The Paradox of Inaccurate Mimicry

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Abstract

Many species gain a selective benefit through mimicry by converging on the phenotypic attributes of another unrelated species which has resulted in many examples of mimics bearing a striking resemblance to their model. It is assumed that natural selection should drive the evolution of accurate mimicry while eliminating inaccurate mimics from the population. Paradoxically, there are many instances of mimics that inaccurately resemble their model. The many hypotheses proposed to explain the occurrence of inaccurate mimics are reviewed, with no one hypothesis providing a complete explanation. This thesis explored a mostly overlooked hypothesis, the *perfecting* hypothesis that predicts that inaccurate mimics are in an intermediate stage in a transition toward accurate mimicry, using ant-mimicking spiders as a model. To investigate this concept, three methods of quantifying mimic accuracy in ant-mimicking spiders were evaluated for their efficacy. Following this, the phylogenies for two subfamilies of ant-mimicking spiders were reconstructed to map the distribution of mimic accuracy and the traits involved in ant mimicry and estimate ancestral states. The results indicate that mimic accuracy, and traits such as the constriction of the body, evolve via an incremental process, supporting the prediction of the *perfecting* hypothesis and further elucidating potential evolutionary processes in mimicry systems.

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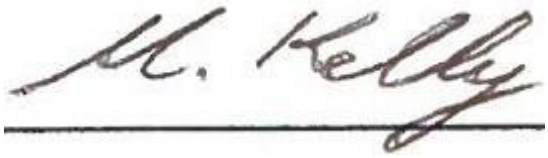

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Chapter 1 - The paradox of inaccurate mimicry: the *perfecting* hypothesis

Abstract

Mimicry is a widespread phenomenon that involves an organism (the mimic) converging on phenotypic characteristics of an unrelated organism (the model), thereby gaining a fitness benefit. It is expected that the more accurate a mimic is in resembling its model the greater the selective benefit, and that the process of natural selection should refine mimic fidelity. Paradoxically, there are many cases of inaccurate mimics that share only a vague resemblance to their model. In this chapter, I review the numerous hypotheses that have been proposed to explain the occurrence of inaccurate mimicry, all of which assume that inaccurate mimics are at an equilibrium state and any further improvement would not increase their fitness, or potentially reduce the fitness of the mimic. None of these hypotheses provide a full explanation even though many of them have been experimentally tested. Here I investigate the *perfecting* hypothesis that predicts alternatively that inaccurate mimics are not at an evolutionary stable state but are in an intermediate step toward accurate mimicry. I discuss ways of testing this hypothesis using a phylogenetic approach on myrmecomorphic (ant-mimicking) spiders to observe the relationships between inaccurate and accurate mimics and better understand evolutionary trends in mimicry systems.

Introduction

Mimicry is considered an excellent example of the power of natural selection to generate adaptations (Kikuchi & Pfennig 2013) and there are many examples of mimics that bear a striking resemblance to the organism they mimic (McIver & Stonedahl 1993). Yet, there are also many widespread cases where the mimic has evolved only a vague or inaccurate resemblance (Sherratt & Peet-Paré 2017). Traditionally it has always been assumed that mimics should experience on-going selection to improve their similarity to their model (Mappes & Alatalo 1997; Gilbert 2005), thus, the paradoxical existence of inaccurate mimics poses a central challenge to mimic theory specifically and to evolutionary theory in general (Azmeh et al. 1998; Edmunds 2000; Sherratt 2002; Ruxton et al. 2004). The paradox of inaccurate mimicry has resulted in the proposition of numerous, yet non-mutually exclusive hypotheses. Many of these hypotheses have been investigated with no one hypothesis providing a full explanation of most instances of inaccurate mimicry (Kikuchi & Pfennig 2013). Thus, further studies are required. The aim of this review is to describe the current state of inaccurate mimicry research and propose the investigation into a mostly overlooked, but potentially underlying, hypothesis: the *perfecting* hypothesis. Contrary to the alternative hypotheses proposed (reviewed in Kikuchi & Pfennig 2013), the *perfecting* hypothesis predicts that inaccurate mimics are in fact not in an evolutionary stable state but in a transitionary or intermediate phase between either an anachoretic

(hiding), masquerade or cryptic phenotype and that of an accurate mimic phenotype (Edmunds 2000; Edmunds 2006; Pekár 2014a; Pekár 2014b). The hypothesis predicts that selection is in the process of driving inaccurate mimics on an evolutionary trajectory toward more accurate mimetic form.

The first part of the review will discuss trait evolution with particular focus on the widespread phenomenon of mimicry, a paradigm of convergent evolution. Next, I review the occurrence and prevalence of inaccurate mimicry and the multiple hypotheses proposed to explain this paradox of inaccurate mimicry. Following this, the *perfecting* hypothesis, the focus of this study, will be examined to establish the importance of exploring this concept to investigate whether, or not, inaccurate mimics are in the process of evolving into a more accurate form. The review will then include a discussion on the use of a phylogenetic approach necessary to investigate this hypothesis using myrmecomorphic spiders as a case study. Finally, an outline of the potential outcomes of this Masters thesis will be presented along with an overview of the chapters that follow.

Mimicry: the convergent evolution of traits

The evolution of a trait may confer selective advantage in a given ecological context. This is facilitated by genetic and developmental mechanisms that allow the trait to vary (Wiens et al. 2006). Traits, or phenotypes, that exhibit higher evolutionary fitness, relative to other traits within a common environment, will persist, while traits that result in a lower evolutionary fitness may be lost. Over evolutionary time, traits are shaped by selection and persist if they carry a selective benefit (Martins 2000). Traits may evolve and accumulate differences between related groups leading to a divergence in phenotype and the formation of distinct novel species (i.e. speciation), termed divergent evolution (Gulick 1888). Alternatively, similar traits may evolve in unrelated species under comparable selective regimes, resulting in phenotypic similarity between independent lineages, known as convergent evolution (Stern 2013; Arbuckle et al. 2014; Maruyama & Parker 2017).

Convergent evolution is considered a key evolutionary process and is often the consequence of adaptation to a comparable niche as distantly related organisms are driven towards the same phenotypic adaptive optima (Arbuckle et al. 2014). Recent adaptive radiations serve as striking examples of convergent systems where distantly related taxa follow parallel evolutionary trajectories, e.g. African lake cichlids (Kocher et al. 1993), Hawaiian long-jawed spiders (Gillespie 2004) and Darwin's finches (Grant et al. 2004). These natural experiments, where comparable selection pressures have led to a convergence in phenotypes, provide compelling evidence for the predictability in evolutionary change (Maruyama & Parker 2017). This contradicts the assumption that evolutionary contingency is ubiquitous (Gould 1990), however it has been suggested that contingency may operate on a completely different time scale to convergence due to selection (Erwin 2006). More recently,

the predictability of evolution over deep timescales has received empirical support (e.g. Maruyama & Parker 2017) and is best illustrated in the convergent evolution observed in mimicry, as similar traits are acquired as adaptations to similar selective regimes (Maruyama & Parker 2017).

Mimicry

Mimicry has long been considered a paradigm of convergent evolution (Reed et al. 2011). It occurs when a species (the mimic) converges on the phenotypic characteristics of a distantly related species (the model) incurring the same selective benefits the model derives from the shared phenotype (Edmunds 1974; Endler 1981; Kikuchi & Pfennig 2013). There is evidence that traits associated with mimicry are under strong selective pressure, such that a closer resemblance to the model is expected to increase the fitness of the mimic (Ruxton et al. 2004; Ceccarelli 2013), with natural selection driving the ever-increasing perfection in mimic accuracy (Taylor et al. 2016). Mimicry has always been viewed as evidence of natural selection's power to generate spectacular adaptations (Ceccarelli & Crozier 2007; Kazemi et al. 2014). It is common in invertebrates, flowering plants, fungi, and in most vertebrate classes and can involve chemical, acoustic, or visual traits (Dalziell & Welbergen 2016), or in some instances, the multimodal combination of numerous cues (Rettenmeyer 1970, Ruxton et al. 2004, Durkee et al. 2011).

At its extremes, we differentiate between Müllerian (after Fritz Müller) and Batesian (after Henry Walter Bates) mimicry (Allen & Cooper 1995). Müllerian mimicry describes a warning signal that is shared between aposematic prey species (i.e. species that have evolved conspicuous traits, such as bright colours, to warn predators of their toxicity and/or unpalatability) (Ihalainen et al. 2012). The classic example of Müllerian mimicry is the unpalatable *Heliconius* butterflies (Bates 1862) whose communities commonly comprise of several groups of species that share a common wing pattern, known as 'mimicry rings' (Mallet & Gilbert 1995). Batesian mimicry is a defensive strategy where a palatable harmless *mimic* species copies the communication signals (e.g. physical, chemical, acoustic, or tactile) of a locally well-defended, noxious or unprofitable model species thereby gaining protection from predatory attack (Edmunds 1974; Gilbert 2004; Charlesworth & Charlesworth 2011; Kikuchi & Pfennig 2013). This phenomenon is found in taxa such as *Papillio* swallowtail butterflies and hoverflies (family Syrphidae) that resemble defended species of noxious butterflies and venomous bees and wasps, respectively (Jamie 2017). The phenotypic resemblance to a noxious model provides the mimic with the same protective benefits as the model, as a consequence of misidentification by the receiver (Vane-Wright 1980), i.e. a predator, without the cost of producing complex defences such as toxins (Mokkonen & Lindstedt 2016). The effectiveness of Batesian mimicry is highly dependent on the mimics sharing a spatial and temporal co-occurrence with their

model (McIver & Stonedahl 1993; Ceccarelli 2007; Pekár & Jarab 2011b) and that models have a higher relative abundance than mimics so that the predator encounters the models more frequently than the palatable mimics (Gilbert 2004).

The paradox of inaccurate mimicry

Batesian mimics that rely on visual signals gain a selective advantage when the signal effectively resembles the signal present in the model and induces an aversion response, either innate or learned, from a visually-oriented predator (Ruxton et al. 2004; Kazemi et al. 2014; Bosque et al. 2018). It therefore follows that more accurate mimics have a selective advantage over less accurate mimics, if those less accurate mimics are not recognised by the predator. As a consequence, natural selection drives an ever-increasing accuracy, or perfection, in mimic resemblance to the model (Mappes & Alatalo 1997; Wickler 2013) and eliminates poor or inaccurate mimics (Edmunds 2000; Gilbert 2004). This idea is supported by many examples of striking resemblance between mimics and their models, such as the swallowtail butterfly, *Papilio polytes*, which closely resembles the unpalatable *Pachliopta aristolochiae* (Mallet 2015). Paradoxically, however, mimicry does not consistently involve the convergence of the full range of traits, resulting in many widespread cases of imperfect or inaccurate mimicry (Sherratt & Peet-Paré 2017). For example, many species of hoverfly are poor mimics of wasps and bees (Edmunds 2000), many species of nonvenomous kingsnakes imprecisely mimic deadly coral snakes (Brodie & Brodie 2004; Kikuchi & Pfennig 2010b), and many myrmecomorphic (i.e. ant-mimicking) spiders inaccurately resemble their ant model (Mclver & Stonedahl 1993; Cushing 1997).

In fact, Mokkonen and Lindstedt (2016) suggest that perfect resemblance may be more an exception than a rule and thus presents a challenge to the theory of mimicry and evolutionary theory more broadly. The evolutionary paradox of widespread inaccurate mimicry in many Batesian mimetic systems (Gilbert 2004) currently remains an intensely debated enigma (Kikuchi & Pfennig 2013; Kazemi et al. 2014; Corcobado et al. 2016) and begs the question why mimic fidelity, or accuracy, has not been improved by natural selection (Edmunds 2000, 2006; Johnstone 2002; Sherratt 2002; Holen & Johnstone 2004; Gilbert 2005; Bain et al. 2007; Penney et al. 2012).

