathidini *s. str.*						
Agathacrista depressifera (van Achterberg & Long)	Thailand		KC556782			BROW
Ag. krataei (Sharkey)	Thailand		KC556781			BROW
Ag. sailomi (Sharkey)	Thailand		KC556780			BROW
Agathis montana Shestakov	Turkey		DQ201900			
A. sp. BM-11				AF078468		
A. sp. STJ1-3				AF078458		
A. sp. 2	Costa Rica		DQ201889			
Aphelagathis genehalli Sharkey	Mexico		KP943601			
Ap. verticalis (Cresson)	USA		KR736258			
Alabagrus arawak Sharkey	Neotropics		DQ201896			
Al. fuscistigma Enderlein	Neotropics		DQ201898			
Al. haenschi (Enderlein)	Neotropics		DQ201891			
Al. masneri Sharkey	Neotropics		DQ201897			
Al. maue Sharkey	Neotropics		DQ201899			

Taxon	Locality				Notes
Al. pachamama Sharkey	Neotropics		DQ201892		
Al. parvifaciatus (Cameron)	Neotropics		DQ201893		
Al. stigma (Brullé)	Neotropics		DQ201894		
Al. tricarinatus (Cameron)	Neotropics		DQ201895		
Al. sp. 1	Neotropics		AJ302790		
Al. sp. 2	USA		NS007	NS149	NS069
Braunsia bilunata Enderlein	Africa Sao, Tome		DQ201903		
Br. burmenis Bhat & Gupta	Malaysia		DQ201930		
Br. nr nigriceps	Africa		DQ201904		
Br. sp. 3	Australia, Northern Territory		NS132_136		
Br. sp. 4	Papua New Guinea		NB133_137		
Camptothlipsis oliveri (Stevens)	Australia, Northern Territory		NS83_95		
Cam. sp. 3	Madagascar		DQ201935		As *Bassus* sp. 3
Cam. sp. 9	Madagascar		DQ201934		*As Bassus* s.l. *Camptothlipsis* sp.
Lytopylus macadamiae Briceño & Sharkey	Costa Rica		DQ201901		As *Bassus macadamiae*
L. nr *macademiae*	Costa Rica		DQ201902		As *B.* nr *macadamiae*
Neothlipsis parysae Sharkey	USA		JF29791		
Pharpa dubiosum (Szépligeti)	Neotropics		DQ201890		
Plesiocoelus bassiformes van Achterberg	Costa Rica		DQ201906		
Pneumagathis brooksi (Sharkey)	Mexico: Sonora		KP943656		
Pn. brooksi	Mexico: Yucatan		KP943711		
Therophilus aalvikorum Stevens	Australia, Western Australia		NS53		BROW
T. nr *festinatus* sp. 1	Australia, New South Wales		NS001		BROW
T. nr *latibalteatus* sp. 1	Australia, Tasmania		NS048		BROW
T. nr *malignus* sp. 1	Australia, Queensland		NS006		
T. nr *martialis* sp. 1	Australia, Queensland		NS010		
T. nr *minimus* sp. 1	Australia, Queensland		NS051		BROW
T. mishae Stevens	Australia, Norfolk Island		NS77_89		
T. nr *ruficeps* sp. 1	Australia, Western Australia		NS82_94		BROW
T. nr *ruficeps* sp. 3	Australia, Western Australia		NS80_92		BROW
T. nr *ruficeps* sp. 4	Australia, Victoria		NS84_96		BROW
T. rugosus (Turner) hap. 1	Australia, New South Wales		NS059		BROW
T. rugosus hap. 3	Australia, New South Wales		NS061		BROW
T. nr *tricolor* sp. 1	Australia, Queensland		NS004		BROW
T. unimaculatus (Turner) hap. 1	Australia, South Australia	NS103	NS002	NS018	BROW

101

Species	Location					Colour pattern
T. unimaculatus hap. 2	Australia, South Australia		NS049	NS100		BROW
T. unimaculatus hap. 3	Australia, Victoria	NS106	NS003	NS020	NS073	BROW
T. unimaculatus hap. 4	Australia, South Australia		NS047			BROW
T. unimaculatus hap. 5	Australia, South Australia	NS107	NS008		NS120	BROW
T. sp. 2	Malaysia		DQ201931			Colour pattern unknown
T. sp. 4	Australia		DQ201939			Colour pattern unknown
T. sp. 5	Australia, New South Wales		AF173217			Colour pattern unknown
T. sp. 6.	Malaysia		AJ302793			Colour pattern unknown
T. sp. 7	UK, Silwood		Z97943			Colour pattern unknown
T. sp. 8	Australia, South Australia	AF003498	AJ245682			Colour pattern unknown
T. sp. 17	Australia, New South Wales		NS009			BROW
T. sp. 23	New Caledonia		NS81_93			
T. sp. 27	Australia, New South Wales		NS140	NS101	NS157	BROW
T. sp. 35	Australia, New South Wales		NS060			BROW
T. sp. 39	Australia, Western Australia		NS135_139			BROW
T. sp. 43	Pakistan		NBS142			
T. sp. 44	Australia, South Australia		NBS143			
T. sp. 46	Australia, Western Australia		NS011			
Zamicrodus sensilis Viereck	Colombia		DQ201911			
Za. sp. 1				NS151	NS163	
Earinini						
Amputoearinus fernandezi Sharkey	Guyana		DQ201946			
Am. matamata Sharkey	Colombia		DQ201928			
Austroearinus chrysokeras Sharkey	Costa Rica		DQ201929			
Au. melanopodes Sharkey	Costa Rica		DQ201948			
Au. rufofemoratus Sharkey	Costa Rica		DQ201950			
Crassomicrodus divisus (Cresson)	Mexico		DQ201945			
Earinus elator (Fabricius)	UK	AF176054	DQ201926			
*E.*sp. 1				NS150	NS162	
Sesioctonus akrolophus Briceño	Costa Rica		DQ201927			
S. nr *areolatus*	Costa Rica		DQ201947			
S. kompsos Briceño	Costa Rica		DQ201949			
Mesocoelini						
Aneurobracon sp. 1	Malaysia		DQ201944			
Mesocoelus sp. 1	Costa Rica		DQ201907			

	New Tribe					
T. nr *conspicuus* 1	Thailand		DQ201908			
T. nr *conspicuus* 2	Costa Rica		DQ201909			
T. dimidiator (Nees)	USA, Michigan		DQ201943			
T. stephensae Stevens.	Australia, South Australia		NS005			BROW
T. sp. 1	Columbia		DQ201910			
Cremnoptini						
Biroia sp. 1*	Tanzania		DQ201933			As *Biroia trifasciata**
Cremnops ferrungiensis (Cameron)	Costa Rica		DQ201922			
Cr. haematodes (Brullé)	USA, Colorado		DQ201941			
Cr. virginiensis (Morrison)	USA, Kentucky		DQ201921			
Cr. sp. 1	Australia		DQ201942			
Cr. sp. 4		DQ022283	DQ022280			As *Isoptronotum*
Zacremnops cressoni (Cameron)	Costa Rica		DQ201925			
Diosophrini						
Amputostypos sp. 1	Malaysia		DQ201932			As *Hypsostypos* sp.
Coccygidium luteum Saussure	Kenya		DQ201938			
Coc. nr *sissoo*	Australia		DQ201940			
Coc. sp. 1	Kenya		DQ201919			
Coc. sp. 4	Australia, Queensland	NS45				
Coc. sp. 5	Australia, Western Australia	NS036	NS052	NS024		
Coc. sp. 6	Australia, Western Australia		NS062		NS127	
Coc. sp. 7					NS131	
Disophrys atripennis (Szépligeti)	Indonesia, Sulawesi		DQ201924			
D. subfasciata (Brullé)	Thailand		DQ201923			
D. sp. 1	Madagascar		DQ201937			
D. sp. 2	Madagascar		DQ201936			
D. sp. 3	Australia, Western Australia	NS46	NS62	NS68	NS127	
Euagathis forticarinata (Cameron)	Thailand		DQ201920			
E. sp. 1	Thailand		DQ201905			
E. sp.CXX-2005					DQ056739	

Zelomorpha tropicola (Szépligeti)	Colombia	DQ201914
Z. nr *tropicola*	Guyana	DQ201918
Z. sp. 11	Neotropics	DQ201916
Z. sp. 25	Colombia	DQ201917
Z. sp. 79	Neotropics	DQ201913
Z. sp. 89	Costa Rica	DQ201915
Z. sp. 98	Colombia	DQ201912

*Referred to in Sharkey *et al.* (2006) as *Biroia trifaciata* but the existence of this species is questionable. The name has been mixed-up with *Braunsia trifasciata* Enderlein.

DNA techniques

The Gentra Systems Puregene DNA Purification Kit was used to extract DNA from specimens preserved in ethanol and from dry specimens that had been collected and mounted for up to 24 years prior to extraction. The primers used and optimal PCR setups for amplifying the markers 16S rRNA, 28S rRNA, CO1 and Long wavelength rhodopsin (LW rh) are presented in Tables 2 and 3, respectively. PCR products were cleaned up using the Ultraclean PCR Clean-up Kit (MOBIO Laboratories Inc.), sequence reactions were performed using ABI Big Dye terminator Chemistry, purified using Clean SEQ Clean-up Kit (MOBIO Laboratories Inc.) and sequences read on an ABI 3700 sequencer. Sequences underwent verification using a BLAST search set to default parameters (www.ncbi.nlm.nih.gov/BLAST/, verified March 2007) of sequences in Genbank to ensure data obtained was homologous and not contaminated. Editing of sequences carried out using BioEdit Sequence Alignment Editor © (1997–2013) (versions from 7.07.07 to 7.2.5; Hall (1999)) and initial alignments performed using Clustal W in BioEdit before manually editing sequences.

The primers used to amplify gene fragments from 16S, 28S, CO1, and LW rh are presented in Table 2. For specimens preserved in ethanol, the primers 28SF and 28SPMR were used to amplify an approximately 800 base pair (bp) fragment from in the D2 expansion region of 28S (Mardulyn and Whitfield, 1999) (Table 2). For dry mounted specimens, an approximate 800 bp fragment was too large a segment to amplify successfully. Therefore, internal primers were designed from agathidine sequences to obtain the required sequence as two segments. The first segment was obtained using 28SF (forward) and new primer G1082 (reverse); second segment was obtained using new primers G1090 (forward) and G1091 (reverse). The resultant catenation of the two 28S segments had 8 to10 bp missing medially. To provide enhanced comparisons with earlier sequenced material from Genbank, including Sharkey *et al.* (2006), the 28S sequence fragment size (~ 800 bps) derived from this study was reduced to 406 bps for the combined morphological and 28S data-set, thereby reducing the amount of missing data present. Regions of ambiguous alignment were omitted from the analyses.

Table 2: Primers used in this study

Gene	Name	Sequence (5'–3')	Reference
16S			
Forward	16S outer	CTTATTCAACATCGAGGTC	Whitfield (1997)
Reverse	16SWb	CACCTGTTTATCAAAACAT	Dowton & Austin (1994)
Reverse	M657	TAGCTGCAGTATTATAACTGTAC	This study
28S			
Forward	28SF	AAGAGAGAGTTCAAGAGTACGTG	Mardulyn & Whitfield (1999)
Forward	G1090	GCGTGCACTTCTCTCTTAGTA	This study
Reverse	28SPMR	TAGTTCACCATCTTTCGGGTCCC	Mardulyn & Whitfield (1999)
Reverse	G1082	TACTAAGAGAGAAGTGCACGC'	This study
Reverse	G1091	ATGTTAGACTCCTTGGTCCG	This study
COI			
Forward	C1-J-1718	GGAGGATTTGGAAATTGATTAGTTCC	Simon *et al.* (1994)
Reverse	CI-N-2329	ACTGTAAATATATGATGAGCTCA	Simon *et al.* (1994)
LW rh			
Forward	OpsFor2	GGATGTASCTCCATTTGGTC	Banks & Whitfield (2006)
Reverse	Ops3'Jon2	AGATGCACTTCATTTTCT	Banks & Whitfield (2006)

Table 3: The optimal PCR setup for amplifying each gene segment with temperature and time (°C – time) given for: Denaturing (initial / normal) Annealing Extension (normal / final) and Number of cycles of Denaturing + Annealing + Extension.

Gene	Denaturing	Annealing	Extension	No. Cycles
16S	94 – 45 sec / 2 min	50 – 45 sec	72 – 1 min / 2 min	35
28S				
Complete seg.	94 – 45 sec / 2 min	50 – 45 sec	72 – 1 min / 2 min	34
Seg. 1 & 2	94 – 45 sec / 2 min	45 – 45 sec	72 – 1 min / 2 min	35
COI	94 – 30 sec / 2 min	50 – 30 sec	72 – 1 min / 2 min	35
Rhodopsin	94 – 45 sec / 2 min	45 – 45 sec	72 – 1 min / 2 min	34

Table 4. Models chosen for data partitions by the Akaike information criterion in Modeltest (Posada and Crandall 1998)

Partition	Model Chosen
Morphology	Standard discrete
16S rRNA	GTR+G
28S rRNA	GTR+I+G
Cytochrome oxidase I (CO1)	
1st codon	GTR+I
2nd codon	F81
3rd codon	GTR+I+G
Long wavelength rhodopsin (LW rh)	
1st codon	HKY+G
2nd codon	GTR+I+G
3rd codon	K80+I+G

Phylogenetic analyses

Bayesian inference (BI) (MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001)) and maximum parsimony (MP) (PAUP* 4.0b10 (Swofford 2003)) were used to analyse four data-sets as follows:

Analysis 1 — morphology only based on Data-set 1 as described above. The aim of this analysis was to investigate the level of phylogenetic signal present and if any Australian groups, including BROW complex members, could be resolved as monophyletic.