Proposed hypotheses to explain inaccurate mimicry

While numerous non-mutually exclusive hypotheses have been proposed to explain the prevalence and maintenance of inaccurate mimics and several studies have been conducted to explore many of these hypotheses (Kikuchi & Pfennig 2013), no consensus has been established on this intensely debated issue (Edmunds 2000; Holen & Johnstone 2004; Jackson & Nelson 2012; Kikuchi & Pfennig

2013; Corcobado et al. 2016). Theoretical and empirical considerations of mimicry suggest that resembling the model more accurately should always be advantageous (Kazemi et al. 2014), yet if inaccurate mimicry is an evolutionary stable state this would suggest that opposing or balancing selective forces must exist (Gilbert 2004).

The non-mutually exclusive hypotheses that have been put forth to explain the persistence and maintenance of inaccurate mimicry mostly fall into three broad categories: 1) Relaxed selection; 2) Constraints and trade-offs; and 3) Intermediate forms.

Relaxed selection – This hypothesis predicts that the persistence of inaccurate mimicry reflects a lack of selection. Inaccurate mimics that vaguely resemble a heavily defended and/or particularly noxious model may be avoided by predators as misidentification would exact a heavy cost (Kikuchi & Pfennig 2010b). Consequently, predators avoid a wider range of inaccurate, but similar, traits thereby relaxing selection resulting in the persistence of inaccurate mimics (Edmunds 2000; Gilbert 2004). Relaxed selection may also occur as a consequence of predator indifference or visual/cognitive system limitations (Kikuchi & Pfennig 2013). The inability to perceive imperfections in mimetic traits may be a consequence of the potential predator either lacking the sensory perception or cognitive processing ability that is required to make finer discrimination between model and mimic (Jackson & Nelson 2012; Jamie 2017) and thus, inaccurate mimicry is as adaptive as accurate mimicry. For example, Kazemi et al. (2014), using wild-caught blue tits, experimentally demonstrated that when some predators learn to discriminate prey, the traits with higher salience, or conspicuousness (e.g. a bright colour), can overshadow other traits. This would allow for inaccurate mimics to succeed as there is little or no selection on less-salient traits (Kikuchi & Pfennig 2010b; Cuthill 2014; Sherratt & Peet-Paré 2017). Either of these processes would mean that once a minimum level of resemblance has been achieved, or a high-salience trait has evolved, further refinement in mimetic accuracy is no longer advantageous and will provide very little fitness benefit to the mimic (Taylor et al. 2016).

Constraints and trade-offs – These hypotheses predict that inaccurate mimics are a consequence of genetic, developmental and/or phylogenetic constraints on body plans (Penney et al. 2012), thereby limiting the evolution of accurate mimicry. For example, the large costs of producing mimetic colours or shapes may constrain the mimic to inaccurate mimicry (Pekár & Jarab 2011b; Pekár et al. 2011). These can include trade-offs (e.g. the trade-off between accurate mimicry and foraging ability, fecundity or thermoregulation), where the inaccurate mimic is locally more adaptive than accurate mimicry. As most constraints can potentially be overcome, given sufficient selective pressures and enough time, constraints are unlikely to offer a universal explanation of inaccurate mimicry (Kikuchi & Pfennig 2013).

Intermediate forms – Hypotheses that fall into this category theorise that inaccurate mimics are the result of mimicking a phenotype that is intermediate between multiple model species (multiple-model hypothesis) (Reiskind 1970) or provide defence against multiple predators (multiple-predator hypothesis) (Pekár et al. 2011). In the case of the multiple-model hypothesis it is postulated that by inaccurately mimicking several closely, but allopatrically distributed models (i.e. being a general mimic), the mimic is protected over a larger area than the narrower niche occupied by an accurate mimic of a single model (Edmunds 2000; Moya-Laraño et al. 2013). The multiple-model hypothesis is consistent with observations of ant-mimicking spiders (Edmunds 1978) but not with hoverfly mimics (Penney et al. 2012). In contrast, the multiple-predator hypothesis predicts that the occurrence of inaccurate mimics is a consequence of selective forces exerted by a range of predators (Pekár et al. 2011). Selection for multiple traits may be influenced by the individual-level behaviour of a predator and/or by the predator community (Kikuchi et al. 2016). Therefore, the optimised mimic phenotype is a compromise between the ability to deceive generalist predators that actively avoid their model (i.e. mimic accuracy) while simultaneously being able to evade specialist predators of their model (i.e. movement speed) (Pekár et al. 2011). This may lead to certain traits being under conflicting selection pressure resulting in an inaccurate mimic.

An alternative hypothesis (known as the 'eye of the beholder' hypothesis) is that inaccurate mimics are in fact an artefact of human perception, with naturally occurring predators not perceiving imperfections in mimics and are therefore not a true case of mimicry (Cuthill & Bennett 1993; Dittrich et al. 1993).

Given the non-mutually exclusive nature of the above-mentioned hypotheses there has been a call for studies to test multiple hypotheses simultaneously in a single system to determine the relative contribution of each of the hypotheses toward the evolution of inaccurate mimicry (Kikuchi & Pfennig 2013). So far only two systems have been subjected to multiple hypotheses testing. One study, concentrating on hoverfly mimics of wasps and bees, found the relaxed selection hypothesis supported inaccurate mimicry in that system (Penney et al. 2012). Furthermore, in a series of studies focused on coral snake mimics at least three hypotheses were found to be consistent with inaccurate mimicry, including relaxed selection (Harper & Pfennig 2007), mimetic breakdown (where inaccurate mimicry reflects a local adaptive peak relative to accurate mimics in the absence of the model) (Harper & Pfennig 2008) and 'eye of the beholder' (Kikuchi & Pfennig 2010a). While there has been comprehensive evaluation in a few mimicry systems for some of the hypotheses mentioned above, with the relaxed selection hypothesis garnering the most support, none of these predictions provide a full explanation for most cases of inaccurate mimicry (Kikuchi & Pfennig 2013; Kazemi et al. 2014).

The '*perfecting*' hypothesis

The above hypotheses mostly assume that inaccurate mimicry is by some process or another maintained in the environment and that there is no necessity for the level of mimic accuracy to progress or evolve any further (i.e. an evolutionary stable state). It may be that the traits in inaccurate mimics are under stabilising selection with natural selection involved with maintaining the traits, or alternatively, some traits may be phylogenetically constrained, possibly due to insufficient genetic variation or constraints exerted by the states of other traits, limiting the evolutionary options for the organism (Blomberg & Garland 2002). However, the mostly overlooked *perfecting* hypothesis predicts that inaccurate mimics are in fact undergoing the process of directional selection evolving toward a more accurate mimic form and are at an intermediate stage (or inaccurate stage) in this evolutionary transition (Edmunds 2000; Edmunds 2006).

Considering the temporal progression of evolution, the many, non-mutually exclusive hypotheses, may help understand why inaccurate mimics are currently observed in the environment. Without determining if the *perfecting* hypothesis is contributing to inaccurate mimicry, tests of the alternative hypotheses may only explain its current maintenance ignoring the fact that the mimic may inevitably evolve those traits that contribute to a higher level of accuracy. To investigate this hypothesis, it is important to understand the potential evolutionary process of those traits involved with mimic accuracy. It is also important to establish whether the traits necessary for accurate mimicry evolve in a punctuated fashion or as a gradual process of acquiring traits over a longer evolutionary period (i.e. the *perfecting* hypothesis).

Much of the debate involving the evolution of Batesian mimicry focuses on determining whether it may evolve through a two-step process or the gradual process of incremental evolution (Kikuchi & Pfennig 2010a; Booker et al. 2015). Fisher's (1930) gradual evolution and Nicholson's (1927) two-step hypothesis have been suggested to help explain how mimetic species might transition from a cryptic phenotype to a mimetic phenotype (Fig. 1) (Helm 2008). Convergence towards a mimic phenotype can be considered conceptually as the movement in phenotypic space across a fitness landscape towards an adaptive peak characterising a particular niche or environment (Arbuckle et al. 2014).

Nicholson's two-step hypothesis states that the evolution of mimics occurs initially as a consequence of a large mutational change causing a relatively close resemblance to the model with smaller mutations at several loci further refining the phenotypic similarity to the model over time (Allen &

Cooper 1995; Helm 2008). In this model, the mimic takes a mutational leap over the adaptive valley associated with inaccurate phenotypes from one high fitness state (crypsis or masquerade) to the next (mimicry). Current opinion is that the two-step mechanism is the most likely explanation of the evolution of Batesian mimicry (Kikuchi & Pfennig 2010a).

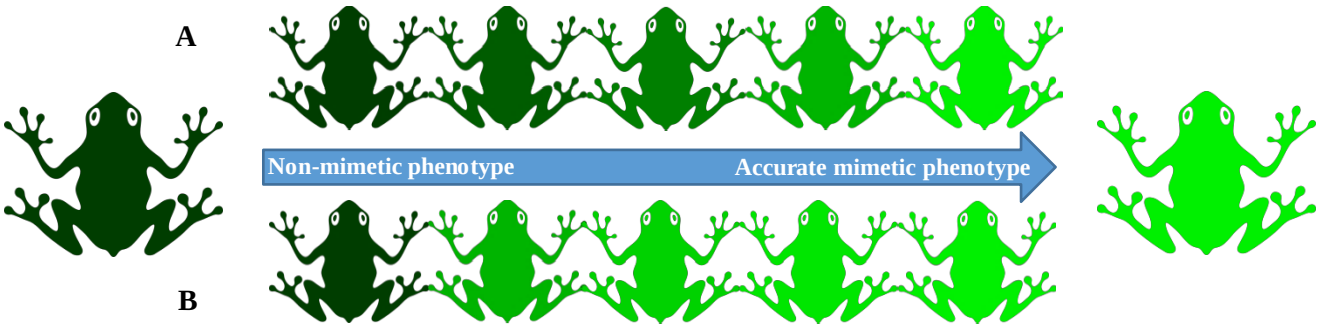

Figure 1. Two possible hypotheses to explain how mimic species make the transition from a cryptic phenotype toward a mimetic phenotype. (A) Fisher's (1930) gradual evolution predicts a process of incremental evolution, whereby a mimic species undergoes small phenotypic changes through evolutionary time to achieve similarity to a model species (Kikuchi & Pfennig 2010b). (B) In contrast, Nicholson's (1927) two-step hypothesis, portrayed as a jump between adaptive peaks, states that mimicry begins with an initial large mutation followed by gradual fine-tuning over time (Balogh et al. 2010).

However, the evolution of mimicry in one taxon may not always apply to the evolution of mimicry in another. The two-step evolutionary transition may be a more likely scenario with simpler forms of mimicry involving the evolution of only a few traits, such as the pattern and colour of a butterfly wing (e.g. *Papillio* swallowtail butterfly, Batesian mimics of defended butterfly species). Yet, when the form of mimicry is more complex, such as involving the integration of multiple extreme morphological and behavioural traits, as those observed in ant-mimicking spiders to create an illusion of ant-likeness, the two-step hypothesis may not seem realistic. The numerous traits required to transition from a spider body to that of an ant-like body includes constrictions and elongation affecting the morphology of the prosoma and abdomen, extension of the pedicel, colour change, thinning of the legs, patches of hairs that reinforce morphological characteristics, and the waving of the first pair of legs in an 'antenna-like' fashion (McIver & Stonedahl 1993; Edmunds 2006; Pekár & Jarab 2011a; Pekár 2014b; Corcobado et al. 2016). The necessity for ant-mimicking spiders to evolve numerous traits to produce an ant-like form makes the gradual process of evolution appear more plausible as the acquisition of these complex character sets by single mutational events is highly unlikely.