Analysis 2 — morphology (Data-set 1) plus 28S data, employing taxa from this study and Sharkey *et al*. (2006) that were complete for both data sources. The main objective of this analysis was to place Australian *Therophilus*, including members of the BROW mimicry complex, within a global context. The 28S sequences were also analysed separately to enable a direct comparison with the influence of combining morphology with the 28S data. The resultant tree is not shown but levels of support for the main agathidine clades are compared in Table 5.

Analysis 3 — 28S DNA sequences from both Genbank and this study for taxa that comprise Agathidini *s. str.* (i.e. clade A1 that was resolved in Analysis 2 (Fig. 2)) to facilitate a broader geographical and taxonomic representation. This analysis also included additional species from the United Kingdom and Malaysia, as well as sequences from four recently erected genera, *Agathacrista* Sharkey, *Aphelagathis* Sharkey, *Neothlipsis* Sharkey, and *Pneumagathis* Sharkey (Table 1). These four genera were erected to deal with the polyphyly of *Therophilus s.l.* (referred to henceforth as '*Therophilus*' to represent the species that cannot be placed in the clade containing the type species). Because of the high level of congruence between BI and MP results for analysis 2, only BI was used for analysis 3. Other representatives from Agathidini *s. str.* genera *Alabagrus*, *Braunsia* and *Pharpa* were used as out-group taxa. The main objective of this analysis was to investigate more closely the relationships of Australian *'Therophilus'*, including BROW members, with non-Australian species to determine if any taxa fell within the recently erected genera.

Analysis 4 — morphology (Data-set 2) plus 16S, 28S, CO1 and LW rh. This analysis was designed to provide resolution at various phylogenetic levels. For a few taxa (not *Therophilus* spp.), sequences were missing for some markers. Therefore, sequences from closely related species were concatenated to provide complete coverage for all

markers. For example, the outgroup taxon *Cardiochiles* sp. was represented by combination of sequence data from *Cardiochiles* sp. 3 and *C.* sp. 5, and is displayed on the phylogenetic tree as *Cardiochiles* sp. 3 / sp. 5. This was also done for *Agathis*, *Ascogaster*, *Coccygidium*, *Euagathis*, *Earinus*, and *Zamicrodus* taxa as specified in Figure 4. The main objective of this analysis was to obtain better resolution of relationships among Australian *'Therophilus'*, particularly among species comprising the BROW mimicry complex. The limited taxonomic and geographical representation of non-Australian *'Therophilus'* species meant that the relationships for Australian *'Therophilus'* was not able to be thoroughly investigated.

Chi square tests of homogeneity of base frequencies across taxa for the 16S, 28S, CO1 and LW rh sequences were performed using PAUP* to identify significant heterogeneity in base composition (Lockhart *et al.* 1994). Appropriate models of evolution for the BI analyses of morphological and sequence data were determined using the Akaike information criterion derived from PAUP* and Modeltest 3.7 (Posada and Crandall 1998).

For the BI of morphology plus 28S two data partitions, morphology and 28S sequences, were implemented with the chosen model of evolution designated for each partition (Table 4). For the morphology plus four genes, nine data partitions were analysed using chosen models of evolution for each partition (Table 4). Model parameters were unlinked and estimated separately for each partition. Bayesian analyses were run across four chains for five million generations sampling every 100 generations. Stationarity was determined from an examination of log-likelihoods and trees sampled before stationarity was reached were excluded from further analysis. Multiple runs were performed to assess that all parameters were not considerably different at stationarity based on alternate prior probabilities.

For MP analyses the heuristic search algorithm with 100 random sequence addition replicates was used to eliminate any bias from taxon ordering in the datasets. All morphological and sequence characters in separate and combined analyses were 'unord' and of equal weight. Multistate characters were interpreted as polymorphisms and gaps treated as missing data. Starting tree(s) were obtained via stepwise addition with the addition sequence random. Number of trees held at each step during stepwise addition was 1. Branch-swapping algorithm was tree-bisection-reconnection (TBR) and steepest descent option was not in effect. No more than 1000 trees of score (length) greater than or equal to 1 was saved for each replicate. The number of rearrangements per addition-sequence

replicate was limited to 1,000,000 to avoid very long computational times. Initial 'MaxTrees' was set to 1000 and automatically increased by 1000. Branches were collapsed (creating polytomies) if maximum branch length was zero. Confidence in the resultant MP trees was assessed from 1000 non-parametric bootstrap pseudoreplications.

For analyses 1, 2 and 4, the BI consensus trees were largely congruent in topology and levels of support for resolved nodes with the corresponding MP consensus trees. Thus, the MP strict consensus tree for each analyses are not shown, however, all bootstrap values equal to or greater than 50% are placed beneath the corresponding nodes on the BI trees (Figs 1, 2 and 4).

Results
Analysis 1: Morphology only
The MP analysis of the 40 parsimony informative characters, generated 10,549 'best trees' with lengths of 231 steps. The resultant strict consensus tree had a consistency index (CI) = 0.249, retention index (RI) = 0.668, and rescaled consistency index (RC) = 0.166. The low parsimony CI and RC values indicated that a high level of homoplasy was present within the character set.

The analysis provided little resolution of the Agathidinae with most tribal and generic relationships receiving insufficient support from either inference method (Fig. 1). Most in-group taxa included could be viewed as falling out in a basal polytomy.

Therophilus was polyphyletic in the absence of any unequivocal synapomorphies and was represented by four separate lineages (Fig. 1). The only lineage containing *Therophilus* taxa that received any support was clade 2 (52%). This weakly supported clade contained all Australian *Therophilus* taxa, except for *T.* sp. 4, and also included all members of the BROW mimicry complex. This clade also contained non-Australian species from Pakistan (*T.* sp. 43) and New Caledonia (*T.* sp 23) and was rendered paraphyletic by the presence of the type species of *Bassus*, *B. calculator* (European species), as well as members of *Lytopylus* (from Europe) and *Camptothlipsis* (from Australia). There was no internal support for relationships depicted in clade 2 and, in the absence of any support, the remaining *Therophilus* lineages are best considered as part of a basal polytomy.
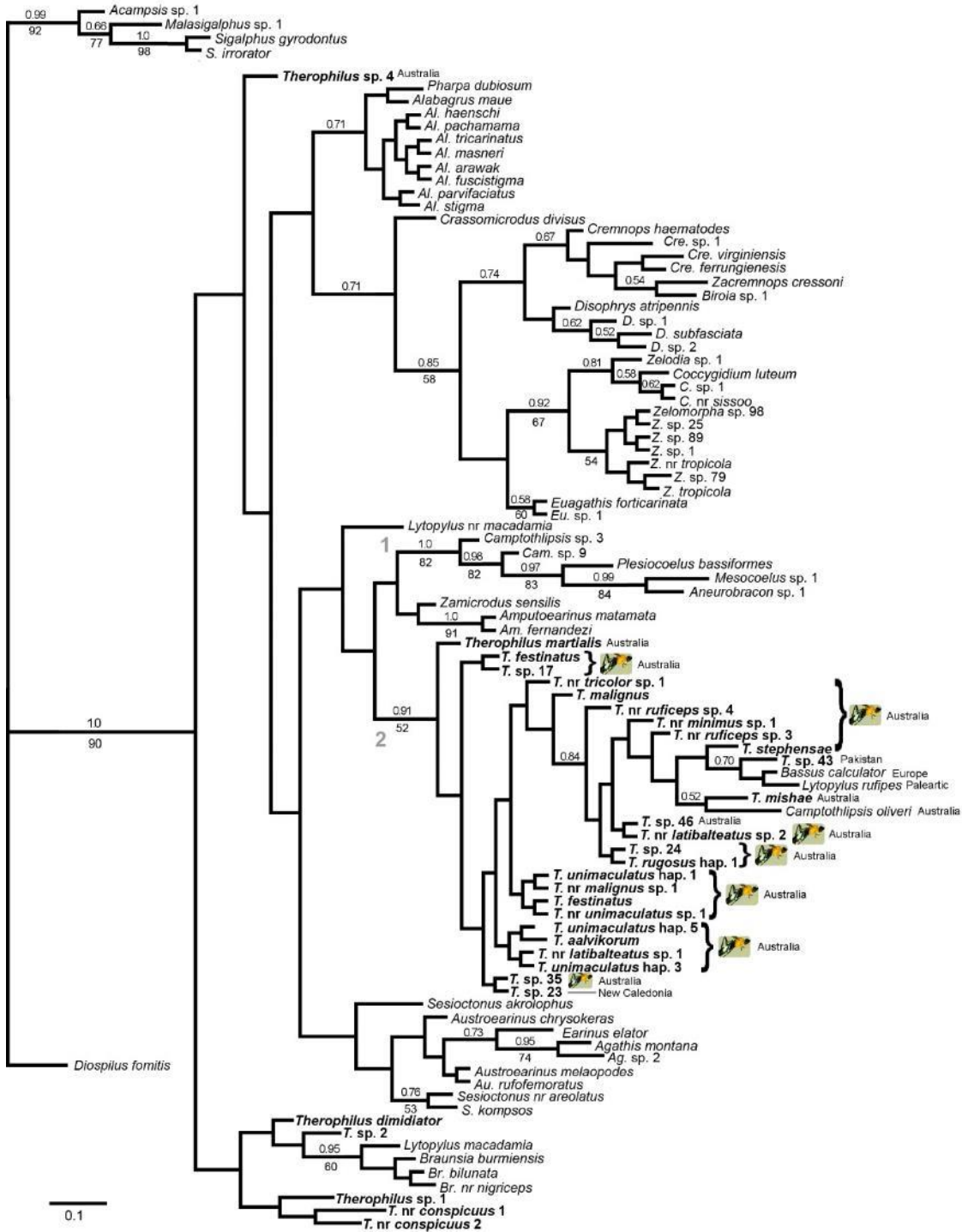
Figure 1. BI concensus tree based on Analysis 1 of 40 morphological characters adopted from Sharkey *et al.* (2006) with minor modifications (refer Appendix B) and the addition of predominantly Australian taxa. Posterior probabilities given above branches; MP bootstrap values below. BROW members denoted by after name.
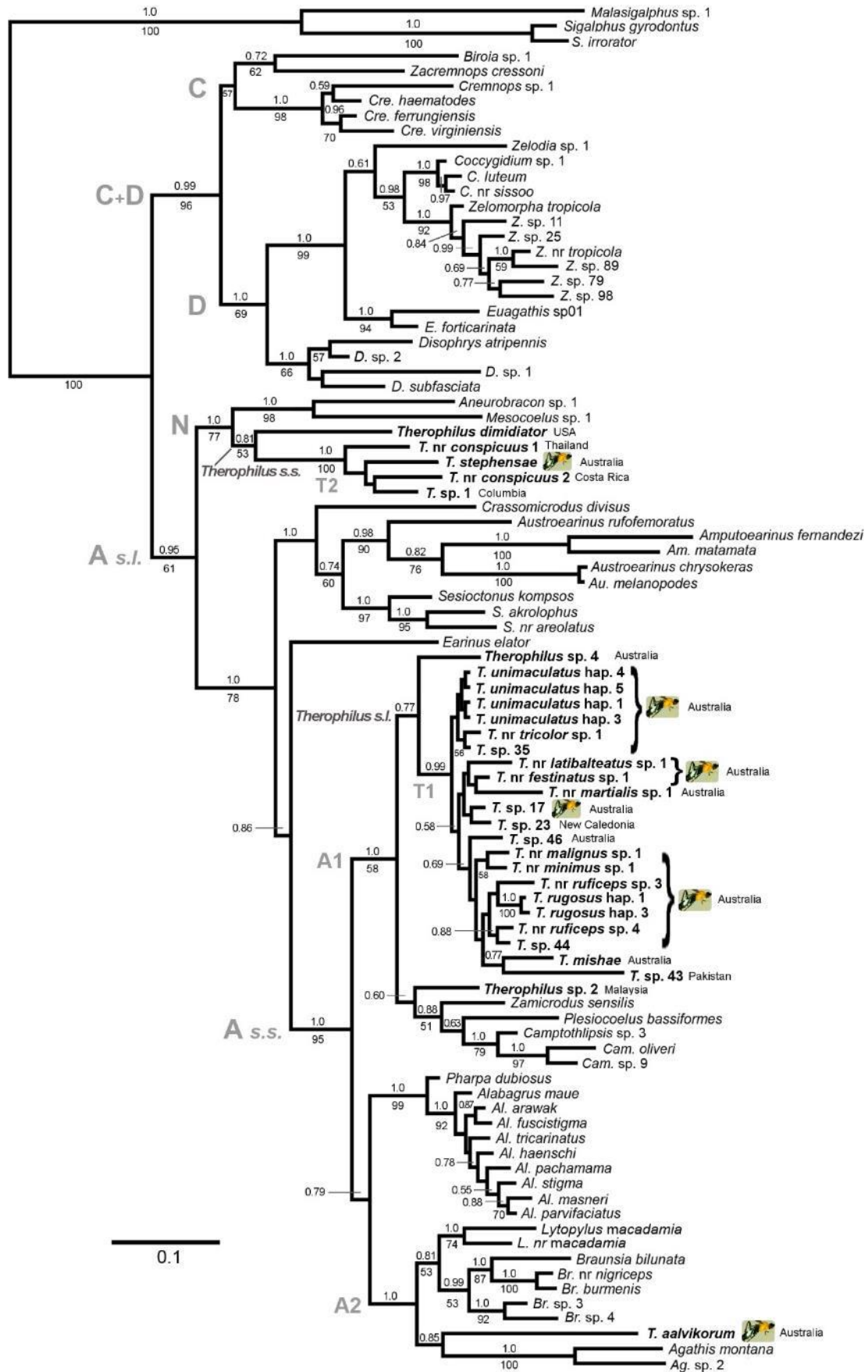
Figure 2. BI concensus tree based on Analysis 2 of morphological and 28S data from Sharkey *et al.* (2006) and this study. Posterior probabilities given above branches; MP bootstrap values below. Labelled nodes denote clades mentioned in text; A *s.l.* = Agathidini *sensu lato*; A *s. str.* = Agathidini *sensu stricto*; N = 'New Tribe' of Sharkey *et al.* (2006); BROW members denoted by 🐝 after name.

Analysis 2: Morphological plus 28S

The analysis of morphological characters plus 28S sequence data with ambiguous regions excluded resulted in 446 characters, with 278 of these being parsimony informative. No significant difference in base composition across taxa was detected (*28S rRNA*: P = 1.0). The MP resultant strict consensus tree of the combined data was derived from 741 trees with tree length 1744 (CI = 0.328, RI = 0.707, RC = 0.232).

The relationships resolved in this analysis were markedly different to that of the morphology only tree and clearly demonstrated the Australian *Therophilus* fauna and the BROW mimicry complex to be polyphyletic (Fig. 2). Australian *Therophilus* species, including members of the BROW mimicry complex, fell out in three main divergent lineages, within the Agathidini *s.l.*: 1) *'Therophilus'* within Agathidini *s. str.*; 2) *Therophilus s. str.* within the 'New Tribe' *sensu* Sharkey *et al.* (2006); and 3) a previously undetected *Therophilus* lineage also within Agathidini *s. str.* but highly divergent to *'Therophilus'*.

All Australian *Therophilus* species, except for *T. aalvikorum* Stevens and *T. stephensae* Stevens (both BROW species), fell within an unsupported *'Therophilus'* clade nested in a well-supported Agathidini *s. str.* (labelled A1 clade, 1.0: 58% (Fig. 2)). Most *'Therophilus'* species were resolved within a terminal clade (T1) which had high BI support (0.99) with the exclusion of *T.* sp. 2 (Malaysia) and *T.* sp. 4. (Australia). This clade hosted most of the Australian species included (with the exception of *T.* sp. 4.) but also included non-Australian species, *T.* sp. 23 (New Caledonia) and *T.* sp. 43 (Pakistan), indicating that this lineage is not unique to Australia. Relationships within the T1 clade were not wellresolved with only three terminal clades, all made up of BROW members only, receiving any support: (*T.* nr *tricolor* sp. 1, *T.* sp. 35) (56%); (*T.* nr *malignus* sp. 1, *T.* nr *minimus* sp.1) (58%); and (*T. rugosus* hap. 1, hap. 3) (1.0: 100%).

'Therophilus' was polyphyletic with respect to *T.* sp. 2 which fell out as a basal member of a lineage comprising the marginally supported (51%) (*Camptothlipsis, Plesiocoelus*, *Zamicrodus*) clade. However, the basal polyphyly of *Therophilus. s.l.* was not supported by either inference methods and should be viewed as forming an extensive basal polytomy made up of *T.* sp. 4, T2 clade, *T.* sp. 2, and (*Camptothlipsis, Plesiocoelus, Zamicrodus*) clade.

Therophilus stephensae was resolved as the lone Australian and BROW representative in the 'New Tribe' *sensu* Sharkey (2009), falling within a well-supported

(1.0; 100%) *Therophilus s. str.* clade (T2) (Fig. 2). *Therophilus s. str.* was resolved as a monophyletic group but with low MP support (53%), with *T. dimidiator* being basal to the more highly derived T2 clade. The well-supported T2 clade represents a widespread group, containing Australian, Central and South American, and south-east Asian species that are divergent from *T. dimidiator*.

Therophilus aalvikorum formed a previously undetected *Therophilus* lineage that fell within the well-supported Agathidini *s. str.* A2 clade along with the *Agathis* and *Braunsia + Lytopylus* lineages (Fig. 2). The long branch lengths indicate that *T. aalvikorum* is highly divergent from other Agathidini *s. str.* A2 clade members and is the only species known in the A2 group that displays the BROW mimicry colour pattern.

The addition of morphological characters to the 28S data generally provided greater resolution and levels of support for basal agathidine relationships, many of which were not resolved by 28S data alone (Table 5). Conversely, the addition of morphological data did erode support for a number of more apical relationships. The monophyly of the Agathidini (0.95; 61%), Cremnoptini (57%), *Therophilus s. str.* (53%) were only supported (albeit at low levels for latter two groups) with the addition of morphological data. For more terminal relationships in the tree, such as *Plesiocoelus + Zamicrodus*, support levels were eroded, particularly bootstrap values in the case of Agathidini *s. str.* clades A1 and A2.

Table 5. Comparison of support levels for the main lineages between 28S only data and morphology + 28S data for taxa in data set 2 shown by BI (posterior probabilities) and MP (bootstrap).

Clade	28S (n = 91)		Morphology & 28S (n = 91)	
	Post. Prob.	Boot strap (%)	Post. Prob.	Boot strap (%)
Agathidini *s.l*	Non-sig. (0.91)	Not supported	0.95	61
Agathidini *s. str.*	1.0	97	1.0	95
Agathidini *s. str.* 1 (A1)	1.0	84	1.0	58
Agathidini *s. str.* 2 (A2)	1.0	62	1.0	Not resolved
Cremnoptini	Not resolved	Not resolved	Non-sig. (0.33)	57
Cremnoptini+Disophrini	0.95	66	0.99	96
Disophrini	0.96	51	1.0	69
New Tribe	1.0	Not resolved	1.0	77
Therophilus s. str.	Not resolved	Not resolved	Non-sig. (0.81)	53
'Therophilus'	Non-sig. (0.5)	Not supported	Not resolved	Not resolved
Zamicrodus+Plesiocoelus	0.99	63	Not resolved	Not resolved

Analysis 3: 28S for Agathidini s. str. Clade A1 only

The alignment of 28S gene sequences from the D2 expansion region with ambiguous regions excluded resulted in an alignment of 675 bp. The BI analysis resolved the A1 clade but support was not quite significant (0.94) (Fig. 3). This clade comprised numerous genera with a poorly supported but intriguing basal split between Old World genera (clade A1a: 0.72) and New World genera (clade A1b: 0.84). Within the Old World A1a clade only convincing support was shown for *Agathacrista* (1.0), *Camptothlipsis* (0.99) and (*T. rugosus*, *T.* sp. 5) (1.0). There was no resolution of a *'Therophilus'* T1 clade as supported in the analysis of Sharkey *et al.* (2006). For the New World clade A1b, the *Aphelagathis -Pneumagathis*) sister group relationship was well supported (1.0) although 28S alone failed to support *Pneumagathis*. Both genera are highly divergent to all other lineages within the A1 clade. In the absence of support this clade and its basal branches, the many internal lineages form an extensive basal polytomy along with *Braunsia*. The Australian *'Therophilus'* species remained unresolved but were clearly shown not associated with the recently erected genera, in particular the south-east Asian genus *Agathacrista* that also contains species that display the BROW colour pattern.

Analysis 4: Therophilus *morphology plus 16S, 28S, CO1 & LW rh*

This analysis resulted in 1617 characters, with 604 being parsimony informative. No significant difference in base composition across taxa for each gene was detected. The MP strict consensus tree of the combined data was derived from two trees of length 2286 (CI = 0.485, RI = 0.895, RC = 0.434).

The analyses, particularly the BI, provided greater resolution of Australian *'Therophilus'* relationships within the A1 clade. Both BI and MP showed good support for the *'Therophilus'* T1 clade (0.99; 70%) with the non-BROW Pakistan species, *T.* sp. 43, falling out basally to a highly supported all-Australian clade (T1a) (1.0; 87%) (Fig. 4). The Australian species predominantly fell within the clade T1b (1.0) that formed a basal polytomy in the T1a clade with a non-BROW species, *T.* nr *martialis* sp. 1, and BROW species, *T.* nr *festinatus*. The T1b clade comprised two clades: the *T. unimaculatus* group (1.0; 87%), potentially composed entirely of BROW members (colour pattern of *T.* sp. 8 is unknown), and the *T. rugosus* group (0.98), composed of both BROW and non-BROW species. Of particular interest in regards to potential evidence for an additional derived origin (or loss) of the BROW mimicry pattern in this tree is the well resolved and highly supported apical clade (1.0; 71%) within the *T. rugosus* group. Within this apical clade, the

non-BROW species, *T*. sp. 44, falls out as the basal member to the well-supported BROW clade (*T*. nr *latibalteatus* sp. 1, *T. rugosus*).
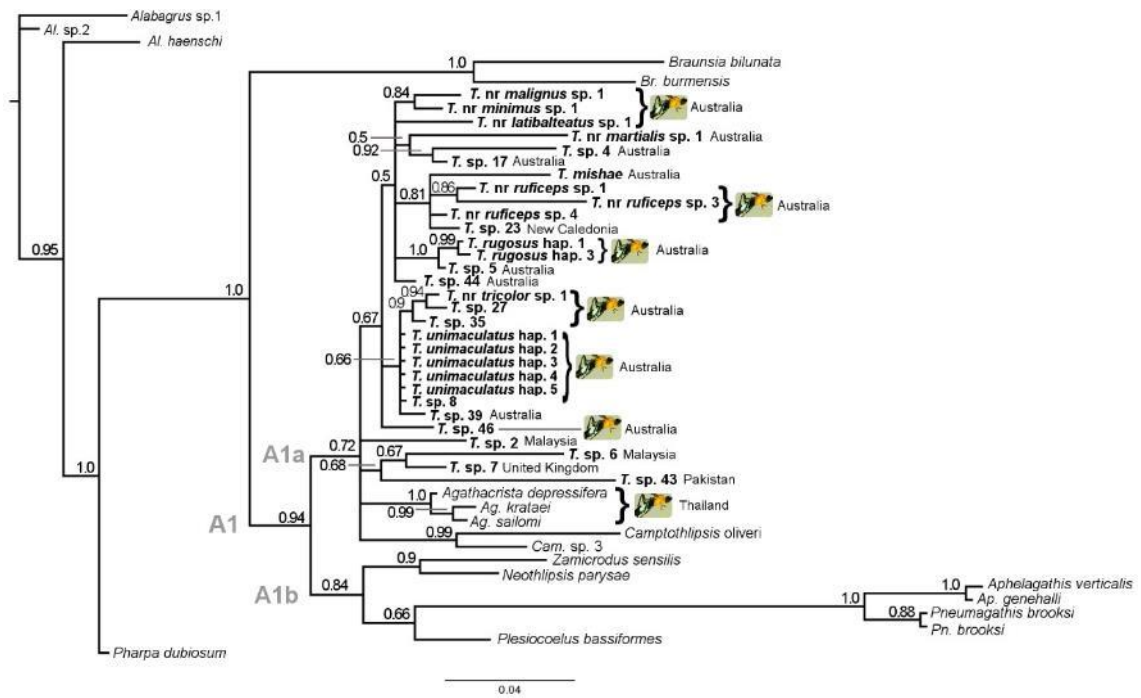
Figure 3. BI concensus tree based on Analysis 3 of Agathidinae Agathidini *s. str.* Clade 1 28S sequence data from Genbank and from this study. BI posterior probabilities given above branches; MP bootstrap values below. BROW members denoted by after name.

Figure 4. BI concensus tree based on Analysis 4 of morphology plus 16S, 28S, CO1 and LW rh data. Posterior probabilities given above branches; bootstrap values below. Labelled nodes denote clades that are mentioned in the text; A *s.l.* = Agathidini *sensu lato*; A *s. str.* = Agathidini *sensu stricto*; BROW members denoted by after name.

Discussion

Australian Therophilus

The Australian *Therophilus* fauna and the BROW mimicry complex are clearly polyphyletic, as demonstrated by analyses 2 and 4, with species falling into at least three main lineages (Figs 2 and 4). The bulk of the Australian species in this study fell within the unresolved *'Therophilus'* tree region that appears to host multiple lineages of Australian species. Analyses 2 (Fig. 2) and 3 (Fig. 3) that included a broader geographical representation compared to Analysis 4 provided no compelling evidence of monophyletic Australian lineages, thus the re-establishment of previously synonymised endemic genera *Agathiella* Szépligeti or *Orgiloneura* Ashmead is not warranted at this stage. The known generic diversity within this lineage is not fully represented here as Thailand species of *Bassus s. str.* and a new undescribed genus have been shown to represent sister groups to the newly erected *Agathacrista* (Sharkey and Stoelb 2013) that fell out in a basal polytomy with many unresolved Australian lineages in Analysis 3 (Fig. 3). These three genera are apparently restricted to the Old World, primarily within the Oriental and eastern Palearctic regions, but with species also occurring within the northern, tropical regions of Australasia (Sharkey and Stoelb 2013). Unfortunately, sequences for *Bassus s. str.* and the new undescribed genus were not available for this study so their relationship with the Australian fauna could not be assessed. In this study the polyphyletic *'Therophilus'* was composed mostly of Australian species which is a likely reflection of a bias in taxon sampling, but it also highlights that the Australian agathidine fauna is dominated by species in this lineage (Stevens *et al.* 2010, 2011). Previous studies of the Oriental agathidine fauna also revealed a polyphyletic *Therophilus* that is as species rich as all other agathidine genera combined (Sharkey *et al.* 2009; van Achterberg and Long 2010) with both *Therophilus s. str.* and *'Therophilus'* well represented (Sharkey and Stoelb 2012).