Nur (1970) suggested the concept that inaccurate mimicry is not an evolutionary stable state, rather that they may eventually evolve closer structural resemblance to the model, achieving a higher level

of accuracy. Fisher's (1930) gradual evolution, indicative of a *perfecting* process, predicts a process of incremental evolution whereby a mimic species achieves similarity to a model via small phenotypic changes over evolutionary time which may better explain the evolution of complex mimicry. Kikuchi and Pfennig (2010a) provided experimental support for the gradual evolution of accurate mimicry in harmless kingsnake mimics of venomous coral snakes. Their study showed that in areas where the toxic model was abundant the attack rate on intermediate phenotypes was reduced relative to areas where the model was not abundant. This suggests that in this system, due to the noxious nature and abundance of the model, selection may act to form a smooth slope rather than an adaptive valley and allow inaccurate mimicry to persist and gradually evolve a higher level of accuracy (Kikuchi & Pfennig 2010a).

Irrespective of the precise mechanism of transition (gradual or step-wise), testing the overall idea of perfecting mimicry over evolutionary time requires a phylogenetic approach to determine if there are observable trends in mimic accuracy and the evolution of the associated traits.

A phylogenetic approach to investigating inaccurate mimicry

Phylogenetic-based approaches have been used to estimate rates of morphological change and provide further insight into the pattern of adaptive morphological evolution (Butler & King 2004; Prudic & Oliver 2008; Adams et al. 2009; Derkarabetian et al. 2010). Phylogenetic studies, for example, have investigated the relationship between shape and size and have revealed varying rates of phenotypic evolution across clades for various traits (Adams et al. 2009). Traits are known to evolve repeatedly over the course of a clade's phylogenetic history. With ongoing improvements in molecular phylogenetic techniques, it has become possible to make inferences on trait evolution, especially inferring adaptation using the multiple repeated origins of a particular trait (Wiens et al. 2006). A phylogenetic study conducted by Maruyama & Parker (2017) uncovered an ancient system of convergent morphological and behavioural evolution in rove beetles (Staphylinidae). They found that multiple rove beetle lineages evolved ant-mimicking body forms following a foreseeable phenotypic trajectory. In this thesis, I will use a phylogenetic approach to shed light on the paradox of inaccurate mimicry using ant-mimicry in spiders (Araneae).

Myrmecomorphic spiders

Ant-mimicry (myrmecomorphy), the resemblance to ants through convergence in morphological characters (Cushing 1997), likely evolved during the Cretaceous when ants first appeared (Pekár 2014b). It is commonly assumed that myrmecomorphy protects otherwise palatable prey species from predators (Batesian mimicry) (Sherratt 2017) through their morphological resemblance to unpalatable

or aggressive ants (Cushing 1997). Ants possess formidable defences such as powerful mandibles, poison-injecting stings and the production of various defensive secretions (Nelson & Jackson 2006) and are known to behave aggressively toward intruders, often attacking collectively (Pekár & Jarab 2011a). These defensive strategies alongside their abundance in all terrestrial habitats make ants highly suitable models for a Batesian mimic (Cushing 1997; Pekár 2014a). Myrmecomorphy is found in numerous taxa, including beetles, mantids, dipterans, phasmids, and is very common in spiders (McIver & Stonedahl 1993; Cushing 1997; Cushing 2012). The evolution of myrmecomorphy in distantly related taxa serves as a striking example of the independent convergence of traits that result in a resemblance to ants (Pekár 2014b) and is a clear illustration of the power of convergent evolution at deep phylogenetic levels.

Spiders (Araneae) alone have experienced multiple parallel occurrences of convergent evolution (e.g. Salticidae and Corinnidae) including a predictable morphological evolutionary trajectory toward myrmecomorphy (McIver & Stonedahl 1993; Cushing 1997; Pekár 2014b). Alongside ants, spiders are abundant features in all terrestrial habitats with myrmecomorphy more frequent in tropical species (Pekár 2014a). There is evidence that myrmecomorphy in spiders protects from predator attacks (e.g. Cutler 1991; Nelson & Jackson 2006; Nelson et al. 2006; Nelson & Jackson 2009; Huang et al. 2010; Durkee et al. 2011). Myrmecomorphy is by far the most frequent form of Batesian mimicry in spiders (Reiskind 1971; Cushing 1997; Pekár 2014a) evolving independently in 16 families and 85 genera (Pekár 2014b) with more than 200 species recognised as myrmecomorphic (Sherratt 2017). However, not all species have reached the same level of ant-like similarity. Accuracy varies from inaccurate, e.g. *Micaria* (Gnaphosidae), to highly accurate, e.g. *Aphantochilus* (Thomisidae) (Edmunds 2006; Moya-Laraño et al. 2013; Nelson & Card 2016). The existence of such a range in accuracy makes spiders an ideal taxon for a phylogenetic investigation of the paradox of inaccurate mimicry and the perfecting hypothesis.

Phylogenetic investigation of myrmecomorphic trait accuracy

While the idea of traits being perfected over evolutionary time is persuasive and intuitive, it is currently inconclusive whether this mechanism can explain the wide spread occurrence of mimetic inaccuracy in ant mimicking spiders. A species-level phylogenetic investigation is required to resolve this question (Pekár 2014b) and determine whether mimic species require more evolutionary time to perfect their phenotypic similarity to their model. Using a molecular phylogenetic approach at a species-level will also provide further insight into morphological convergence in spiders, trends of morphological change, and whether accurate mimicry is phylogenetically constrained (Pekár 2014b). Myrmecomorphic studies (both ecological and evolutionary) can lead to a broader understanding of

systematics and predator-prey relationships (McIver & Stonedahl 1993), as well as providing critical insight into the limitations of natural selection in the production of complex adaptations (Kikuchi & Pfennig 2013). Ultimately, a comprehensive species-level phylogeny of mimetic trait accuracy would contribute to resolving the paradox of inaccurate mimicry.

Overview of following chapters

In this thesis, methods for the assessment of mimetic quality, or accuracy, were evaluated (Chapter 2) to determine their suitability in quantifying differences amongst mimetic species. One method involved the scoring of myrmecomorphic spiders based on the accumulation of multiple traits known to contribute to ant-like morphology and was compared to an alternate method (geometric morphometrics) of quantifying mimic shape accuracy. Following this assessment, a phylogenetic analysis of a set of myrmecomorphic spider species was performed to observe the relative phylogenetic relationships between inaccurate and accurate mimics (Chapter 3). This allowed us to identify the phylogenetic trends in mimetic accuracy and estimate the likely evolutionary history of the traits required for ant-mimicry (i.e. is there evidence of a step-wise process of myrmecomorphic evolution in spiders).

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Chapter 2: Quantifying mimic accuracy in myrmecomorphic spiders

Abstract

Mimicry is often noted as a prime example of natural selection, with the convergent evolution of traits in a mimic resulting in a similarity to its model. However, the similarity between the mimic and model is not always precise. Historically, the assessment of mimic accuracy has been based on qualitative or subjective measures. The quantitative assessment of mimic accuracy has rarely been attempted. Here I introduce a method of quantifying the accuracy of myrmecomorphic (ant-mimicking) spiders using the accumulation of specific traits noted in the literature as contributing to the ant-like appearance of myrmecomorphs. The accumulation of traits was scored in a mimic quality assessment table (MQAT) with the assumption that individuals that possessed numerous traits would be more accurate than an individual possessing few. The MQAT method of mimic assessment was evaluated and compared to an alternative method of mimic assessment, geometric morphometrics, to evaluate the efficacy and efficiency of these methods. It was concluded that geometric morphometrics are best suited to taxa that rely on the shape of their body or wings for mimetic accuracy but the MQAT is suitable for complex forms of mimicry where accuracy is influenced by numerous traits in addition to body shape, such as the width and length of appendages.

Introduction

Mimicry is an example of an adaptation requiring numerous distinct, but integrated, traits (Charlesworth & Charlesworth 2011). It could be expected that related species sharing environmental characteristics, and therefore similar selective regimes, would share traits adaptive for those regimes (Blomberg & Garland 2002; Maruyama & Parker 2017). However, many mimics do not possess the full suite of traits observed in accurate mimics, even of the same model species (Edmunds 2006; Sherratt & Peet-Paré 2017). Myrmecomorphy (or ant-mimicry) has evolved numerous times independently in spiders (Araneae) (McIver & Stonedahl 1993; Cushing 1997; Pekár 2014b) and involves the integration of multiple traits. There are many striking examples of this complex form of mimicry such as *Myrmaplata plataloides*, an ant-mimicking spider of the Asian Weaver Ant, *Oecophylla smaragdina*, which is, to the human eye at least, an incredibly convincing mimic (Edmunds 2006; Cushing 2012). Simultaneously, there are many widespread cases of mimics that exhibit very few morphological adaptations or possess only colour similarities to their ant model and are generally thought to be inaccurate mimics, for example *Micaria sociabilis* an ant-mimic of the ant *Liometopum microcephalum* (Pekár & Jarab 2011; Moya-Laraño et al. 2013).

Selection for accurate mimicry involves dramatic alterations to the phenotype, including behaviour, colour pattern, body size and body shape (Holen & Johnstone 2004). Morphologically, myrmecomorphy in spiders requires a transformation from a stocky arachnid with two body segments and eight legs into a thin insect with three body segments consisting of narrow constrictions, two antennae and six legs (Shamble et al. 2017). The challenges for a spider to produce an accurate illusion of ant-like form can include extreme morphological adaptations such as an elongation of the prosoma and/or opisthosoma and/or constriction of the prosoma and/or opisthosoma that increases the resemble of a petiole or the three body segments seen in ants (McIver & Stonedahl 1993; Pekár & Jarab 2011; Corcobado, Herberstein & Pekár 2016).

It is generally acknowledged that the degree of mimic similarity is very difficult to quantify (Turner 1984; Bain et al. 2007; Penney et al. 2012). The identification of mimics has nearly exclusively been based on visual resemblance according to human perception without any experimentation or further quantification, which will not correspond to the visual and cognitive systems found in many potential predators of mimetic species (Gilbert 2004). One explanation for inaccurate mimicry is that mimetic similarity is perceived differently by natural predators than by human vision (Bain et al. 2007). In fact, it has been suggested that mimic imperfection is merely an anthropocentric perception and that predators do not distinguish between inaccurate mimics and their model, known as the "eye of the beholder" hypothesis (Cuthill & Bennett 1993; Dittrich et al. 1993).

Many experiments have demonstrated the protective capacity of numerous myrmecomorphic species, including mimics with varying degrees of accuracy, providing evidence that myrmecomorphy in spiders is a case of Batesian mimicry (e.g. Cutler 1991, Nelson & Jackson 2006b, Nelson et al. 2006, Nelson & Jackson 2009; Huang et al. 2010; Durkee, Weiss & Uma 2011; Nelson 2012). These experiments have mostly focused on mimics that are considered relatively accurate mimics (e.g. *Myrmarachne* spp.) and thus little is known about the protection from predation of less accurate mimics.

To investigate the spectrum of mimicry, from inaccurate through to highly accurate, the first challenge becomes assessing the quality of mimics. The accuracy of mimics has been mainly assessed through subjective means (Pekár & Jarab 2011), which introduces the potential for human bias in determining the level of accuracy of a given mimic. The ability to objectively quantify the level of accuracy among a group of mimics is essential to test predictions such as their relative level of protection from predators, the rarity of inaccurate mimics (Gilbert 2005), or the phylogenetic relationships between inaccurate and accurate mimics. The most ecologically relevant way to

determine the efficacy of a mimic would be to assess mimic-quality based on predator perception through predator/prey style experiments (e.g. Nelson 2012).

Prior studies have evaluated predatory perception, relative to human assessment of mimic accuracy, by observing the activity of predators when confronted with mimics possessing varied levels of similarity to their model. Dittrich et al. (1993), using pigeons as a proxy for avian vision, attempted to determine if pigeons could distinguish between inaccurate and accurate mimics consistent with human evaluation. While pigeons themselves are not insectivorous, the avian visual system is thought to be highly conserved and the qualitative results of such a study should be relevant to a range of avian species (Bain et al. 2007). The study conducted by Dittrich et al. (1993) found that, while a few mimics viewed as inaccurate by human perception were categorised as accurate by pigeons, the overall results of the experiment corresponded with the order of mimetic quality assessed by the human eye. The conclusions of this study and other prior bird predation studies (e.g. Mostler 1935), coupled with the fact that birds are visually guided predators, suggest that birds are capable of perceiving inaccurate mimics with the extent of protection dependent on the level of similarity of the mimic phenotype to its' model. Additionally, a study conducted by Nelson (2012) tested the response of visually based spider predators to mimics of varying accuracy. This study determined that the aversive behaviour of the predators to mimics increased with the mimics' visual similarity to the model, suggesting that invertebrate predators are perfectly capable of generating selective pressure for accurate mimicry (Gilbert 2004; Morris & Reader 2016). The results of these studies suggest that predators perceive levels of mimic accuracy comparable to human perception. Thus, it is likely that some, if not all, of the traits that contribute to mimic accuracy to the human eye are also perceived by natural predators. The remarkable convergence of traits observed in many mimetic species can be considered evidence of the ability of specialised and experienced predators exerting selective pressure for precise mimicry (Estrada & Jiggins 2008). However, to quantify mimic accuracy on a large scale across numerous individuals/species using a predator-trial type approach involves ethical implications, as well as, the time required to obtain the specimens of interest (predators, models, and mimics) and conduct the predator study. This type of approach is beyond the scope of this study.

More recently, objective tools for measuring visual and behavioural components of mimicry have been developed, including motion tracking software and image analysis (Pekár & Jarab 2011). Colour analysis has been used to measure mimetic similarity (e.g. Pekár & Jarab 2011), as well as, comparison of movement (i.e. trajectories) (McLean & Skowron Volponi 2018). These methods are relatively computationally intensive and laborious.

The idea of using a table of known traits to assess mimic quality is not unique. Edmunds (2006), for example, examined the morphology of salticid and corinnid ant-mimicking spiders. He subjectively assessed traits to determine whether some traits contributed to a general ant-like appearance, while other traits were specific to a given model. Some traits were considered general adaptations to ants, such as the elongation of the pedicel, presence of white hairs on the prosoma, and constrictions to the prosoma, while traits such as the elongation of the prosoma and opisthosoma and body colour were adaptations to specific ants. Bain et al. (2007) investigated inaccurate mimicry in hoverflies by developing and evaluating a numerical model based on measures of a wide range of biometrical attributes to identify the key features, or traits, important for avian assessment of mimetic similarity. They found that some traits were more relevant than others in avian assessment of mimic accuracy (such as antennal length, the number of visible stripes, and the presence of colourful patches). The study conducted by Bain et al. (2007) provides testable insights into how predators use the presence of morphological traits to inform their discriminative decisions.

Penney et al. (2012), in a study of hoverfly mimetic fidelity, used biometric measures to conduct a comparison of multivariate analysis of trait values (i.e. morphometrics) with avian rankings of mimic accuracy. They found a positive correlation between the morphometric measures and human rankings. Later Pekár (2014b) used a checklist of traits to assess accuracy in myrmecomorphic spiders. Taking data from taxonomic papers, Pekár (2014b) scored the accuracy of myrmecomorphic spiders into four categories based on the accumulation of certain morphological traits, such as size and overall colouration, thin legs, improved colouration (e.g. bands of lightly coloured hairs), constrictions on the prosoma or abdomen, an abdominal scutum, or eye spots). He then used these scores of mimic accuracy to determine whether inaccurate mimicry is ancestral to accurate mimicry. He found that the estimated ancestral state for Corinnidae was inaccurate myrmecomorphy, while the ancestral state for Salticidae was non-mimetic.

Here I introduce and evaluate a combined modified version of the trait accumulation method used by Pekár (2014b) with biometric measures of traits (*sensu* Penney et al. 2012) to objectively assess mimic accuracy in myrmecomorphic spiders as an alternative to existing, more complex methods. This method involves the use of a mimic quality assessment table (MQAT) to score traits that have been noted to contribute to the ant-like morphology of myrmecomorphic spiders. Trait scoring was performed via biometrical measurements of specific anatomical features contributing to ant-likeness in myrmecomorphic spiders within the subfamilies Castianeirinae (Family: Corinnidae) (number of specimens, n = 28) and Myrmarachninae (Family: Salticidae) (n = 25). Outgroup and non-mimic species for the Castianeirinae (n = 4) and outgroup species for the Myrmarachninae (n = 7) were also

measured. Two alternative methods were employed: 1) a binary (absence/presence) scoring method; and 2) a continuous scoring method (representing the characters' state). An individual that possesses more of these traits (binary), or to a greater degree (continuous), can be considered more accurate relative to an individual that has evolved only a few of the traits (binary), or have evolved the trait to a lesser degree (continuous).

An analysis comparing the two techniques of scoring mimic accuracy (i.e. binary and continuous) was conducted to evaluate their performance. Finally, a subset of species assessed using the MQAT were compared with the mimic accuracy assessment using geometric morphometric analysis, established in a prior study (D.J. McLean, unpublished data), to evaluate the efficacy and efficiency of these methods at producing a reasonable assessment of myrmecomorphic accuracy.

Methods

Focal groups

The largest number of myrmecomorphic spiders is found in Salticidae, the largest family of spiders, and Corinnidae (Cloudsley-Thompson 1995; Pekár 2014a). This study focused on a subfamily from each of these two families known for including species with varying degrees of myrmecomorphic accuracy: Myrmarachninae (family Salticidae) and Castianeirinae (family Corinnidae) (Pekár 2014b). Myrmarachninae is the most speciose clade of ant-like salticids with the majority of species found in the large genus *Myrmarachne* (Wanless 1978; Maddison 2015), of which all 182 described species (World Spider Catalog 2018) are specialised ant mimics (Nelson 2012; Benjamin 2015). Comparison between different species of *Myrmarachne* demonstrates how even species living sympatrically have morphologically differentiated, likely due to strong selective pressures and ecological differences as they adapt to the microhabitat inhabited by their respective ant model (Ceccarelli 2010). Recent changes proposed by Prószyński (2016) have transferred multiple species from the genus *Myrmarachne* into 19 new genera (e.g. *Myrmaplata*, *Myrmapeni*, and *Myrmapana*). Other genera that make up this monophyletic natural group (Myrmarachninae) include *Judalana*, *Ligonipes*, *Rhombonotus*, and *Damoetas* (Davies & Zabka 1989; Maddison 2015). Many of the spiders belonging to the well-defined monophyletic subfamily Castianeirinae (Reiskind 1969; Bosselaers & Jocqué 2002; Raven 2015) present diverse morphological and/or behavioural adaptations for myrmecomorphy (Oliveira 1986; Cushing 1997; Raven 2015; Candiani & Bonaldo 2017). The most sophisticated myrmecomorphs in this subfamily are known to be equal to, or even surpass, the level of accuracy seen in Salticidae (Deeleman-Reinhold 2001).

Acquisition of spider samples

Specimens of spiders were obtained from multiple sources (Table S1). Australian spider samples were sourced from field collections performed at various locations along the east coast of Australia (*n* = 18), supplied by the Queensland Museum (*n* = 16), as well as, donated preserved specimens from a prior study (*n* = 11; Jim McLean, Macquarie University, Sydney, Australia). Additionally, specimens with a wider global distribution outside of Australia were sourced from the collection maintained at the Museum of Comparative Zoology (MCZ), Harvard University (Cambridge, Massachusetts) (*n* = 18) and a donated preserved specimen (*n* = 1, Stano Pekár, Masaryk University, Brno, Czech Republic).

Sampling from the field involved the visual inspection of tree trunks (including underneath bark), branches, foliage, vegetation, leaf litter, as well as the use of beat trays. Specimens were contained individually in plastic vials and then returned to Macquarie University alive and filmed both dorsally and laterally using a Basler Ace 640×480pix USB 3.0 high speed video camera (Basler AG, Ahrensburg, Germany) for later biometrical measurement and analysis of specific traits contributing to ant-like resemblance *in vivo*. The field caught spiders were filmed within a rectangular prism constructed from microscopic slides adhered together with glue. Ruled paper was adhered to the surface of the prisms' base, acting as a scale for later measurement of anatomical features. Spiders were released gently into the prism from tubes and allowed to move freely throughout the area during filming. Spiders obtained from field collection were identified using multiple sources (e.g. Davies & Zabka 1989; Dankittipakul & Singtripop 2013; Whyte & Anderson 2017).

Specimens obtained from MCZ were imaged both dorsally and laterally using a VHX-6000 digital microscope (Keyence Corporation, Osaka) for the later biometrical measurement of selected traits. For those specimens obtained from alternative preserved collections, such as the Queensland Museum (where only a single leg was acquired), a literature search (e.g. Raven 2015) was performed to obtain measurements of selected traits. The analysis and measurement of traits in specimens, either filmed, imaged, or sourced from prior publications, was conducted using ImageJ v1.5 (Schneider, Rasband & Eliceiri 2012). All specimens included in evaluating accuracy were considered to be in good condition. Any specimens possessing obvious damage or distorted morphology were excluded from analysis.

Trait selection and evaluation

To avoid subjectivity as much as possible, myrmecomorphic accuracy was defined via the assessment and scoring of morphological traits noted by previous authors to contribute to ant-like morphology in

spiders. Each of the traits was quantified using biometrical measurements (with the exception of the presence of improved colouration, discussed below).

The selected traits were assessed in two ways to determine which best captured the quantification of mimic accuracy. One method was scoring using an absence/presence binary system, the second assessment used continuous values that can be viewed as an expression of the traits' state. The resulting values thus represent proportional distances between phenotypic character states.

Scoring using binary method (absence/presence)

Each of the traits were quantified using biometrical measurements and then scored based on an absence/presence binary methodology (with the exception of constrictions to the prosoma and opisthosoma, discussed below). Following prior studies (e.g. Pekár 2014b) and characteristics used in taxonomic keys for spider species identification (e.g. Davies & Zabka (1989) - (Salticidae) and Raven (2015) - (Corinnidae)), the presence of each trait is defined as follows:

1) Thin legs more closely resembling the thin legs observed in ant anatomy (Cloudsley-Thompson 1995; Deeleman-Reinhold 2001; Durkee, Weiss & Uma 2011) were determined using the femoral width/length ratio of leg III. Following Pekár (2014b), species with a third leg femoral width to length ratio < 0.25 were considered having a thin leg. Measurements included the length of the femur and the width (at the widest point); 2) The presence of an elongated, slender prosoma (Edmunds 2006; Durkee, Weiss & Uma 2011; Nelson 2012; Ceccarelli 2013; Candiani & Bonaldo 2017) was quantified using characteristics used in spider taxonomy. Measurements for length of the prosoma were taken from the dorsal profile between the anterior limit of carapace (not including chelicerae) to the posterior extent (not including the pedicel, if present). Spiders are described as having an elongated prosoma (or ant-like habitus) when it was >1.5x longer than it is wide (e.g. Davies & Zabka 1993; Raven 2015); 3) An elongated, slender opisthosoma (Edmunds 2006; Durkee, Weiss & Uma 2011; Nelson 2012; Ceccarelli 2013) is treated in accord with elongated, prosoma. Hence an opisthosoma that is measured from the anterior limit of the opisthosoma, not including the pedicel (if present), to the posterior tip, not including spinnerets (if present), was >1.5x longer than wide it was considered elongated; 4) The presence of a clearly visible elongated pedicel simulating the petiolus of an ant (Cloudsley-Thompson 1995; Edmunds 2006; Durkee, Weiss & Uma 2011; Ceccarelli 2013; Pekár 2014b) was determined by its easily observable presence in either the lateral or dorsal view (Ceccarelli 2013; Pekár 2014b); 5) The presence of improved colouration that reinforces morphological resemblance involves specific characteristics and does not include general overall colouration nor colouration that does not contribute to ant-like appearance in the spider. An individual was scored as possessing improved colouration if they possess either: a) a transverse band/stripe of

lightly coloured setae on the abdomen and/or prosoma imitating the 'neck' of an ant or increasing the deceptive appearance of a body division/petiole (Pekár & Jarab 2011) and/or b) the appearance of 'eye' spots, including the darkening of the area surrounding the posterior lateral eye (PLE) creating the illusion of an ants' compound eye (Pekár 2014b); 6) A constriction on the prosoma to enhance the appearance of a 'neck' or pronotum, characteristic of ant morphology (Edmunds 2006; Pekár 2014b; Candiani & Bonaldo 2017). As the constriction of the prosoma can occur either laterally or dorsally and result in an alteration of the body shape of the spider when viewed from different angles these were scored separately and considered separate traits. Additionally, as the illusion of ant-like morphology increases with the constriction depth/width on the spider these traits were scored to reflect this increase in accuracy. In the case of a lateral constriction, where the constriction did not fall below 90% of the total prosomal height (i.e. from base of carapace to highest point on carapace) this was considered a slight constriction and given a score of one. Constrictions between 80 and 90% where considered intermediate and were given a score of 2, while extreme constrictions below 80% of the total carapace height were given a score of 3; and 7) Constriction on the opisthosoma to enhance the appearance of a body division (Deeleman-Reinhold 2001; Pekár 2014b) is considered in the same context as constrictions on the prosoma, i.e. the lateral and dorsal constrictions were scored independently, and scoring was based on the severity of the constriction.

Finally, the sum of the present traits was then converted to a proportion of the total number of possible traits (or trait states in the case of constrictions) included in the MQAT. These resulting values would then indicate mimic quality with higher numbers representing highly accurate mimics and low numbers representing lower quality mimics. To include specimens that did not have all the trait data available a conservative measure was used by assuming the trait was not present (i.e. scored as 0), then calculated the same way as those specimens that had all traits available.

Scoring using continuous method

All measurements were obtained using the same methodology described in the binary scoring method above. Biometrical measurements were utilised to obtain a continuous value for each character to evaluate whether this method more precisely measures the differences between traits and variation in accuracy. For the continuous assessment the following methodology was implemented:

1) Thin legs – the width/length ratio of the third leg femur was used as the measure of this condition. Therefore, by subtracting this ratio from 1, leg dimensions with higher values indicates thinner third legs; 2) Elongated prosoma – the width/length ratio was subtracted from 1 so that higher values represented an increased elongation of the prosoma; 3) Elongated opisthosoma – the width/length ratio was subtracted from 1 so that higher values represented an increased elongation of the

opisthosoma; 4) Elongation of the pedicel – this trait was measured by taking the resulting value of the pedicel length/total body length ratio. Higher values indicate a more elongated pedicel relative to total body length; 5) Improved colouration – as this trait had three potential components, i.e. a) bands or stripes on the prosoma; b) bands or stripes on the opisthosoma; and c) eye spots or darkening of the PLE, the individual was scored based on the proportion of these three traits it possessed (i.e. an individual with one of the three characteristics received a measure of 0.334, an individual with two received 0.667, and an individual with all three traits received a score of 1; 6) Constriction of the prosoma – lateral measure was taken by measuring the distance from the base of the carapace to the lowest point on the constricted region and dividing this by the measurement taken from the base of the carapace to the highest point on the carapace. This value was then subtracted from 1 to result in higher values indicating more extreme constrictions. The same was repeated for the dorsal profile with measurements taken from the widest point of the carapace and the width at the point of the prosomal constriction; 7) Constriction of the opisthosoma – lateral measure was taken by measuring the distance from the base of the opisthosoma to the lowest point on the constricted region and dividing this by the measurement taken from the base of the opisthosoma to the widest point on the opisthosoma. This value was then subtracted from 1 to result in higher values indicating more extreme constrictions. The same was repeated for the dorsal profile with measurements taken from the widest point of the opisthosoma and the width at the point of the opisthosomal constriction.

Finally, the sum of the values calculated for each of the traits was then converted to a proportion of the total number of possible traits included in the MQAT. Higher resulting values indicate a highly accurate mimic while low numbers represent lower quality or inaccurate mimics. To include specimens that did not have all the trait data available (i.e. incomplete specimens, limited access to specimens (sourced from museum), or data not recorded in current literature, a conservative measure was used by assuming the trait was not present (i.e. scored as 0), then calculated the same way as those specimens that had all traits available.

It should also be noted that sexual dimorphism is common in many myrmecomorphic spiders. For example, the male of *Myrmarachne* species possess enlarged porrect chelicerae (Davies & Zabka 1989; Edmunds 2006; Nelson 2012). This trait is likely the result of sexual selection (i.e. female preference or male –male competition) (Pollard 1994; Joron & Mallet 1998; Ceccarelli 2010). It has been stipulated that the enlarged chelicerae on the male create the illusion of an encumbered ant, i.e. an ant carrying a nest mate or food, making *Myrmarachne* a compound mimic (Edmunds 2006; Nelson & Jackson 2006a; Ceccarelli 2010). However, while it has been shown to still be effective at deterring visually based predators, it experiences reduced protection relative to the female (Nelson

2012). To assess the highest potential mimic state of the species this trait was excluded as it is not representative of the species in its entirety (i.e. the trait is not found in the female). However other traits, such as the dimensions of the opisthosoma, can also differ. Females of some species often have slightly less exaggerated elongation or constrictions of the opisthosoma relative to the male, likely related to fecundity in the female. The sex of the specimens used in the scoring of traits is indicated in Table S1.

Geometric morphometrics

To determine the validity of the MQAT in assessing mimic accuracy in myrmecomorphic spiders, the results were compared to the accuracy assessment evaluated by an alternative strategy, geometric morphometrics (D.J. McLean, unpublished data). Geometric morphometrics, the mathematical analysis of shape, can play a key role in a variety of biological studies and can illustrate and help to describe the differences, or similarities, between shapes (Zelditch, Swiderski & Sheets 2012). Morphometric tools are therefore capable of assessing and comparing body outlines between a mimic and their model (Pekár & Jarab 2011). This prior geometric morphometric study used elliptical Fourier analysis, as the anatomies of ants and spiders do not share consistent structural homology, i.e. non-mimic spiders do not possess a 'neck', nor a clearly visible petiole, characteristic of ant anatomy, therefore the traditional use of "landmarks" for morphometric analysis was not appropriate. The body outlines of several species of ants, mimics and non-mimic spiders were analysed. The shape outline of each specimen was prepared in Adobe Photoshop CS2 using photographic images taken from both the dorsal and lateral angle. These shape outlines were then converted into outline co-ordinates, and an elliptical Fourier transform applied, producing a set of points for each outline. Following this, a linear discriminant analysis was applied to identify variation that can discriminate between different object classes (i.e. ant body shape and spider body shape) in a set of multi-dimensional points. The resulting value calculated by this linear discriminant analysis quantifies how 'ant-like' a body shape outline is. Calculations were performed in R (R Development Core Team 2016) using the Momocs package (Bonhomme et al. 2014). This analysis provided a value indicating the ant-likeness for both the dorsal and lateral profile. A linear regression using R was then performed to examine the correlation between the results of both the dorsal and lateral linear discriminant analysis (the product of the geometric morphometric analysis) and the results of the MQAT.

Results

In the subfamily Castianeirinae, the mimic species assessed by the MQAT binary method of scoring as being the more accurate mimic species included *Sphecotypus niger*, *Serendib suthepica*, *Aetius*

nocturnus, *Myrmecotypus rettenmeyeri*, *Mazax pax*, and *Mazax spinosa* (Table 1). Species such as *Poecilipta gloverae*, *Castianeira trilineata*, *Nucastia supunnoides*, *Iridonyssus kohouti*, *Corinnomma* sp., *Castianeira longipalpa*, and *Castianeira cingulata* were scored intermediate between the more accurate species and the inaccurate species. The least accurate of the Castianeirinae evaluated included *Serendib volans*, *Nyssus semifuscus*, *Nyssus luteofinis*, *Leptopicia bimaculata*, *Leichhardteus albofasciatus*, *Nyssus paradoxus*, *Leichhardteus conopalpis*, *Copa kabana*, *Castianeira gertschi*, *Battalus byrneae*, *Nyssus avidus*, *Iridonyssus formicans* and *Disnyssus helenmirrenae*.

Table 1. The results of the mimic quality assessment table (MQAT) using the binary scoring method for the subfamily Castianeirinae and outgroup (n = 32). Specimens are ordered from lowest to highest level of mimic accuracy. The traits and their method of scoring are described in the methods section above. 'No data' indicates where there were no data available to measure the trait. *Indicates samples considered as outgroups or non-ant-mimic spiders.

Unique I.D.	Species	Thin legs	Elongated prosoma	Elongated opisthosoma	Elongated pedicel	Improved colouration	Degree of lateral prosomal constriction	Degree of dorsal prosomal constriction	Degree of lateral opisthosomal constriction	Degree of dorsal opisthosomal constriction	Mimic Accuracy
Q80326	*Disnyssus helenmirrenae*	No data	0	0	0	0	No data	0	No data	0	0
Q84598	*Iridonyssus formicans*	0	0	0	0	0	0	0	0	0	0
Q84821	*Nyssus albopunctatus**	0	0	0	0	0	0	0	0	0	0
Q00016	*Nyssus avidus*	0	0	0	0	0	No data	0	No data	0	0
SHS116	*Nyssus coloripes**	0	0	0	0	0	0	0	0	0	0
TBS504	*Nyssus coloripes**	0	0	0	0	0	0	0	0	0	0
Q00002	*Battalus byrneae*	1	0	0	0	0	0	0	0	0	0.06
MCZ137106	*Castianeira gertschi*	0	0	0	0	1	0	0	0	0	0.06
Q00013	*Copa kabana*	0	0	1	0	0	0	0	No data	0	0.06
Q87362	*Creugas gulosus**	1	0	0	0	0	No data	0	No data	0	0.06
Q95115	*Leichhardteus conopalpis*	0	0	1	0	0	No data	0	No data	0	0.06
Q00017	*Nyssus paradoxus*	0	0	1	0	0	No data	0	No data	0	0.06
MCZ34637	*Paradiestus giganteus**	0	0	1	0	0	0	0	0	0	0.06
Q00014	*Leichhardteus albofasciatus*	0	0	1	1	0	0	0	No data	0	0.12
Q98191	*Leptopicia bimaculata*	0	0	1	1	0	No data	0	No data	0	0.12
Q50886	*Nyssus luteofinis*	0	0	1	0	1	0	0	0	0	0.12
Q84600	*Nyssus semifuscus*	No data	No data	1	No data	1	0	0	0	0	0.12
MCZ44162	*Serendib volans*	0	1	0	1	0	0	0	0	0	0.12
MCZ136939	*Castianeira cingulata*	0	1	1	0	1	0	0	0	0	0.18
MCZ126957	*Castianeira longipalpa*	0	1	1	0	1	0	0	0	0	0.18
MCZ95991	*Corinnomma sp.*	1	1	No data	0	0	0	0	1	No data	0.18
JMS109	*Iridonyssus kohouti*	0	0	1	1	0	0	0	1	0	0.18
Q84637	*Iridonyssus kohouti*	0	0	1	1	0	0	0	1	0	0.18
Q44095	*Nucastia supunnoides*	0	1	1	0	1	0	0	No data	0	0.18
MCZ142573	*Castianeira trilineata*	0	1	1	0	1	0	0	1	0	0.24
Q84597	*Poecilipta gloverae*	0	1	1	1	1	0	0	No data	1	0.29
MCZ28153	*Mazax spinosa*	1	1	1	1	1	0	0	1	0	0.35
MCZ79128	*Mazax pax*	No data	1	1	1	1	0	0	2	1	0.41
MCZ67875	*Myrmecotypus rettenmeyeri*	No data	1	1	1	1	1	1	1	2	0.53
MCZ16734	*Aetius nocturnus*	1	1	1	1	1	3	0	1	2	0.65
MCZ96085	*Serendib suthepica*	No data	1	1	1	0	3	2	1	3	0.71
MCZ28119	*Sphecotypus niger*	0	1	1	1	0	2	3	2	3	0.76

The more accurate mimic species within Castianeirinae evaluated by the MQAT continuous value method (Table 2) included S*phecotypus niger*, *Aetius nocturnus*, *Nucastia supunnoides*, *Poecilipta gloverae*, *Myrmecotypus rettenmeyeri*, and *Mazax spinosa*. Species such as *Castianeira trilineata*, *Castianeira cingulata*, *Serendib suthepica*, *Castianeira longipalpa*, *Nyssus luteofinis* and *Mazax pax* were measured as being intermediate between the more accurate species and the inaccurate species. The least accurate Castianeirinae were evaluated to be *Castianeira gertschi*, *Leptopicia bimaculata*, *Copa kabana*, *Leichhardteus conopalpis*, *Serendib volans*, *Iridonyssus kohouti*, *Battalus byrneae*,

Leichhardteus albofasciatus, *Corinnomma* sp., *Nyssus paradoxus*, *Iridonyssus formicans*, *Nyssus avidus*, *Nyssus semifuscus* and *Disnyssus helenmirrenae*.