With an increase in taxon sampling, this study further demonstrated the polyphyly of *Therophilus* with the revelation of a previously undetected lineage from Australia (Figs 2 and 4). This lineage, represented by *T. aalvikorum,* which fits within the morphological limits of *Therophilus* (Fig. 1), was found to be genetically divergent from both *Therophilus s. str.* and *'Therophilus'*. *Therophilus aalvikorum* fell within the Agathidini *s. str.* A2 clade along with *Agathis*, *Braunsia and Lytopylus*. *Agathis* is a speciose and widespread genus in the northern Hemisphere (van Achterberg and Long 2010) whose non-exemplar members have traditionally been difficult to differentiate from *Therophilus* (as *Bassus*) species (Simbolitti and van Achterberg 1992; Sharkey 2004). Although *T. aalvikorum* is

genetically distant from the *Agathis* lineage, we are reluctant to recognise this species as a new genus until much denser taxon sampling is undertaken to more comprehensively define the limits of *Agathis* and to ensure that other lineages, not included in this study, are included.

Therophilus stephensae was the only Australian species to be resolved in *Therophilus s. str.* nested within the New Tribe of Sharkey and Stoelb (2012) (equivalent to the "n. tribe?" of Sharkey *et al.* (2006)) (Figs 2 and 4). The issues in defining *Therophilus s. str.* morphologically are well summed up in the opening line of the diagnosis provided by Sharkey and Stoelb (2012): "*There is neither one character nor a specific combination of characters that distinguishes members of* Therophilus [*s. str.*] *from all other agathidines*". The morphological phylogeny demonstrated this with *Therophilus stephensae* falling out along with *'Therophilus'* taxa as well as *T. aalvikorum* within a paraphyletic *Therophilus*. Despite the difficulties in reliably defining the morphological limits of *Therophilus s. str.*, Sharkey and Stoelb (2012) considered another Australian species, *T. antipoda* (Ashmead), to also belong to this clade. Evidence from this study suggested that *Therophilus s. str.* may represent multiple genera with *T. stephensae* falling within a well-supported, geographically widespread clade, that was highly divergent to *T. dimidiator* (Fig. 2). Sharkey and Chapman (2015) depicted a highly divergent basal spilt within the *Therophilus s. str.* supporting this notion but this was not discussed in their paper as the focus was the erection of new Neartic genera within the *'Therophilus'*.

How should *'Therophilus'* be treated in light of this rampant polyphyly? The next obvious step would be to begin defining each genetically well-supported divergent lineage as a separate genus, a process already underway with the recognition of New World genera *Aphelagathis*, *Pneumagathis* and *Neothlipsis* (Sharkey *et al.* 2011; Sharkey and Chapman 2015), and the Oriental genus *Agathacrista* (Sharkey and Stoelb 2012). However, not all genera can be reliably defined morphologically because of the high degree of character convergence among the widely separated lineages and the high level of polymorphic characters within each group. Therefore, a greater representation from a broader geographic area of *'Therophilus'* taxa, as well as the elucidation of existing and synonymised lineages (e.g. *Agathiella*, *Agathis*, *Bassus* and *Orgiloneura*) is required to better understand and define the evolutionary relationships present.

BROW mimicry complex

This study clearly demonstrates that the BROW pattern has evolved multiple times independently within the Australian *'Therophilus'* fauna, as well as being present within the widely divergent *T. aalvikorum* and *Therophilus s. str.* lineages. Within the *'Therophilus'* T1 clade, the basal taxon within a number of the supported groups were often non-BROW species with BROW taxa comprising more apical lineages. The colour pattern is also known to occur in other regions with some *Bassus* and *Agathacrista* species from Vietnam and Thailand also displaying the BROW colour pattern (van Achterberg and Long 2010; Sharkey and Stoelb 2013). The convergent evolution of the BROW pattern across a range of non-hymenopteran insect orders (Diptera, Hemiptera and Lepidoptera) have also been associated with convergence in the braconid wasp body form (Naumann 1991; pers. obs.) suggesting that the models may be derived from any number of braconid BROW species within the Agathidinae, Braconinae, Doryctinae, and/or Helconinae (Quicke *et al.* 1992; Belokobylskij *et al.* 2004; Iqbal *et al.* 2006). Are there aspects of convergence of body form as well as colour pattern that represent a factor in confounding the morphological recognition of the polyphyletic *Therophilus*? This would be difficult to determine as there would be many possible model / co-model forms operating across such a broad geographical area which polyphyletic *Therophilus* are known to occur. At a gross morphological level (e.g., over-all body form / 'appearance', degree of sculpturing present), such a factor may be operating to some degree. However, it would be considered less of an influence at a finer character scale commonly used to differentiate genera (e.g., various mouth-part, leg, and/or wing characteristics) that are likely to be under other more demanding host or feeding related natural selection pressures.

Conclusion

The use of multiple genes in this study, not surprisingly, provided better resolution of relationships among Australian *'Therophilus'* taxa compared to 28S alone. However, to better understand the evolution of *'Therophilus'* taxa a broader taxonomic and geographical representation is required to better define and test the monophyly of lineages and determine if, or how many, uniquely Australian clades are present. A more robust phylogenetic understanding of *'Therophilus'* would benefit further investigation of the BROW mimicry complex, not just from a phylogenetic perspective, but also in providing an evolutionary framework to support physiological and/or behavioural studies that might investigate a number of unknown aspects (e.g., model versus mimic, Batesian versus Müllerian) concerning the BROW mimicry complex.

Acknowledgements

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Chapter 5: General Discussion

5.1 Overview

This systematic study of the Australian Agathidinae revealed the generic and species diversity of the subfamily throughout the various biogeographical realms within the continent and elucidated the evolutionary relationships of the dominant and polyphyletic genus, *Therophilus*. The taxonomic component of the study aimed to document and define the generic diversity of the Australian agathidine fauna (**Chapter 2**), with particular emphasis on the taxonomy and diversity of the largest genus present, *Therophilus* (**Chapter 3**). A complete taxonomic revision of *Therophilus* for Australia was beyond the scope of this study given the large number of undescribed taxa found to be present. Instead, all 17 *Therophilus* species were redescribed as previous descriptions, the most recent being 1918, were outdated and inadequate to define species limits. This also involved the documentation of the presence and variation in the BROW colour pattern displayed by many species involved in the mimicry complex. Four new species were also described to support the first phylogenetic investigation of *Therophilus* relevant to the Australia fauna, including members of the BROW mimicry complex (**Chapter 4**). The findings of this study placed the evolution of Australian *Therophilus* into a world context for the first time and revealed that at least three main divergent lineages are present, one of which represents a new previously undetected lineage. This work also demonstrated that the BROW complex has evolved multiple times independently within the Australian fauna.

5.2 Diversity of Australian Agathidinae

Following the examination of available material from major collections, the Australian agathidine fauna was found to naturally comprise nine genera: *Camptothlipsis* Enderlein (as junior synonym of *Baeognatha* Kokujev in Chapter 2, later reinstated by van Achterberg and Long, 2010), *Biroia* Szépligeti, *Braunsia* Kriechbaumer, *Coccygidium* Saussure (including *Amputostypos* Sharkey in Chapter 2, later treated as junior synonym by van Achterberg and Long, 2010, with Australian species transferred to *Zelodia* van Achterberg), *Cremnops* Foerster, *Disophrys* Foerster, *Euagathis* Szépligeti, *Therophilus* Wesmael and *Zelodia*. A tenth genus, *Lytopylus* Foerster, was also found to be present in Australia but is known only from a single introduced species, *L. rufipes* Nees von Esenbeck. Also, following examination of the relevant type, an endemic genus *Platyagathis* was proposed as a new junior synonym of *Disophrys*. This study also recorded two genera, *Camptothlipsis* and *Coccygidium*, from the continent for the first time. The generic diversity is considerably less than recorded in modern taxonomic treatments of the Oriental and Nearctic faunas, that have identified 20 and 15 genera,

respectively (van Achterberg and Long 2010; Sharkey and Clutts 2011; Sharkey and Stoelb 2012; 2013). The Australian fauna has strong Oriental affinities with all genera recorded also represented in the Oriental region.

Two genera previously considered to be cosmopolitan and to occur in Australia, *Agathis* Latreille and *Bassus* Fabricius, were deemed absent from Australia, with *Agathis dimidiata* Brullé declared a *nomen dubium*, and all other Australian species previously considered to belong to *Agathis* and *Bassus* transferred to *Therophilus*. The modern treatments of *Agathis* define the genus as mostly confined to the more temperate regions of the northern Holarctic but with some species present in the Oriental region (van Achterberg and Long 2010; Sharkey *et al.* 2009). With the current generic concept proposed for *Bassus* the genus is considered to possess an Old World distribution (Sharkey *et al.* 2009).

The Australian fauna is dominated by tropical genera with the northern tropical to sub-tropical regions of the continent hosting the greatest generic diversity (**Chapter 2**). Only the polyphyletic *Therophilus* was found to be widespread throughout the Australian continent (including Tasmania). *Therophilus* is the most diverse genus comprising about two–thirds of all agathidine species on the continent, with over 150 species present, 20 of which are described (**Chapter 3**). *Therophilus* is more diverse in the temperate regions of the south-east and the south-west of the continent. Although this pattern may be accentuated by a sampling artefact it does indicate that the Australian fauna has evolved to exploit hosts across all climatic conditions throughout the continent. *Coccygidium* is the next most speciose genus with an estimated 20 to 25 species for the continent, none of which are described. *Cremnops* and *Disophrys* are the only other genera to be well represented in Australia, with the remaining genera comprising relatively minor components of the fauna.

5.3   Phylogeny of Australian *Therophilus*

The evolutionary affinities among the Australian *Therophilus* species and with the fauna globally were not known previously. This study investigated the evolutionary relationships of Australia members of the genus and placed them in a global context using a combination of morphological data and sequence information for multiple mitochondrial and nuclear genetic markers (**Chapter 4**). In total, 41 *Therophilus* species, including 32 Australian species, were included in the analyses along with 92 non-*Therophilus* species, nine of which were Australian.

The main finding of this phylogenetic study was that Australian *Therophilus* does not represent a monophyletic radiation. Instead, the results demonstrated that the two main *Therophilus* lineages, *Therophilus s. str.* (within the 'New Tribe'), and '*Therophilus*' (within the Agathidini *s. str.*), recognised in previous studies (Sharkey *et al.* 2006; Sharkey and Stoelb 2012) were both represented within the Australian fauna. However, with an increase in taxon sampling, the polyphyly of *Therophilus* was further demonstrated with the revelation of a previously undetected lineage, represented by *T. aalvikorum,* which fits within the morphological limits of *Therophilus* but is genetically divergent from both *Therophilus s. str.* and '*Therophilus*'. The polyphyly of Australian *Therophilus* was in contrast to other Australian species that were placed within existing genera *viz. Braunsia, Camptothlipsis, Coccygidium, Cremnops,* and *Disophrys*, all of which were monophyletic.

All but two Australian *Therophilus* species sequenced fell within the unresolved '*Therophilus*' highlighting that the fauna is dominated by species in this polyphyletic group. '*Therophilus*' has been found to be diverse in the Oriental and Nearctic regions with multiple well-represented genera known to occur that have been recently resurrected (*Camptothlipsis*, (Sharkey *et al.* 2009), redefined (*Bassus s. str.* (Sharkey *et al.* 2009)), proposed (*Neothlipsis* (Sharkey *et al.* 2011); *Agathacrista* (Sharkey and Stoelb 2013); *Aphelagathis* and *Pneumagathis* (Sharkey and Chapman 2015)), or recognised but undescribed (Sharkey and Stoelb 2013), in response to treating the numerous polyphyletic branches comprising '*Therophilus*'. The multi-gene analysis provided better resolution of '*Therophilus*' relationships but lacked the taxonomic and geographic representation to thoroughly elucidate the relationships among Australian species and to other closely related non-Australian taxa that precluded the recognition of new genera.

The previously undetected *T. aalvikorum* lineage fell within the Agathidini *s. str.* branch along with *Agathis*, *Braunsia and Lytopylus*. *Agathis* is a speciose and widespread genus in the northern Hemisphere (van Achterberg and Long 2010) that has traditionally been difficult to differentiate from *Therophilus* (as *Bassus*) species (Simbolitti and van Achterberg 1992; Sharkey 2004). Although *T. aalvikorum* is genetically distant from the *Agathis* lineage, it would be premature to recognise this lineage as a new genus because much denser taxon sampling is required to more comprehensively define the limits of *Agathis* and to ensure that other lineages, not represented in the analysis, are included.

Therophilus stephensae was the only Australian species to be resolved within the *Therophilus s. str.* group (which includes the type species) in the New Tribe of Sharkey and Stoelb (2012). The issues in defining *Therophilus s. str.* given that it has no recognisable synapomorphies are summarised by Sharkey and Stoelb (2012). The morphological phylogeny demonstrated these issues with *T. stephensae* falling out along with *Therophilus s.l.* taxa, as well as *T. aalvikorum,* within a paraphyletic *Therophilus* branch. Evidence from this study suggests that *Therophilus s. str.* may represent multiple genera with *T. stephensae* falling within a well-supported, geographically widespread clade that is highly divergent to *T. dimidiator*. Sharkey and Chapman (2015) also depicted a highly divergent basal spilt within the *Therophilus. s. str.* supporting this notion.

How should the Australian *Therophilus* be treated in light of this rampant polyphyly? With no single character or suite of distinguishing morphological features known to enable the reliable identification of the various branches of the polyphyletic *Therophilus*, it will be very difficult to divide the whole group up into monophyletic genera without an almost complete reliance on molecular data (Sharkey *et al.* 2009; Stevens *et al.* 2010; Stevens *et al.* 2011; Sharkey and Stoelb 2012; Sharkey and Chapman 2015). This process has commenced to some degree with the recognition of several small groups of species, i.e. the New World genera *Aphelagathis*, *Pneumagathis* and *Neothlipsis* (Sharkey *et al.* 2011; Sharkey and Chapman 2015), and the Oriental genus *Agathacrista* Sharkey and Stoelb (2012), which have been recognised through molecular phylogenetics. However, each genus is not always able to be reliably defined morphologically because of the high degree of character convergence among the widely separated lineages constituting these genera and the high level of polymorphic characters within each group. To date these new genera have represented relatively easy clades to 'carve off' as new genera. The process will increasingly be far more difficult for the highly species rich and potentially geographically widespread '*Therophilus*' lineages. This will require a much greater representation of '*Therophilus*' taxa from a broader geographic scope, as well as the inclusion of type species from existing and synonymised genera (e.g. *Agathiella*, *Agathis*, *Bassus* and *Orgiloneura*). In addition, it is important that future molecular studies incorporate a multi-gene analysis to improve the robustness of the phylogenetic results to better resolve '*Therophilus*' relationships.

5.4   BROW mimicry complex

The phylogenetic results clearly demonstrated that the BROW pattern has evolved on multiple occasions within Australian '*Therophilus*', as well as being present within the

widely divergent *T. aalvikorum* and *Therophilus s. str.* lineages. The colour pattern is also known to occur outside of Australia within *Therophilus s.l.* with some *Bassus* and *Agathacrista* species from Vietnam and Thailand also displaying the BROW pattern (van Achterberg and Long 2010; Sharkey and Stoelb 2013). The convergent evolution of the BROW mimicry colour pattern across a range of non-hymenopteran insect orders (Diptera, Lepidoptera, and Hemiptera) has often also been associated with a convergence in the braconid wasp body form (Naumann 1991; pers. obs.) suggesting that the models may be derived from any number of braconid BROW species within the Agathidinae, Braconinae, Doryctinae, and/or Helconinae subfamilies (Quicke *et al.* 1992; Belokobylskij *et al.* 2004; Iqbal *et al.* 2006).

Does the BROW colour pattern represent an aposematic signal? Aposematic (warning) signals are a common antipredatory defence within the animal kingdom, particularly the Insecta. Essentially, aposematic signals are the development of bright and contrasting colours (but not always; see Wuster *et al.* 2004) that warn predators that an otherwise potential prey item is unprofitable for them to attack because of the possession of defences, such as distastefulness/unpalatibility, toxicity, or painful or venomous bites or stings. The successful signalling of unprofitability reduces the level of predation after 'initial predator education relative to profitable prey that may rely on other predator avoidance mechanisms such as crypsis or evasion (Gibson 1980; Sword *et al.* 2000; Sword 2002). Commonly studied examples are the brightly coloured unpalatable tropical butterflies (e.g. Mallet and Barton 1989; Mallet and Gilbert 1995; Joron and Mallet 1998), the black and yellow or black and red stinging wasps, and the highly coloured poisonous frogs (Symula *et al.* 2001).

There has not been any empirical evidence gathered to date to demonstrate that the bright and contrasting BROW colour pattern displayed within the Braconidae and other insect groups represents an aposematic signal, a notion first proposed by Naumann (1991) and Quicke *et al.* (1992). However, there is considerable corroborating evidence that the combination of these colours do represent an aposematic signal. There are many non-hymenopteran insects both in and outside Australia that display a variety of black, red-orange and white colour motifs but which have quite different patterns compared with the BROW pattern, as described above (see Chapter 2). Arguably the most commonly known examples would be the well-studied monarch butterfly, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae), a native of north America that now occurs in Australia where it is referred to as the wanderer butterfly, and its co-model the viceroy butterfly, *Limenitis*

archippus (Cramer) (Nymphalidae**)** (Ritland and Brower 1991; Mallet and Joron 1999). Other non-Australian lepidopteran examples include members of *Delias* Hübner (Lepidoptera: Peiridae) and their recorded mimics made up of other peirid species, *Appias lyncida* (Cramer), *Cepora nerissa* (F.), and *Prioneris thestylis* (Doubleday) (Canfield and Pierce 2010). In each of these cases the black, red-orange and white patterns displayed were demonstrated to be aposematic to avian predators.

In Australia, there are many examples of native non-hymenopteran insect species that display black, red-orange and white patterns including many lepidopteran species from a number of families (e.g. Noctuidae: *Comocrus behri* (Angas) (mistletoe moth); Nymphalidae: *Heteronympha merope merope* (Fabricius) (common brown); *Vanessa kershawi* (McCoy) (Australian painted lady); and Papilionidae *Papilio anactus*, Macleay (dainty swallowtail butterfly)), as well as dipteran and hemipteran species. There are also instances of lepidopteran species that display relatively small black, red-orange and white colour patches. For example, the lycaenid *Jalmenus evagoras* Hübner (common imperial blue butterfly) has distinct black, red-orange and white colour arrangements along the dorsal posterior margin of the hind wings that are in sharp contrast to the remaining dorsal colours and pattern displayed. Other lycaenid species, (e.g. *Ogyris amaryllis meridionalis* (Bethune-Baker) (coastal amaryllis azure)) display a black, red-orange and white motif medially on the underside of the forewings. Further evidence that the colour combination of black, red-orange and white arranged in the BROW pattern represent a warning of unprofitability to potential predators is the evolutionary convergence of lepidopteran, dipteran and hemipteran species on the BROW pattern including the braconid wasp body form.

Mimicry of aposematic colours and patterns has long been recognised (Poulton 1890 in Rowe *et al.* 2004). Mimicry is defined as the simulation by an organism (the mimic) of signal properties of another living organism (the model) which are perceived as signals of interest by a third living organism (the operator or signal-receiver), such that the mimic gains in fitness as a result of the operator identifying it as an example of the model (Vane-Wright 1980). This is in contrast to crypsis that has evolved as a predator avoidance mechanism in which an organism avoids detection because no signal is perceived by a potential predator (Stevens and Cuthill 2006). Some insects may employ both anti-predator measures whereby camouflage is used and aposematic colours are concealed (e.g. on the inside of hind legs (e.g. some mantids) or hind wings (e.g. some moths)) but can be flashed

to deter predation if the former strategy fails to avoid detection (Edmunds 1972; Rothschild 1985; Hogue 1993).

Do the *Therophilus* BROW species represent Müllerian or Batesian mimics? Müllerian mimicry, first discussed by Muller (1879), is when the mimic is also unprofitable to a predator which then strengthens the aposematic signal and both model and mimic benefit (Mallet and Joron 1999; Turner and Speed 1999; Joron 2003). In cases of proven or suspected Müllerian mimicry, the species involved are referred to as co-models. Batesian mimicry, first discussed by Bates (1862), is when a profitable mimic resembles an unprofitable species, the model (Mallet and Joron 1999; Turner and Speed 1999; Joron 2003). Many lepidopteran mimicry complexes have been studied and are considered to consist predominately of Müllerian mimics (e.g. Mallet and Barton 1989; Mallet and Gilbert 1995; Joron and Mallet 1998; Kapan 2001). This stands to reason as Müllerian co-models would provide a benefit to each other by sharing the burden of educating predators (Speed 1993; MacDougall and Dawkins 1998). Batesian mimics would theoretically reduce the effectiveness of the signal so such mimics would be selected to exist at a lower density in the environment than their models (Edmunds 1974; Turner 1987; Speed *et al.* 2000; Kuchta 2005). However, Batesian mimics are known to occur in allopatric populations to their models but may exist as rare mimetic phenotypes compared to more common non-mimetic phenotypes such that apostatic (i.e. frequency dependant) predation occurs (Pfennig and Mullen 2010). In addition, mimicry can be facultative whereby insects are able to adjust their participation in a mimicry complex dependant on environmental cues, such as seasonal changes, that could herald increases in predatory pressures during peak periods of a facultative mimic's activity (Canfield and Pierce 2010).

The BROW colour pattern amongst braconines was hypothesised by Quicke *et al.* (1992) to be predominantly Müllerian mimicry based on the observation that some of the species were able to give a painful sting and exude a pungent odour when handled. It is not known if *Therophilus* species possess venom, are distasteful, or can produce a pungent odour when threatened. It is also not known what the main predator/s of *Therophilus* are; Araneae, Insecta, and/or avian? *Therophilus* species do possess long ovipositors that can be over 1.5 times their own body length which might be capable of delivering a painful rebuke to a potential predator. If this were so, then *Therophilus* members of the BROW complex would be considered Müllerian co-models. It is interesting to note that if *Therophilus* BROW species were indeed models, solely on the basis that the female wasp

could use her ovipositor defensively, then conspecific males would contribute to weakening the aposematic signal.

An important aspect of determining if *Therophilus* members of the BROW complex represent Müllerian co-models or not would be to investigate if species contain chemical compounds that may act as deterrents to predation. If so, are these potentially defensive chemicals synthesised *de novo* or are *Therophilus* larvae able to sequester the required compounds from their herbivorous host then retain them for defensive purposes through to adulthood? It has been documented from at least six insect orders that herbivores are able to sequester a broad range of biosynthetic compounds from their host plants to produce, in a more energy efficient manner compared to *de novo* biosynthesis, unpalatable or toxic substances as an effective chemical defence from predation, including parasitism (Duffey *et al.* 1986; Nishida 2002; Opitz *et al.* 2010). Numerous studies on the impacts of plant secondary chemical compounds on the tritrophic interactions among host plant, host invertebrate, and their invertebrate predators and parasitoids have shown that some invertebrate predators and parasitoids are better adapted for overcoming, tolerating or avoiding the chemical defences of their prey or hosts (Reichstein *et al.* 1968; Rothschild *et al.* 1973; Whitman 1988; Ode *et al.* 2004; Smilanich *et al.* 2009; Lampert *et al.* 2010; Bixby-Brosi and Potter 2012). However, the sequestration of defensive chemicals by terrestrial invertebrate predators and parasitoids from their plant feeding prey for their own defensive purposes has received less attention. It was first demonstrated by Witte *et al.* (1990) that the aphid predator, *Coccinella septempunctata* L. (Coleoptera), was able to sequester defensive alkaloid chemicals from its prey that had originally been derived from the host plant. An interesting line of further research would be to determine if parasitic wasp larvae are able to sequester defensive compounds from their herbivorous host that are retained for later defensive purposes during their adult stage.

5.5   Future research directions

This study has provided an important contribution to the taxonomic and phylogenetic knowledge of the Australian Agathidinae. The generic taxonomic framework presented for Australia also provided an accurate assessment of the diversity of each genus present and the number of undescribed species remaining in each. The updated descriptions for 18 species and new descriptions of four species involved in the taxonomic quagmire of the polyphyletic '*Therophilus*', has provided a firm basis for the continuing task of identifying and describing the many undescribed and undiscovered *Therophilus* species present in Australia. It is important that future taxonomic work on the Australian *Therophilus* fauna

be conducted in conjunction with molecular analyses to not only assist in the determination of species limits but to also investigate further the generic level relationships. There are smaller numbers of undescribed species for other Australian genera that are also in need of further taxonomic treatment, but it is less imperative that such studies also include molecular data as their generic and species limits are better defined compared to *Therophilus*.

The phylogenetic analyses conducted as part of this study has highlighted further the polyphyly of *Therophilus* and the importance and need to use multiple genetic markers, in conjunction with a broader taxonomic and geographical representation, to better understand and robustly define the evolutionary relationships present. An important development to greatly enhance future phylogenetic outcomes will be the use of next generation sequencing. Such an advancement in sequencing large numbers of genes and subsequent phylogenetic analysis, coupled with a broader taxonomic and geographic representation, will play a pivotal role in further elucidating the phyogeny of Agathidinae and specifically defining the multiple lineages of the polyphyletic assemblage comprising what is currently referred to as '*Therophilus*'.

Many benefits in other facets of research on the Agathidinae would stem from a more robust phylogenetic understanding of the Agathidinae and '*Therophilus*'. One facet might include gaining a better understanding of host associations and relationships, that may also assist in future investigations of the potential use of *T. unimaculatus* and/or *T. rugosus* in the biological control of the native Australian lepidopterans *Etiella behrii* Zeller (Pyralidae) and *Epiphyas postvittana* (Walker) (Tortricidae) that have become significant pests in several countries, as well as in southern and eastern Australia. Another fruitfull line of research would be further investigation of the BROW mimicry complex, not just from a purely phylogenetic perspective (i.e. how many independent convergences have arisen), but also in providing an evolutionary framework to support physiological and/or behavioural studies that might investigate a number of unknown aspects (e.g., model versus mimic, Batesian versus Müllerian).

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Appendices

Appendix 1: Chapter 4

Morphological characters and character states adapted from Sharkey *et al*. 2006) for Analysis 1 and Analysis 2.

Character 1   Length of third labial palpomere
    0 = absent or reduced (less than half as long as both palpomeres 2 and 4
    1 = subequal in length relative to palpomeres 2 and 4

Character 2   Length of labio-maxillary complex.
    0 = of normal proportions, galea shorter than mandible
    1 = elongate, galea longer than mandible

Character 3   Lateral carinae of frons
    0 = present
    1 = absent

Character 4   Sculpture between antennae
    0 = two carinae.
    1 = one or no carinae

Character 5   Posterior area of vertex
    0 = excavated.
    1 = not excavated

Character 6   Apical flagellomere shape
    0 = blunt
    1 = acute
    2 = with apical nipple

Character 7   Presence of basal lobe on foreclaw
    0 = present
    1 = absent

Character 8   Shape of basal lobe of foreclaw
    0 = small, sharp but not curved
    1 = quadrate
    2 = curved and sharp (claws bifid, cleft)

Character 9   Size of basal tooth of hind claw (cleft claws only)
    0 = small or absent
    1 = normal

Character 10   Long setae at apex of spur of fore tibia
    0 = present
    1 = absent

Character 11   Non-apical spines on mid tibia
    0 = present
    1 = absent

Character 12   Hind trochanter shape
    0 = elongate
    1 = not elongate

Character 13   Prominence (swelling) of propleuron
    0 = present
    1 = absent

Character 14   Notauli presence
   0 = present
   1 = absent

Character 15   Notauli sculpture
   0 = present
   1 = absent

Character 16   Post-scutellar depression
   0 = present
   1 = absent

Character 17   Propodeal sculpture
   0 = with 1 to 3 closely aligned median longitudinal carinae, anterior transverse
       carinae present or absent posterior transverse carinae always absent
   1 = areolate posterior transverse carina present
   2 = two median longitudinal carinae bordering a spindle-shaped area
   3 = lacking macrosculpture
   4 = scattered rugae

Character 18   Size of medio-posterior areola of propodeum
   0 = large
   1 = normal

Character 19   Rugose sculpture of propodeum
   0 = present
   1 = absent.