Mimic assessment scoring using continuous values showed variation to the results of the binary assessment. Obvious differences when comparing the continuous assessment with the binary assessment include *Serendib suthepica*, *Mazax pax*, *Corinnomma* sp., *Nyssus semifuscus,* and *Iridonyssus kohouti* ranking higher in the binary assessment of mimic accuracy than in the continuous assessment. While *Castianeira longipalpa*, *Castianeira gertschi*, and *Nucastia supunnoides* ranked lower in the binary assessment of mimic accuracy relative to the continuous assessment.

Table 2. The results of the mimic quality assessment table (MQAT) scored using the continuous scoring method for the subfamily Castianeirinae and outgroup (n = 32). Specimens are ordered from lowest to highest level of mimic accuracy. The traits and their method of scoring are described in the methods section above. 'No data' indicates where there were no data available to measure the trait. *Indicates samples considered as outgroups or non-ant-mimic spiders.

Unique I.D.	Species	Thin legs	Elongated prosoma	Elongated opisthosoma	Elongated pedicel	Improved colouration	Degree of lateral prosomal constriction	Degree of dorsal prosomal constriction	Degree of lateral opisthosomal constriction	Degree of dorsal opisthosomal constriction	Mimic Accuracy
Q80326	*Disnyssus helenmirrenae*	No data	0.22	0.30	0	0	No data	0	No data	0	0.06
Q84600	*Nyssus semifuscus*	No data	No data	0.43	No data	0.33	0	0	0	0	0.08
Q00016	*Nyssus avidus*	0.66	0.26	0.20	0	0	No data	0	No data	0	0.12
Q84598	*Iridonyssus formicans*	0.70	0.23	0.20	0	0	0	0	0	0	0.12
SHS116	*Nyssus coloripes**	0.66	0.21	0.30	0	0	0	0	0	0	0.13
TBS504	*Nyssus coloripes**	0.65	0.22	0.31	0	0	0	0	0	0	0.13
Q84821	*Nyssus albopunctatus**	0.68	0.26	0.28	0	0	0	0	0	0	0.14
Q00017	*Nyssus paradoxus*	0.70	0.21	0.39	0	0	No data	0	No data	0	0.14
MCZ95991	*Corinnomma sp.*	0.76	0.52	No data	0	0	0	0	0.05	No data	0.15
Q87362	*Creugas gulosus**	0.78	0.24	0.31	0	0	No data	0	No data	0	0.15
Q00014	*Leichhardteus albofasciatus*	0.65	0.26	0.37	0.05	0	0	0	No data	0	0.15
Q84637	*Iridonyssus kohouti*	0.65	0.24	0.37	0.05	0	0	0	0.01	0	0.15
Q00002	*Battalus byrneae*	0.75	0.32	0.28	0	0	0	0	0	0	0.15
JMS109	*Iridonyssus kohouti*	0.67	0.24	0.38	0.05	0	0	0	0.01	0	0.15
MCZ34637	*Paradiestus giganteus**	0.75	0.18	0.44	0	0	0	0	0	0	0.15
MCZ44162	*Serendib volans*	0.70	0.35	0.24	0.11	0	0	0	0	0	0.16
Q95115	*Leichhardteus conopalpis*	0.63	0.30	0.50	0	0	No data	0	No data	0	0.16
Q00013	*Copa kabana*	0.71	0.26	0.46	0	0	0	0	No data	0	0.16
Q98191	*Leptopicia bimaculata*	0.72	0.28	0.43	0.07	0	No data	0	No data	0	0.17
MCZ137106	*Castianeira gertschi*	0.66	0.33	0.22	0	0.33	0	0	0	0	0.17
MCZ79128	*Mazax pax*	No data	0.44	0.48	0.10	0.33	0	0	0.13	0.10	0.18
Q50886	*Nyssus luteofinis*	0.64	0.26	0.40	0	0.33	0	0	0	0	0.18
MCZ126957	*Castianeira longipalpa*	0.61	0.38	0.42	0	0.33	0	0	0	0	0.19
MCZ96085	*Serendib suthepica*	No data	0.51	0.44	0.02	0	0.24	0.19	0.01	0.37	0.20
MCZ136939	*Castianeira cingulata*	0.70	0.40	0.43	0	0.33	0	0	0	0	0.21
MCZ142573	*Castianeira trilineata*	0.74	0.40	0.37	0	0.33	0	0	0.03	0	0.21
MCZ28153	*Mazax spinosa*	0.76	0.44	0.33	0.08	0.33	0	0	0.03	0	0.22
MCZ67875	*Myrmecotypus rettenmeyeri*	No data	0.65	0.36	0.03	0.67	0.08	0.08	0.01	0.19	0.23
Q84597	*Poecilipta gloverae*	0.70	0.47	0.50	0.03	0.33	0	0	No data	0.07	0.23
Q44095	*Nucastia supunnoides*	0.73	0.40	0.52	0	0.67	0	0	No data	0	0.26
MCZ16734	*Aetius nocturnus*	0.78	0.41	0.52	0.03	0.33	0.29	0	0.04	0.18	0.29
MCZ28119	*Sphecotypus niger*	0.70	0.70	0.58	0.07	0	0.17	0.35	0.15	0.35	0.34

The more accurate mimic species within Myrmarachninae evaluated by the MQAT binary method (Table 3) were *Myrmaplata plataleoides*, *Myrmarachne smaragdina*, *Myrmarachne macaulayi*, *Myrmarachne helensmithae*, and *Myrmapana parallela*. Species measured as being intermediate between the more accurate species and the inaccurate species included *Rhombonotus gracilis*, *Myrmarachne macleayana*, *Myrmarachne bicolor*, *Myrmapana centralis*, *Ligonipes semitectus*, *Myrmarachne erythrocephala*, *Ligonipes lacertosus*, and *Myrmarachne luctuosa*. Less accurate

mimic species of this subfamily were *Myrmapeni chickeringi*, *Myrmarachne formicaria*, *Judalana lutea*, and *Damoetas nitidus*.

Table 3. The mimic quality assessment table (MQAT) scored using the binary scoring method for the subfamily Myrmarachninae and outgroup (n = 32). Specimens are ordered from lowest to highest level of mimic accuracy. The traits and their method of scoring are described in the methods section above. 'No data' indicates where there were no data available to measure the trait. *Indicates samples considered as outgroups or non-ant-mimic spiders.

Unique I.D.	Species	Thin legs	Elongated prosoma	Elongated opisthosoma	Elongated pedicel	Improved colouration	Degree of lateral prosomal constriction	Degree of dorsal prosomal constriction	Degree of lateral opisthosomal constriction	Degree of dorsal opisthosomal constriction	Mimic Accuracy
MCZ126911	*Neon nellii**	No data	0	0	0	0	0	0	0	0	0
SHS108	*Opisthoncus quadratarius**	0	0	0	0	0	No data	0	No data	0	0
JMS102	*Apricia jovialis**	0	0	1	0	0	0	0	0	0	0.06
JMS103	*Astia hariola**	0	0	1	0	0	0	0	0	0	0.06
TBS101	*Opisthoncus sp.**	0	0	1	0	0	0	0	0	0	0.06
TBS402	*Abracadabrella elegans**	0	1	1	0	0	0	0	0	0	0.12
KWS603	*Helpis minitabunda**	1	1	1	0	0	0	0	0	0	0.18
JMS108	*Damoetas nitidus*	0	1	1	1	1	0	0	1	0	0.29
JCS304	*Judalana lutea*	1	1	1	1	0	1	0	0	0	0.29
JMS111	*Judalana lutea*	1	1	1	1	0	1	0	0	0	0.29
CRS101	*Myrmarachne formicaria*	1	1	1	1	1	1	0	1	0	0.41
MCZ125078	*Myrmapeni chickeringi*	No data	1	1	1	1	1	0	1	2	0.47
MUS401	*Myrmarachne luctuosa*	1	1	1	1	1	2	0	1	1	0.53
SRS102	*Myrmarachne luctuosa*	1	1	1	1	1	2	0	1	1	0.53
KWS605	*Ligonipes lacertosus*	1	1	1	1	1	1	0	3	1	0.59
SHS102	*Ligonipes lacertosus*	1	1	1	1	1	1	0	3	1	0.59
JMS115	*Myrmarachne erythrocephala*	0	1	1	1	1	2	1	1	2	0.59
JMS119	*Myrmarachne erythrocephala*	0	1	1	1	1	2	1	1	2	0.59
WWS101	*Ligonipes semitectus*	1	1	1	1	1	2	0	2	3	0.71
SHS103	*Ligonipes semitectus*	1	1	1	1	1	2	0	2	3	0.71
MCZ93522	*Myrmapana centralis*	1	1	1	1	1	1	2	2	2	0.71
JCS303	*Myrmarachne bicolor*	1	1	1	1	1	2	1	2	2	0.71
JMS112	*Myrmarachne bicolor*	1	1	1	1	1	2	1	2	2	0.71
JMS120	*Myrmarachne macleayana*	1	1	1	1	1	3	2	1	1	0.71
JMS122	*Myrmarachne macleayana*	1	1	1	1	1	3	2	1	1	0.71
JMS126	*Rhombonotus gracilis*	1	1	1	1	1	2	1	2	2	0.71
MCZ108610	*Myrmapana parallela*	1	1	1	1	1	2	2	1	3	0.76
TPS103	*Myrmarachne helensmithae*	1	1	1	1	1	2	3	1	2	0.76
TPS104	*Myrmarachne macaulayi*	1	1	1	1	1	3	3	2	3	0.94
TPS310	*Myrmarachne macaulayi*	1	1	1	1	1	3	3	2	3	0.94
TPS307	*Myrmarachne smaragdina*	1	1	1	1	1	3	3	3	2	0.94
MCZ128934	*Myrmaplata plataleoides*	1	1	1	1	1	3	3	3	3	1

The more accurate mimic species within Myrmarachninae evaluated by the MQAT continuous values method (Table 4) were *Myrmaplata plataleoides*, *Myrmarachne smaragdina*, *Myrmarachne macaulayi*, *Myrmarachne macleayana*, *Myrmarachne luctuosa*, *Myrmarachne helensmithae*, and *Myrmarachne bicolor*. Species measured as being intermediate between the more accurate species and the inaccurate species included *Rhombonotus gracilis*, *Myrmarachne erythrocephala*, *Ligonipes semitectus*, *Myrmapana parallela*, *Myrmapana centralis*, and *Ligonipes lacertosus*. Less accurate mimic species of this subfamily were *Myrmarachne formicaria*, *Damoetas nitidus*, *Myrmapeni chickeringi*, and *Judalana lutea*.