Character 20   Coriarious sculpture of propodeum
   0 = present
   1 = absent

Character 21   Hind coxal cavities
   0 = open, sharing common foramen with metasoma
   1 = closed, separated from metasoma

Character 22   Elevated ridge between hind coxal cavities and metasomal foramen
   0 = present
   1 = absent

Character 23   Longitudinal ridge of setae on hind basitarsus
   0 = present
   1 = absent

Character 24   Longitudinal carinae on hind trochantellus
   0 = present
   1 = absent

Character 25   Fore wing vein 1Rs+M
   0 = complete
   1 = incomplete

Character 26   Last abscissa of RS of fore wing
   0 = curved towards wing apex
   1 = straight
   2 = curved towards anterior wing margin (coding mistake with this character state,
   entered as 1 also in Sharkey *et al.* (2006); refer to discussion in modifications made
   regarding this matter).
   3 = absent

Character 27   Last abscissa of Rs of fore wing
    0 = complete
    1 = incomplete

Character 28   Marginal cell of fore wing
    0 = long and narrow
    1 = normal

Character 29   Fore wing vein 2RS2
    0 = present
    1 = absent

Character 30   Second cubital cell of fore wing
    0 = wider than long
    1 = not wider than long

Character 31   Rs and r-m veins of fore wing
    0 = converging anteriorly
    1 = not converging anteriorly square or rectangular

Character 32   Second cubital cell of fore wing
    0 = present
    1 = absent

Character 33   Last abscissa of Cu of hind wing
    0 = contiguous with penultimate abscissa of Cu
    1 = not contiguous with penultimate abscissa of Cu or absent

Character 34   2r-m of hind wing
    0 = complete (as an unsclerotized vein)
    1 = incomplete
    2 = absent

Character 35   Pair of longitudinal carinae on first metasomal tergum
    0 = present
    1 = absent

Character 36   Median longitudinal swelling of first metasomal tergum
    0 = present
    1 = absent

Character 37   Sculpture of first median tergite
    0 = coriarious
    1 = striate
    2 = smooth
    3 = granulostriate
    4 = rugosostriate
    5 = rugose

Character 38   Sculpture of second median tergite
    0 = smooth
    1 = coriarious
    2 = striate
    3 = granulostriate
    4 = rugosostriate
    5 = rugose

Character 39   Sculpture of third median tergite
     0 = smooth
     1 = striate
     2 = coriarious
     3 = granulostriate
     4 = rugosostriate
     5 = rugose

Character 40   Ovipositor shape
     0 = short and decurved
     1 = long and straight

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Appendix 2: Chapter 4

Morphological data matrix for Analysis 1 and Analysis 2. The coding in Sharkey *et al.* (2006) begins with first character state = 1. Mr Bayes recognises the first character state (ancestral) = 0. Therefore, all coding from Sharkey *et al.* (2006) was modified accordingly; e.g. 1 changed to 0, 2 changed to 1, etc. In addition, in Sharkey *et al.* (2006) the data matrix contains numerous letters that denote the presence of various polymorphic states. Unfortunately, the meaning of this coding is not provided in that publication and so is undecipherable. However, Sharkey (pers. comm.) provided an earlier draft of the paper in which the letter coding was described (outlined below). Because Mr Bayes cannot recognise letter coding as meaning the presence of 2 or more states, i.e. a taxon being polymorphic for a given character, the polymorphic states are instead presented in parenthesis.

The polymorphic letter code used by Sharkey *et al.* (2006) but omitted from the final publication is as follows: a = states 0 & 1; b = states 1 & 2; c = states 2 & 4; d = states 2 & 5; e = states 0 & 5; f = states 1 & 4.

Characters

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Acampsis sp. 1	1	0	1	1	1	0	0	1	-	1	1	1	1	0	0	0	2	1	0	1	0	1	1	1	0	1	0	1	1	1	1	0	1	2	0	1	5	4	4	0
A. sp. 2	0	0	1	1	0	1	0	0	-	1	0	1	1	0	1	1	0	1	0	0	0	1	1	1	1	1	0	1	1	1	0	0	0	2	0	1	4	0	0	1
Agathis montana	1	1	1	1	0	1	0	0	-	1	0	1	1	0	0	1	0	1	0	1	0	1	1	1	1	1	0	1	1	1	0	0	0	2	0	1	4	0	0	1
Alabagrus haenschi	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	2	0	0	1
Al. arawak	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	2	0	0	1
Al. fuscistigma	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	2	0	0	1
Al. masneri	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	2	0	0	1
Al. maue	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1	1	0	2	0	0	1
Al. pachamama	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	2	0	0	1
Al. parvifaciatus	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	[01]	[01]	2	0	0	1
Al. stigma	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	0	0	[25]	[05]	0	1
Al. tricarinatus	0	0	0	1	1	1	0	1	-	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	2	0	0	1
Amputoearinus fernandezi	1	0	1	0	1	1	0	1	-	1	0	1	0	1	-	1	3	2	1	1	1	1	1	1	0	1	0	1	1	1	0	0	1	2	1	1	2	0	0	?
Am. matamata	1	0	1	0	1	1	0	1	-	1	0	1	0	1	-	1	3	2	1	1	1	1	1	1	0	1	0	1	1	1	0	0	1	2	1	1	2	0	0	?
Amputostypos sp. 1	1	0	1	0	1	2	0	2	1	1	1	1	1	0	0	1	1	[01]	0	1	1	1	0	0	1	1	0	0	0	1	0	0	1	2	1	1	2	0	0	0
Aneurobracon sp. 1	0	0	1	1	1	1	0	0	-	1	0	0	1	0	0	0	0	1	1	0	1	1	1	1	1	3	1	2	1	2	2	1	1	2	1	1	0	0	0	1
Austroearinus chrysokeras	1	0	1	1	1	1	0	1	-	1	0	1	1	1	-	1	2	1	1	1	0	1	1	1	1	1	0	1	1	1	0	0	1	2	0	1	2	0	0	1
Au. melanopodes	1	0	1	1	1	1	0	1	-	1	0	1	1	1	-	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	0	0	1	2	0	1	2	0	0	1
Au. rufofemoratus	1	0	1	1	1	1	0	1	-	1	0	1	1	1	-	1	0	1	1	1	0	1	1	1	1	1	0	1	1	1	0	0	1	2	0	1	2	0	0	1
Bassus calculator	0	0	1	1	0	1	0	1	-	1	0	1	1	0	0	0	4	2	0	1	1	0	1	1	1	0	0	1	1	0	0	0	1	2	0	1	1	[02]	0	1
Biroia sp. 1	1	1	0	0	1	1	0	2	1	1	1	1	1	1	-	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	0	1	1	2	0	1	1
Braunsia bilunata	0	0	1	[01]	1	1	0	1	-	1	0	1	1	0	1	1	1	1	0	1	1	0	1	1	1	0	1	0	1	1	0	0	1	2	0	1	1	2	1	1
Br. burmenis	0	0	1	1	1	1	0	1	-	1	0	1	1	0	0	1	1	1	0	1	1	0	1	1	1	0	1	0	1	1	0	0	1	2	0	1	1	2	1	1
Br. nr nigriceps	0	0	1	1	1	1	0	1	-	1	0	1	1	0	1	1	0	1	0	1	1	0	1	1	1	0	1	0	1	1	0	0	1	2	0	1	1	2	1	1
Camptothlipsis oliveri	0	1	1	1	1	1	0	1	-	1	0	1	1	0	0	0	4	2	0	1	1	0	1	0	1	0	1	1	0	1	-	-	1	2	1	1	3	0	0	1
Cam. sp. 3	0	0	1	1	1	1	0	1	-	1	0	1	1	0	0	1	3	2	1	1	1	1	1	1	1	1	0	1	1	2	2	1	1	2	1	1	2	0	0	1
Cam. sp. 9	0	0	1	1	1	1	0	1	-	1	0	1	0	0	0	1	3	2	1	0	1	1	1	1	1	1	0	1	1	2	2	1	1	2	1	1	0	0	0	1
Coccygidium luteum	1	0	0	0	1	2	0	2	1	0	1	1	1	0	0	1	1	1	0	1	1	1	0	0	1	1	0	0	1	1	0	0	1	2	1	1	2	0	0	0
Coc. nr *sissoo*	1	0	0	0	1	2	0	2	1	0	1	1	1	0	0	1	1	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0	1	2	1	0	2	0	0	0
Coc. sp. 1	1	0	0	0	1	2	0	2	1	0	1	1	1	0	0	1	1	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0	1	2	1	0	2	0	0	0
Cremnops ferrungiensis	1	1	0	0	1	1	0	2	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	0	1	0	0	1	[01]	1	1	0	1	0	1	2	0	0	[01]
Cr. haematodes	1	1	0	0	1	1	0	2	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	[01]	1	0	0	1	1	1	1	0	1	1	1	1	2	0	0	1
Cr. sp. 1	1	1	0	1	1	1	0	2	1	1	1	1	1	0	1	1	0	2	0	1	1	1	1	1	1	[01]	0	1	1	1	1	0	1	1	1	1	2	0	0	1
Cr. virginiensis	1	1	0	0	0	1	0	2	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	0	0	1	[01]	1	1	0	1	0	1	1	2	0	0	[01]
Crassomicrodus divisus	1	0	1	0	1	1	1	-	-	1	0	1	1	0	0	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	0	0	1	2	1	1	2	0	0	0

Taxon	Character states
Disophrys atripennis	1 1 0 0 1 1 0 2 1 1 1 1 1 0 0 1 1 1 0 1 1 1 1 0 1 1 0 1 0 1 1 0 1 1 1 1 2 0 0 0
D. sp. 1	1 1 0 0 1 1 0 2 0 1 1 1 1 0 0 1 1 0 0 1 1 1 1 0 1 [01] 0 1 1 1 1 0 1 1 1 1 2 0 0 0
D. sp. 2	1 1 0 0 1 1 0 2 0 1 1 1 1 0 0 1 1 0 0 1 1 1 0 0 1 1 0 0 0 1 1 0 1 [12] 1 1 2 0 0 0
D. subfasciata	1 1 0 0 1 1 0 2 0 1 1 1 1 0 1 1 1 0 0 1 1 1 0 1 1 1 0 1 0 1 1 0 1 1 1 1 2 0 0 0
Diospilus fomitis	1 0 1 1 1 2 1 0 - 1 1 1 1 0 0 0 1 0 0 0 1 0 1 1 1 0 1 0 1 1 1 1 0 1 2 0 1 5 0 0 1
Earinus elator	1 0 1 0 1 1 0 0 - 1 0 1 0 1 - 1 0 1 0 1 0 1 1 1 0 1 0 1 1 1 0 0 0 2 0 1 2 0 0 1
Euagathis forticarinata	1 0 1 0 1 1 0 2 1 1 1 1 1 0 1 1 1 1 0 1 1 1 0 1 1 1 0 1 0 1 0 0 1 1 1 1 2 0 0 0
E. sp. 1	1 0 1 0 1 1 0 2 1 1 1 1 1 0 1 1 1 1 0 1 1 1 0 1 1 1 0 1 0 1 0 0 1 1 1 1 2 0 0 0
Lytopylus macadamiae	0 0 1 1 1 1 0 1 - 1 0 1 1 0 1 0 1 1 1 1 1 0 1 1 1 0 1 1 1 0 0 1 2 0 1 1 [24] [14] 1
L. nr *macademiae*	0 0 1 1 1 1 0 1 - 1 0 1 1 0 1 1 3 2 1 1 1 1 0 1 1 1 1 0 1 1 0 0 1 2 0 1 2 2 0 1
L. rufipes	0 0 1 1 0 1 0 1 - 1 0 1 1 0 0 0 4 2 0 1 1 0 1 1 1 0 0 1 1 0 0 1 2 0 1 1 2 1 1
Malagsigalphus sp. 1	1 0 0 1 1 0 1 - - 1 1 1 1 0 0 0 3 2 0 1 0 1 1 1 0 1 0 1 1 0 1 0 1 2 0 1 5 4 4 0
Mesocoelus sp. 1	0 0 1 1 1 1 1 - - 1 0 0 1 [01] 1 0 0 2 1 0 1 1 1 1 1 3 1 2 1 2 2 1 1 2 0 1 [01] [12] 0 1
Pharpa dubiosum	0 0 0 1 1 1 0 1 - 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 0 1 0 1 0 0 1 1 0 1 2 0 1
Plesiocoelus bassiformes	0 0 1 1 1 1 0 1 - 1 1 1 0 0 0 1 3 2 0 0 1 1 1 1 1 3 1 2 1 2 2 1 1 2 1 1 0 1 0 1
Sesioctonus akrolophus	0 0 1 1 1 1 1 - - 1 1 0 1 1 - 1 1 1 1 1 1 0 1 1 1 1 0 1 0 1 0 0 1 2 0 1 2 0 0 1
S. irrorator	1 0 0 1 1 0 0 1 - 1 1 1 1 0 0 0 2 1 0 1 0 1 1 1 0 1 0 1 1 0 1 0 0 0 0 1 5 5 5 0
S. kompsos	0 0 1 1 1 0 1 - - 1 0 1 0 1 - 1 0 1 1 1 1 0 1 1 1 1 0 1 1 1 0 0 1 2 0 1 2 0 0 1
S. nr *areolatus*	0 0 1 1 1 0 1 - - 1 0 1 1 1 - 1 2 1 1 1 1 0 1 1 1 1 0 1 1 1 0 0 1 2 0 1 2 0 0 1
Sigalphus gyrodontus	1 0 0 0 1 0 0 1 - 1 1 1 1 0 0 0 2 1 0 1 0 1 1 1 0 1 0 1 1 0 1 0 0 0 0 1 5 5 5 0
Therophilus aalvikorum	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 4 2 0 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. dimidiator	0 0 1 1 1 1 0 1 - 1 0 1 0 0 0 0 1 1 0 1 1 1 1 1 1 0 1 1 1 0 0 1 2 1 1 1 2 0 1
T. festinatus	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. malignus	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 0 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. martialis	0 0 1 1 0 1 0 1 - 1 0 1 1 0 1 0 3 2 1 1 1 ? 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. mishae	0 0 1 1 0 1 0 1 - 1 0 1 1 0 1 0 4 2 0 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 3 3 0 1
T. nr *conspicuus* 1	0 0 1 0 [01] 1 0 0 - 1 0 1 1 0 0 0 1 [01] 0 1 0 1 1 1 1 0 0 1 1 1 0 0 0 2 1 1 1 2 0 1
T. nr *conspicuus* 2	0 0 1 1 1 0 1 - 1 0 1 1 0 1 0 1 1 0 [01] 1 1 1 1 0 0 1 1 1 0 0 0 2 0 1 1 0 0 1
T. nr *festinatus* sp. 1	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. nr *latibalteatus* sp. 1	0 0 1 1 0 1 0 1 - 1 0 1 1 1 1 0 3 2 1 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. nr *latibalteatus* sp. 2	0 0 1 1 0 1 0 0 - 1 0 1 1 1 - 0 4 2 0 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. nr *malignus* sp. 1	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 4 2 0 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. nr *martialis* sp. 1	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. nr *minimus* sp. 1	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 4 2 0 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 4 0 0 1
T. nr *ruficeps* sp. 3	0 0 1 1 0 1 0 0 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. nr *ruficeps* sp. 4	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 4 2 0 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 [23] 0 0 1
T. nr *tricolor* sp. 1	0 0 1 1 1 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. rugosus hap. 1	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 4 2 0 1 1 0 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1

Taxon	Character states
T. sp. 1	0 0 0 1 1 1 1 0 1 - 1 0 1 ? 0 0 0 1 1 0 1 0 1 1 1 1 1 0 1 0 1 0 0 0 2 1 1 1 0 0 1
T. sp. 17	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. sp. 2	0 [01] 1 1 1 1 0 1 - 1 0 1 0 0 0 1 1 1 0 0 1 1 1 1 1 1 0 1 1 1 0 0 1 2 0 1 1 2 0 1
T. sp. 23	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. sp. 35	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 1 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. sp. 4	0 0 1 1 1 1 0 1 - 1 0 1 0 1 - 1 1 1 0 1 1 1 1 1 1 1 0 1 1 1 0 0 1 2 1 1 2 0 0 ?
T. sp. 43	2 0 1 0 0 1 0 1 - 1 0 1 1 0 0 0 4 2 0 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 0 1 1 0 0 1
T. sp. 46	0 0 1 1 0 1 0 1 - 1 0 1 1 0 0 0 4 2 0 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. stephensae	0 0 1 0 0 1 0 1 - 1 0 1 1 1 - 0 4 2 0 1 1 1 1 1 1 1 0 0 1 1 0 0 1 2 1 1 1 0 0 1
T. unimaculata hap.1	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. unimaculata hap.5	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. unimaculatus hap. 3	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
T. unimaculatus hap. 4	0 0 1 1 0 1 0 1 - 1 0 1 1 1 - 0 3 2 1 1 1 0 1 1 1 1 0 0 1 1 0 0 1 2 1 1 2 0 0 1
Zelomorpha tropicola	1 0 0 0 1 2 0 2 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 0 1 1 0 0 0 1 0 0 1 1 1 0 2 0 0 0
Z. nr *tropicola*	1 0 0 0 1 2 0 2 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 0 1 1 0 0 0 1 0 0 1 2 1 0 2 0 0 0
Z. sp. 11	1 0 0 0 1 2 0 2 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 0 1 1 0 0 ? 1 0 0 1 1 1 1 2 0 0 0
Z. sp. 25	1 0 0 0 1 2 0 2 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 0 1 1 0 0 ? 1 0 0 1 1 1 1 2 0 0 0
Z. sp. 79	1 0 0 0 1 2 0 2 1 1 1 1 1 1 - 1 1 1 1 1 1 1 0 0 1 1 0 0 ? 1 0 0 1 1 1 0 2 0 0 0
Z. sp. 89	1 0 0 0 1 2 0 2 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 0 1 1 0 0 ? 1 0 0 1 1 1 1 2 0 0 0
Z. sp. 98	1 0 0 0 1 2 0 2 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 0 1 1 0 0 ? 1 0 0 1 1 1 1 2 0 0 0
Zacremnops cressoni	1 1 1 0 1 1 0 2 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 0 0 1 0 1 1 0 0 1 1 1 2 0 0 1
Zamicrodus sensilis	0 0 1 1 1 1 0 1 - 1 0 1 0 0 0 1 1 3 2 1 1 1 1 1 1 1 1 0 1 1 1 0 0 1 2 1 1 2 0 0 1

Appendix 3: Chapter 4

Modifications made to morphological data matrix of Sharkey *et al.* (2006) used in Analysis 1 and Analysis 2.

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1   *Diospilus fomitis* is coded as second cubital absent (32:0), and yet it is coded for other characters (e.g. 30:1 and 31:1) indicating that this state is present.

2   The bulleted points below refer to the reduced wing venation of *Pleiocoelus*, *Mesocoelus*, and *Aneurobracon:*

- Character 26: coded -, -, ? respectively; have given given another state = 3 for absent; therefore the coding here has been changed accordingly for each of the relevant taxa to 3,3,3. It should be noted that 3 character states were originally proposed but character state 2 (cs2) was erroneously written as 1 and this code was therefore used throughout the matrix in Sharkey *et al.* (2006) resulting in both cs1 and 2 being coded as 1. This is an error that I have not been able to rectify for taxa coded by Sharkey. I suspect cs2 would only concern the out group taxa so it should not have much influence on the relationships of the ingroup taxa. Because of this oversight cs3 was created in case the error above is ever rectified.

- Character 28: all 3 genera coded as 'normal' = 1. They are far from normal as they can be open or absent. Therefore an additional state has been created cs2 = open or absent.

- Character 29: coded as ?, ?, 1 when they all should be cs1 (i.e. vein 2rs2 absent).

- Character 30: coded as ?,?,- created cs1 = second cubital cell absent. This also relates to *Therophilus* sp. 9 and *T*. sp. 3.

- Character 31 coded as − for all. This is ambiguously character but hinges on the presence of the first submarginal cell (if understood correctly). Therefore, an additional state has been created cs2 = absent. This also relates to *Therophilus* sp. 9 and *T*. sp. 3.

3   Character 18 (C18) should have cs2 = absent. All taxa that are coded 3 or 4 in C17 should have a code of 2 for C18. This includes *Therophilus* nr *macadamiae* which was coded as lacking macrosculpture on the propodeum (C17: cs3), yet Sharkey *et al.*

(2006) coded it as having a large medio-posterior areola on the propodeum (C18: cs0). Coding has been changed to C18: cs2.

4   *Malasigalphus* which is coded as not having basal lobes (where??) is coded as ? for C8; this has been changed to -. Also this taxon has been coded with a ? for C6; this has been changed to 0, as for other sigalphines.

5   *Mesocoelus* for C8 recoded from ? to −.

6   *Agathis montana* for C16 recoded from ? to 1.

7   Interestingly, Pucci and Sharkey (2004) discuss the absence of a basal lobe on the tarsal claws as an autapomorphy for *Crassomicrodus* yet *Crassomicrodus divisus* is coded in Sharkey *et al.* (2006) as having C7:cs0 and a cleft tarsal claw in C8 (cs2). Therefore, *Crassomicrodus divisus* has been recoded to C7:cs1, and C8:cs- . In addition C11 has been recoded from ? to 1.

8   Simbolotti and van Achterberg (1992) redescribed *T. dimidiator* as having only one carina/crest between the toruli. However, Sharkey *et al.* (2006) have coded the specimen identified as *B. dimidiator* as having two carinae. It is unknown whether the species is polymorphic for this character or whether it has been incorrect identified. Because of the uncertainty involved the original coding applied by Sharkey *et al.* (2006) it has not been changed.

9   Several *Zelomorpha* taxa were coded with ? for C29. Why they were not able to be coded is not known. This has not been resolved.

10   *Earinus elator* for C37 recoded from ? to 3.

Appendix 4: Chapter 4

Morphological characters and character states for Analysis 4.

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Character 1   Carina/ae between toruli in anterior view
     0 = 2 carinae or crests present.
     1 = 1 carina or crest present.
     2 = no carinae present, region flat or broadly rounded elevation instead.
     3 = no carinae present, region marked by groove instead.

Character 2   ante-ocellar area. Simbolotti & van Achterberg (1999) refers to the small and usually triangular area in front of the medial ocellus that may be occupied by a real (pit like) triangular depression formed by carinae that most often converge anteriorly. This area is mostly flat with hardly a noticeable impression, or is totally flat.
     0 = occupied by triangular depression formed by carinae that converge anteriorly; can be faint.
     1 = strong or faint carinae or impressions may be present but do not form a triangular depression
     2 = totally smooth.

Character 3   Lateral carinae of frons
     0 = present.
     1 = absent.

Character 4   Distance of lateral ocelli from medial ocellus
     0 = greater than or equal to 1 MOD.
     1 = less than 1 MOD.

Character 5   Concavity of posterior head
     0 = greater than or equal to 1 MOD.
     1 = less than 1 MOD.
     2 = not concave, straight or convex instead.

Character 6   Apical flagellomere shape
     0 = blunt.
     1 = acute.
     2 = with apical nipple.

Character 7   Head shape below eyes in anterior view
     0 = rounded, lateral margins curving rapidly inwards so that genal height is less than or equal to 0.5 clypeal width.
     1 = triangular, lateral margins strongly convergent (but less so than above) so that genal height is 0.5 – 0.8 clypeal width.
     2 = elongate, lateral margins parallel or relatively marginally convergent so that genal height is nearly equal to (0.9) or greater than clypeal width.

Character 8   Posterior ventral extension of gena
     0 = present.
     1 = absent.

Character 9   Length of labio-maxillary complex
     0 = normal, galea shorter than or as long as mandible.
     1 = elongate, galea longer than mandible.

Character 10   Labial palpomere 3 (LP3) length
  0 = long, equal to or greater than 0.5 length of LP4.
  1 = intermediate in length, less than 0.5 but greater than or equal to 0.3 length of LP4.
  2 = short in length, less than 0.3 length of LP4 (may appear absent except at high magnification).

Character 11   Subpronope
  0 = carina (distinct or faint) bordering posterior margin.
  1 = carinae on both postjerior and anjterior margins.
  2 = simple, no carinae associated.
  3 = indistinct or absent.

Character 12   Opening associated with medio-posterior and medio-anterior margins of the pronotum and scutum respectively
  0 = absent.
  1 = present.
  2 = absent, but dorsal pit (pronope) on or associated with pronotum only.

Character 13   Notauli
  0 = present and sculptured (scrobiculate).
  1 = absent or indistinct, not sculptured, maybe slight impression posteriorly only.
  2 = present, or partly so, i.e. anteriorly only, and not sculptured.

Character 14   Scutellar sulcus sculpturing
  0 = distinct medial and one pair lateral carinae present.
  1 = distinct medial and 2 or more pairs of lateral carinae present, may appear scrobiculate.
  2 = distinct medial carina present only.
  3 = no distinct carina present.

Character 15   Scutellar sulcus walls
  0 = both anterior and posterior walls steep, vertical or nearly so.
  1 = anterior wall sloped, not nearly vertical.

Character 16   Scutellar sulcus posterior margin
  0 = entire posterior margin curved or arched inwards.
  1 = posterior margin straight, or mostly so, may have medial protuberance associated with medial carina.

Character 17   Posterior margin of the scutellar raised triangular region
  0 = carinate or scrobiculate.
  1 = rugose, may be faintly so.
  2 = rounded and smooth.

Character 18   Sternalus
  0 = Present; distinct, deeply impressed and scrobiculate.
  1 = Present; not as distinct, relatively shallow impression, faintly scrobiculate.
  2 = Indistinct or absent, faint, short, non-scrobiculate impression at most.

Character 19   Wing colouration and patterns
  0 = distinct orange and /or yellow and black pattern present.
  1 = no pattern present but wings infuscate.
  2 = no pattern present, wings clear.

Character 20   Fenestra (break in vein where wing folds)
  0 = present.
  1 = absent.

Character 21 Marginal cell
    0 = long and continually narrow; vein RS terminating relatively far basal to wing apex.
    1 = not as above; if vein RS terminates far basal to wing apex, then cell relatively broader.
    2 = absent or open.

Character 22 Second submarginal cell (2R2)
    0 = present but not petiolate.
    1 = present & petiolate.
    2 = present & petiolate, but reduced, relatively small in size.
    3 = absent.