There were few differences between the binary and continuous assessments of the subfamily Myrmarachninae. These differences included *Myrmapana parallela* and *Myrmapana centralis* which ranked higher in the binary assessment than in the continuous assessment, while *Myrmarachne luctuosa* ranked lower in the binary assessment relative to the continuous assessment.

Table 4. The mimic quality assessment table (MQAT) scored using the continuous scoring method for the subfamily Myrmarachninae and outgroup (*n* = 32). Specimens are ordered from lowest to highest level of mimic accuracy. The traits and their method of scoring are described in the methods section above. 'No data' indicates where there were no data available to measure the trait. *Indicates samples considered as outgroups or non-ant-mimic spiders.

Unique I.D.	Species	Thin legs	Elongated prosoma	Elongated opisthosoma	Elongated pedicel	Improved colouration	Degree of lateral prosomal constriction	Degree of dorsal prosomal constriction	Degree of lateral opisthosomal constriction	Degree of dorsal opisthosomal constriction	Mimic Accuracy
MCZ126911	*Neon nellii**	No data	0.23	0.25	0	0	0	0	0	0	0.05
SHS108	*Opisthoncus quadratarius**	0.70	0.13	0.31	0	0	No data	0	No data	0	0.13
JMS103	*Astia hariola**	0.73	0.09	0.46	0	0	0	0	0	0	0.14
JMS102	*Apricia jovialis**	0.68	0.31	0.42	0	0	0	0	0	0	0.16
TBS101	*Opisthoncus sp.**	0.67	0.26	0.51	0	0	0	0	0	0	0.16
TBS402	*Abracadabrella elegans**	0.75	0.36	0.58	0	0	0	0	0	0	0.19
KWS603	*Helpis minitabunda**	0.80	0.35	0.58	0	0	0	0	0	0	0.19
JCS304	*Judalana lutea*	0.75	0.42	0.51	0.02	0	0.06	0	0	0	0.20
JMS111	*Judalana lutea*	0.77	0.43	0.52	0.02	0	0.04	0	0	0	0.20
MCZ125078	*Myrmapeni chickeringi*	No data	0.38	0.51	0.05	0.67	0.08	0	0.05	0.19	0.21
JMS108	*Damoetas nitidus*	0.73	0.43	0.53	0.04	0.33	0	0	0.03	0	0.23
CRS101	*Myrmarachne formicaria*	0.76	0.52	0.52	0.05	0.33	0.05	0	0.04	0	0.25
KWS605	*Ligonipes lacertosus*	0.77	0.51	0.51	0.06	0.33	0.02	0	0.31	0.08	0.29
SHS102	*Ligonipes lacertosus*	0.76	0.50	0.56	0.05	0.33	0.04	0	0.31	0.10	0.29
MCZ93522	*Myrmapana centralis*	0.77	0.47	0.46	0.02	0.33	0.10	0.16	0.14	0.18	0.29
MCZ108610	*Myrmapana parallela*	0.77	0.46	0.52	0.05	0.33	0.12	0.15	0.02	0.25	0.30
WWS101	*Ligonipes semitectus*	0.76	0.41	0.52	0.05	0.33	0.17	0	0.16	0.26	0.30
SHS103	*Ligonipes semitectus*	0.76	0.43	0.53	0.07	0.33	0.18	0	0.13	0.28	0.30
JMS115	*Myrmarachne erythrocephala*	0.71	0.50	0.41	0.05	0.67	0.11	0.07	0.05	0.18	0.31
JMS119	*Myrmarachne erythrocephala*	0.71	0.52	0.46	0.04	0.67	0.12	0.05	0.02	0.17	0.31
JMS126	*Rhombonotus gracilis*	0.75	0.38	0.42	0.04	0.67	0.16	0.04	0.14	0.17	0.31
JMS112	*Myrmarachne bicolor*	0.75	0.54	0.54	0.04	0.33	0.19	0.09	0.17	0.19	0.32
JCS303	*Myrmarachne bicolor*	0.77	0.57	0.53	0.06	0.33	0.16	0.06	0.20	0.18	0.32
TPS103	*Myrmarachne helensmithae*	0.76	0.47	0.64	0.05	0.33	0.19	0.23	0.09	0.11	0.32
MUS401	*Myrmarachne luctuosa*	0.81	0.51	0.59	0.04	0.67	0.16	0	0.09	0.09	0.33
SRS102	*Myrmarachne luctuosa*	0.84	0.52	0.59	0.05	0.67	0.15	0	0.09	0.08	0.33
JMS120	*Myrmarachne macleayana*	0.79	0.52	0.50	0.07	0.67	0.24	0.14	0.04	0.08	0.34
JMS122	*Myrmarachne macleayana*	0.76	0.51	0.52	0.08	0.67	0.23	0.14	0.04	0.10	0.34
TPS104	*Myrmarachne macaulayi*	0.81	0.52	0.59	0.06	0.67	0.23	0.32	0.11	0.30	0.40
TPS310	*Myrmarachne macaulayi*	0.83	0.43	0.62	0.07	0.67	0.26	0.34	0.12	0.31	0.40
TPS307	*Myrmarachne smaragdina*	0.84	0.55	0.58	0.09	0.67	0.34	0.21	0.25	0.16	0.41
MCZ128934	*Myrmaplata plataleoides*	0.85	0.53	0.51	0.13	0.33	0.48	0.31	0.32	0.46	0.44

To compare the level of consistency between the accuracy assessments of these two methods (i.e. binary and continuous), a linear regression was performed using R v3.5.1 (R Development Core Team 2018). The two scoring methods were not as strongly aligned for the Castianeirinae (Fig. 1; Table 5) as was seen with the Myrmarachninae (Fig. 2; Table 6) as indicated by the lower R^2-value.

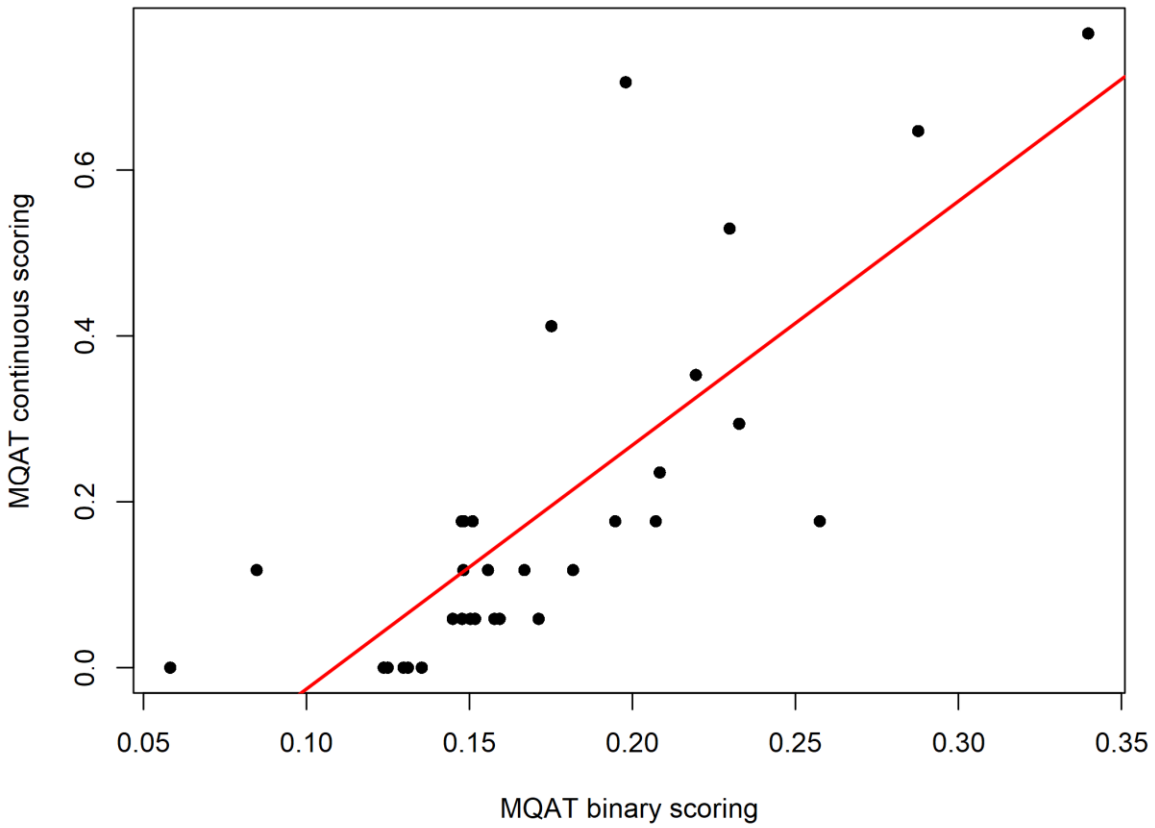

Figure 1. Linear regression comparing the two different mimic assessment methods (i.e. binary and continuous) for the subfamily Castianeirinae and outgroup (n = 32).

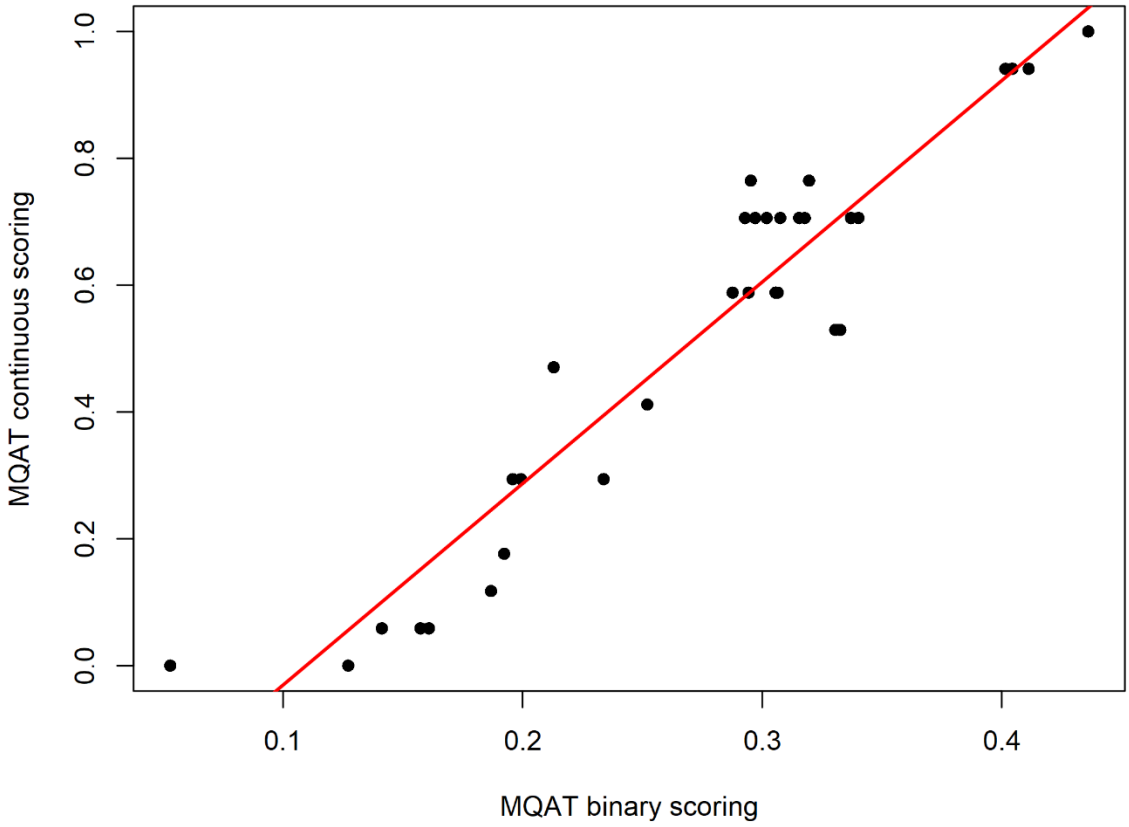

Figure 2. Linear regression comparing the two methods of mimic assessment (i.e. binary and continuous) for the subfamily Myrmarachninae and outgroup (n = 32).