Character 23 Shape of second submarginal cell
    0 = more or less rectangular, length much greater than width.
    1 = quadrate, width and length close to equal.
    2 = triangular, may be considerably reduced in size, in which case may appear more rounded.

Character 24 Vein RS2
    0 = present.
    1 = absent.

Character 25 Fore wing vein RS+M
    0 = complete.
    1 = incomplete.

Character 26 Fore wing vein M+CU pigmentation
    0 = entirely pigmented or nearly so.
    1 = not entirely pigmented, most of, if not all of basal third unpigmented.

Character 27 Fore wing vein M+CU formation
    0 = entirely tubular.
    1 = not entirely tubular.

Character 28 Fore claws
    0 = cleft.
    1 = not cleft.

Character 29 Base of fore claws
    0 = smooth, no structure present.
    1 = pectinate.
    2 = basal lobe.

Character 30 Long setae at apex of spur of fore tibia
    0 = present.
    1 = absent.

Character 31 Pre-apical mid tibial spines
    0 = present, extending along the anterior margin of the mid-tibia for greater than or equal to 0.5 times tibial length from the distal end.
    1 = present, but extending less than 0.5 times length along the anterior margin of the mid-tibia from the distal end.
    2 = absent.

Character 32 Hind claws
    0 = cleft.
    1 = non-cleft.

Character 33   Hind claw base
    0 = smooth, no structure present.
    1 = pectinate.
    2 = basal lobe present.

Character 34   Longitudinal ridge of setae on hind basitarsus
    0 = present.
    1 = absent.

Character 35 Spines on and near apical anterior margin of hind-tibia
    0 = short, stout spines present on apical margin only.
    1 = short stout spines present on apical margin and pre-apically also.
    2 = long, thin spines present on apical margin only.
    3 = absent.

Character 36   Longitudinal carinae of hind trochantellus
    0 = present.
    1 = absent.

Character 37 Separation of hind coxal cavities (hcc) from propodeal foramen (mf)
    0 = wide, transverse carina/ae present between hcc and mf.
    1 = wide, but transverse carina/ae absent or incomplete.
    2 = narrow, thin non-carinate sclerite present only.
    3 = intermediate in width, minimal flat surface between carinate cavity margins present, so carinae nearly fused to form single thin carina.
    4 = absent, hcc open to mf.

Character 38   Setal field on metapleuron
    0 = present, density and thickness of setae such that a distinct white reflectance is given off, particularly when viewed anterior-laterally; surface of sclerite largely obscured.
    1 = present, density of thicken setae less so that surface of sclerite readily visible.
    2 = absent, density and thickness of setae similar to that present on mesopleuron and a distinct white reflectance is not achieved; surface of sclerite not obscured.

Character 39   Propodeal sculpturing
    0 = with 1 to 3 closely aligned median longitudinal carinae, anterior transverse carinae present or absent posterior transverse carinae always absent.
    1 = areolate, but not forming a spindle-shaped region medially.
    2 = areolate, forming spindle-shaped region medially, often with a rugulose background.
    3 = distinctly & extensively rugulose or rugulose-punctate.
    4 = extensively granulose.
    5 = not extensively rugulose or rugulose-punctate, mostly restricted to medial regions.
    6 = absent or largely so, may have some indistinct punctate markings medially at most.

Character 40   Median T1 sculturing
    0 = striate or striate-rugose, in part at least.
    1 = granulose or granulose-rugose, in part at least.
    2 = entirely rugose.
    3 = absent or indistinct.

Character 41   Pair of longitudinal carinae on median T1
      0 = present.
      1 = absent.

Character 42   T2 sculpturing
      0 = striate or striate-rugose, in part at least.
      1 = granulose or granulose-rugose, in part at least.
      2 = entirely rugose.
      3 = absent or indistinct.

Character 43   T3 sculpturing
      0 = entirely rugose.
      1 = absent or indistinct.

Character 44   Ovipositor length
      0 = very long, greater than or equal to entire body length.
      1 = long, greater than metasomal length but less than body length.
      2 = medium, greater than 0.5 times metasomal length but less than or equal to
            metasomal length.
      3 = short, equal to or less than 0.5 times metasomal length.
_____

Appendix 5: Chapter 4 — Morphological data matrix for Analysis 4.

Characters

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Agathis montana	2	0	1	0	0	1	2	0	1	0	0	1	0	1	0	0	2	0	1	1	0	0	1	1	1	0	1	1	2	1	1	1	2	1	1	1	4	2	0	0	0	3	1	1
Agathis sp.2	1	0	1	0	0	1	2	0	1	0	0	1	0	1	0	0	2	0	1	1	0	1	2	1	1	1	1	1	2	1	1	1	2	1	1	1	4	2	0	0	0	3	1	1
Alabagrus sp.2	3	0	0	0	1	1	2	1	0	2	0	1	2	3	1	1	0	0	0	1	0	0	2	1	1	0	1	1	2	1	1	1	2	1	0	1	1	2	3	0	0	0	1	0
T. nr *festinatus* sp.1	1	0	1	1	1	1	1	1	0	0	1	0	1	1	2	1	0	2	1	1	1	0	1	2	1	1	0	1	2	1	1	1	2	1	0	1	1	1	6	3	1	3	1	1
T. unimaculatus hap.1	1	0	1	1	1	1	1	1	0	0	2	0	1	1	2	1	1	2	2	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	3	1	6	3	1	3	1	1
T. sp.17	1	0	1	1	1	1	0	0	0	1	0	1	1	3	1	1	2	2	0	1	0	1	2	1	1	0	1	2	1	1	1	2	1	1	1	1	2	2	6	3	1	3	1	1
T. unimaculatus hap.2	1	0	1	1	1	1	0	0	0	2	0	1	1	0	1	0	2	2	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	1	1	2	6	3	1	3	1	1
T. stephensae	0	1	1	0	0	1	0	0	0	2	0	1	1	1	0	0	1	0	2	1	0	1	2	1	1	1	1	1	2	1	1	1	2	1	1	1	2	2	3	0	1	3	1	2
T. unimaculatus hap.3	1	0	1	1	1	1	1	0	0	1	0	1	1	2	1	1	2	2	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	3	1	6	3	1	3	1	1	1
T. unimaculatus hap.5	1	0	1	1	1	1	1	0	0	1	0	1	1	0	0	1	2	2	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	3	1	6	3	1	3	1	1	1
T. nr *tricolor* sp.1	1	0	1	1	1	1	1	0	0	1	0	1	1	2	1	1	2	2	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	3	1	6	3	1	3	1	0	
T. nr *malignus* sp.1	1	0	1	1	1	1	1	0	0	1	0	1	1	0	1	1	2	2	1	1	0	1	2	1	1	1	1	1	2	1	1	1	2	1	1	1	1	1	5	3	1	3	1	1
T. nr *martialis* sp.1	2	1	1	1	1	1	1	0	0	2	0	1	1	3	1	1	2	1	1	1	0	1	2	1	1	0	1	2	1	1	1	2	1	1	1	1	1	2	6	3	1	3	1	1
T. nr *minimus* sp.1	1	0	1	1	0	1	1	0	0	1	0	1	1	0	1	1	2	1	1	1	0	2	2	1	1	0	1	2	1	1	1	2	1	1	1	1	1	1	3	0	1	3	1	1
T. sp.46	1	0	1	1	0	1	1	0	0	1	0	1	1	0	1	0	2	2	1	1	0	2	2	1	1	1	1	1	2	1	0	1	2	1	1	1	1	0	5	1	1	3	1	1
T. aalvikorum hap.1	1	1	1	0	0	1	1	0	1	0	0	1	1	2	0	1	2	0	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	1	0	1	6	3	0	3	1	1
T. sp.27	1	0	1	1	0	1	1	0	0	2	0	1	1	2	1	1	2	2	1	1	0	2	2	1	1	0	1	2	1	0	1	2	1	1	1	3	1	6	3	1	3	1	1	
T. aalvikorum hap.2	1	1	1	1	0	1	1	0	1	0	0	1	1	0	0	1	2	0	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	1	1	6	3	0	3	1	1	
T. nr *latibalteatus* sp.1	1	0	1	0	0	1	1	0	0	2	0	1	1	0	1	0	2	0	1	1	0	2	2	1	1	1	1	1	2	1	1	1	2	1	1	1	1	1	3	3	1	3	1	1
T. rugosus hap.1	1	0	1	1	0	1	1	0	0	1	0	1	1	1	1	1	2	0	1	1	0	1	2	1	1	1	1	1	2	1	1	1	2	1	1	1	0	0	3	1	1	3	1	1
T. sp.35	1	0	1	1	1	1	1	0	0	1	0	1	1	2	1	1	2	2	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	3	1	5	0	1	3	1	1	
T. rugosus hap.3	1	0	1	1	0	1	1	0	0	1	0	1	1	0	1	1	2	0	1	1	0	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	3	3	1	3	1	1	
T. sp.43	0	1	1	1	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	1	0	1	2	1	1	1	1	1	2	1	1	1	2	1	1	1	0	0	3	0	0	1	1	1
T. sp.44	1	0	1	1	0	1	0	0	0	2	0	1	1	1	0	0	2	0	2	1	0	2	2	1	1	1	1	1	2	1	1	1	2	1	0	1	3	2	3	0	1	1	1	1
T. sp.8	1	0	1	1	1	1	1	0	0	1	0	1	1	2	1	0	2	2	1	1	0	1	2	1	1	0	1	2	1	0	1	2	1	1	1	1	6	3	1	3	1	0		
Coccygidium sp.5/7	0	1	1	1	1	0	1	0	0	0	1	0	0	2	1	1	0	0	0	1	0	0	2	1	1	0	1	0	0	0	2	0	0	0	0	1	2	1	3	1	3	1	3	
Coc. sp.6	0	1	1	1	1	1	0	0	0	0	2	0	0	2	0	1	0	0	1	1	0	0	2	1	1	1	1	0	0	0	2	0	0	0	0	2	2	2	3	1	3	1	3	
Disophrys sp.3	0	1	0	1	0	1	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	1	0	1	0	0	1	2	0	0	0	0	0	2	1	3	1	3	1	3	
Earinus elator	0	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	2	2	2	1	0	0	2	1	0	1	1	1	2	1	0	1	2	1	1	1	4	2	5	0	1	0	0	1
Euagathis sp.1	0	2	1	1	1	1	1	1	0	0	1	2	2	2	1	1	0	0	1	1	0	0	2	0	1	0	1	0	0	1	2	0	0	0	0	1	1	2	2	3	1	3	1	3
Zamicrodus sensilis	1	2	1	1	2	1	2	1	1	0	1	1	1	3	1	0	2	0	0	1	0	1	2	1	1	1	1	1	2	1	0	1	2	1	0	1	1	2	6	3	1	3	1	1
Cremnops sp.4	0	1	0	0	0	1	2	0	1	0	1	2	1	0	1	1	2	0	1	1	0	0	1	1	1	0	1	0	0	1	2	0	0	1	0	1	1	0	2	3	1	3	1	1
Sigalphus	3	1	0	1	0	0	0	0	0	2	0	0	1	0	1	2	0	1	0	1	0	0	1	0	0	0	1	2	1	2	1	2	1	2	1	4	2	2	0	0	2	0	3	
Cardiochiles sp.3	2	1	1	1	1	2	0	0	0	2	0	0	1	1	0	2	0	1	0	2	0	0	1	0	0	0	1	1	1	2	1	1	1	2	1	4	2	2	3	1	3	1	3	
Ascogaster sp.2	1	1	1	0	1	1	0	0	0	0	3	0	1	3	1	1	2	2	2	0	1	0	0	1	0	0	0	1	0	1	2	1	0	1	3	1	4	2	3	2	1	2	0	3

Appendix 6: Conference presentations given as part of this study

Stevens, N.B., Murphy, N.P., Austin, A.D, & Jennings, J.T., 2007, "The many lineages of an un-natural grouping: the evolution of the parasitoid wasp genus *Bassus* (Braconidae: Agathidinae), including a colourful mimicry complex, in Australasia". Oral presentation, 5th Southern Connections Conference, Adelaide.

Stevens, N.B., Murphy, N.P., Austin, A.D, & Jennings, J.T., 2006, "The many lineages of an un-natural grouping: the evolution of the parasitoid wasp genus *Bassus* (Braconidae: Agathidinae), including a colourful mimicry complex, in Australasia". Oral presentation, Australian & New Zealand Entomological Societies Conference, Adelaide. (First prize, student competition).

Stevens, N.B., Murphy, N.P., Austin, A.D, & Jennings, J.T., 2006, "An investigation of the evolution of the little known agathidine fauna (Hymenoptera: Braconidae) of Australia". Oral presentation, 6th International Congress of Hymenopterists, Sun City, South Africa.

Stevens, N.B., Murphy, N.P., Austin, A.D, & Jennings, J.T., 2005, "The Agathidinae (Hymenoptera: Braconidae) of Australia; parasitoids of lepidopteran larvae". Oral presentation, Combined Australian Entomological Society's 36th AGM and Scientific Conference, 7th Invertebrate Biodiversity and Conservation Conference, and Australian Systematic Biologists Conference, Canberra.

Stevens, N.B., Austin, A.D. & Jennings, J.T., 2004, "The Agathidinae (Hymenoptera: Braconidae) of Australia; parasitoids of lepidopteran larvae". Poster presentation, XXII International Congress of Entomology, Brisbane.

Stevens, N.B., Austin, A.D. & Jennings, J.T., 2003, "Investigating the systematics of Australian agathadine wasps (Insects: Hymenoptera: Braconidae); solitary endo-parasitoids of lepidopteran larvae". Poster presentation, 34th Australian Entomological Society Conference, Hobart.