To evaluate the effect of missing data, all species with missing data were removed from the regression analyses. This greatly improved the correlation for the Castianerinae, which contained the largest number of specimens with missing data (n = 15) (Table 5; Fig. S1). The removal of specimens with missing data had little effect on the analysis of the Myrmarachninae as there was only three specimens with missing data (Table 6; Fig. S2).

Table 5. Linear regression results for the comparison between the MQAT binary method of scoring with the MQAT continuous method of scoring in the Castianeirinae subfamily. Results are shown for the entire dataset, the dataset with specimens containing missing data excluded, and the dataset with the constriction traits graded as strictly absent/present, not graded in accordance to the degree of constriction.

Castianeirinae			
	Complete dataset	Exclusion of missing data	Constrictions not graded
Adjusted-R^2	0.597	0.917	0.544
p-value	<0.0001	<0.0001	<0.0001
F-statistic	$F_{1,30} = 47$	$F_{1,15} = 177.8$	$F_{1,30} = 38.02$

Table 6. Linear regression results for the comparison between the MQAT binary method of scoring with the MQAT continuous method of scoring in the Myrmarachninae subfamily. Results are shown for the entire dataset, the dataset with specimens containing missing data excluded, and the dataset with the constriction traits graded as strictly absent/present, not graded in accordance to the degree of constriction.

Myrmarachninae			
	Complete dataset	Exclusion of missing data	Constrictions not graded
Adjusted-R^2	0.901	0.907	0.751
p-value	<0.0001	<0.0001	<0.0001
F-statistic	$F_{1,30} = 283.8$	$F_{1,27} = 274.9$	$F_{1,30} = 94.43$

To evaluate the suitability of using the graded score method for evaluating constrictions rather than a strict absence/presence (binary) score an additional linear regression was performed. The application of the strict absence/presence scoring of the constrictions in the Castianeirinae changed the result only slightly relative to the graded scoring of constriction (Table 5; Fig. S3), as only a few of this group possessed constrictions (n = 11). A slightly larger improvement was seen in the Myrmarachninae (Table 6; Fig. S4), as more species had constrictions in this group (n = 25).

Comparison of MQAT with geometric morphometric analysis

The scoring of mimic accuracy using the MQAT (both binary and continuous scores) were compared to the values resulting from a linear discriminant analysis (a product of the geometric morphometric analysis) of ant-like similarity for both the dorsal and lateral shape profiles using species common to both studies (raw data: Table S2). The sample size for this comparison was smaller than the entire dataset of the MQAT (n = 11). Additional linear regressions were performed comparing both the geometric morphometric dorsal and ventral shape values with the MQAT mimic accuracy score (both binary and continuous methods) excluding traits from the MQAT scores that did not affect the dorsal (in the dorsal comparison) or ventral shape (in the lateral comparison), e.g. thin legs, improved colour, and lateral constrictions on the prosoma and opisthosoma.

Comparison of MQAT with dorsal shape values

Comparing the mimic accuracy assessment using the MQAT binary scores (using all traits) with the dorsal shape values was not strongly correlated, with the model only explaining 56% of the observed variation (Table 7; Fig. S5). Using the MQAT continuous scoring (using all traits) with the dorsal shape morphometric scores explained even less of the variation (Table 7; Fig. S6). Removing irrelevant traits (i.e. thin legs, improved colouration, and the lateral constrictions of the prosoma and opisthosoma) from the MQAT binary scoring or the continuous scoring did not improve the regression outcomes (Table 7; Fig. S7; Fig S8).

Comparison of MQAT with lateral shape values

The comparison of the lateral shape values with the MQAT binary scores, explained 70% of the variation (Table 7; Fig. S9) while the MQAT continuous scoring explained 66% of the variation (Table 7; Fig. S10). Removing the traits that do not affect lateral shape (i.e. thin legs, improved colouration, and the dorsal constrictions of the prosoma and opisthosoma) increased R^2 to 0.76 for the binary scoring (Table 7; Fig. S11) but did not noticeably affect the regression with the continuous scoring (Table 7; Fig. S12).

Table 7. Results of linear regression comparing the dorsal and lateral morphometric scores of the linear discriminant analysis with both the binary and continuous MQAT scoring using the Mimic accuracy score (including all traits) and the score excluding traits not affecting dorsal (in the dorsal comparison) and lateral shape (in the lateral comparison).

Dorsal Shape vs.	**Binary (All traits)**	**Binary (Dorsal traits)**	**Continuous (All traits)**	**Continuous (Dorsal traits)**
Adjusted-R^2	0.560	0.557	0.434	0.422
p	0.005	0.005	0.02	0.02
$F_{1,9}$	13.72	13.57	8.68	8.3
Lateral Shape vs.	**Binary (All traits)**	**Binary (Dorsal traits)**	**Continuous (All traits)**	**Continuous (Dorsal traits)**
Adjusted-R^2	0.695	0.756	0.661	0.552
p	0.0009	0.0003	0.001	0.005
$F_{1,9}$	23.82	31.95	20.51	13.33

Discussion

The aim of this chapter was to evaluate the most effective and efficient method of quantifying mimic accuracy using myrmecomorphic spiders. Historically, the assessment of mimic fidelity was assessed only via qualitative or subjective means that do not allow comparison across different studies investigating mimic accuracy (Pekár & Jarab 2011). Three methods were evaluated, two methods utilising biometrical measures (the MQAT binary and continuous methods) and a third, more computationally intensive method, geometric morphometrics. The MQAT (using both a binary and continuous mode of scoring) was modified from two prior studies, one in which the mimic accuracy was scored based upon the accumulation of specified traits (Pekár 2014b), the other was based on using biometrical measures to indicate the character state of the selected trait (Penney et al. 2012). As these methods have been used for different taxa (i.e. spiders and hoverflies) it was the intent of this study to evaluate these methods of mimic assessment within one taxonomic group (i.e. myrmecomorphic spiders) for a more direct comparative analysis.

The ranking of the Myrmarachninae mimic accuracy using the MQAT binary method of scoring correlated strongly with the MQAT continuous method of scoring mimic accuracy. The relationship was however not as strong with the Castianeirinae subfamily because of the large effect of missing data on the continuous scoring method. When specimens with missing data were excluded from the Castianeirinae analysis the result greatly improved showing that obtaining all data for each specimen

of a study is more crucial when using the continuous scoring method. The loss of data did not have as large an effect on the binary assessment. This suggests that when all data are available an assessment using either format (binary or continuous) will result in a similar mimic accuracy score.

The comparison of the MQAT assessments of mimic accuracy (using all the selected traits) and those of the geometric morphometric analysis did not show a strong correlation. This is potentially because the geometric morphometric analysis does not account for the additional traits included in the MQAT, such as thin legs, improved colouration, and the constriction values from the alternative view (i.e. dorsal vs. lateral). However, the removal of these traits did not consistently improve the results. This may be because the MQAT does not capture all the data for the entirety of the body shape as geometric morphometrics does (Zelditch, Swiderski & Sheets 2012) but instead captures specific elements of the shape that have been subjectively deemed important (Edmunds 2006; Durkee, Weiss & Uma 2011; Nelson 2012; Ceccarelli 2013; Candiani & Bonaldo 2017).

It seems that the use of geometric morphometrics is highly suitable if the extent of similarity a study hopes to identify is based solely on the shape of the body. This approach may be best suited to studies of mimics where mimic accuracy is mostly influenced by relatively fewer traits such as body or wing shape, e.g. butterflies (Jones et al. 2013). However, if the assessment of mimic accuracy involves taxa where the mimic accuracy is influenced by traits not involved with body shape, e.g. presence of patches of hair and leg size (Cloudsley-Thompson 1995; Deeleman-Reinhold 2001; Durkee, Weiss & Uma 2011; Pekár & Jarab 2011), then the MQAT is better suited to this task. The MQAT approach also provides the flexibility to further refine the assessment of mimic accuracy by adding additional traits. Such traits could include shifted forelegs (i.e. first two legs separated widely from the hind two legs), presence of an abdominal scutum, length of the fourth leg (i.e. is often lengthened in ant-mimicking spiders), and behaviour (i.e. the waving of the first legs to resemble the antennal movement of an ant and the bobbing up-and-down of the opisthosoma) (Deeleman-Reinhold 2001; Pekár 2014b). Furthermore, given that geometric morphometrics requires many computationally complex steps to arrive at a final accuracy score coupled with the fact that it only assesses the body shape and no other features that contribute to the accuracy of a mimic it is proposed that the MQAT is a simpler, faster method of quantifying the level of mimic accuracy. Another consideration is that geometric morphometrics is based on the distance in morphospace between the mimic's body shape and the body shape of an ant model with shorter distances from the ant model indicating a closer resemblance to an ant. The MQAT does not focus on the characteristics of the ant *per se* but rather the traits thought to contribute to an ant-like body in myrmecomorphic spiders. The assumption being

that the accuracy of a mimic is dependent on the spider possessing many of the targeted traits or having a more extreme modification to a particular trait.

For future studies, the MQAT could be further improved by ensuring that traits used in the assessment have a relatively equal weighting. For example, not all characters used in the MQAT contain the same predictive value and information content for assessing ant-likeness in myrmecomorphic spiders. The thin leg trait, which is also common in non-myrmecomorphic spiders (Pekár 2014b), scored in the range of 0.61 to 0.85 in the continuous method, while the elongated pedicel, proposed to be a major contributor to accurate mimicry (Pekár 2014b) ranged from 0.02 to 0.13. Thus, the potential values in the continuous scoring method, does not contribute an equivalent weight between different traits. It might be more useful to grade the elongated pedicel trait in the binary method, or multiply the traits value by a factor, and in the continuous method normalise the data for all traits.

Overall, the MQAT assessment method, both binary and continuous method, provide a useful measure of mimic accuracy. The assessment of mimic accuracy across the different species aligned well with the author's own objective view of accuracy. Furthermore, the results also concur with descriptions of the species from the literature. For example, the genus *Castianeira* has been noted as being an inaccurate myrmecomorph (Edmunds 2006) and were scored as such in the MQAT assessment. Species noted for possessing a higher level of mimic accuracy, including the genera *Sphecotypus* (Deeleman-Reinhold 2001; Candiani & Bonaldo 2017) and the *Myrmarachne* species *M. plataloides* and *M. smaragdina* (Nelson 2012; Benjamin 2015) were scored as highly accurate in the MQAT assessment. Both the binary and continuous methods of assessing mimic accuracy appear capable of reflecting the continuum of mimic accuracy from inaccurate to highly accurate in myrmecomorphic spiders with the advantage of assessing the level of accuracy not being dependent on the perceptual abilities of scientists and allows for a more consistent and comprehensive way of comparing accuracy across other myrmecomorphic studies.

In the following chapter I will map onto a phylogeny the scores for mimic accuracy, as well as, the individual traits used in the MQAT to observe the evolutionary relationships of these characteristics. This approach should reveal whether mimic accuracy and the associated traits do indeed evolve via a step-wise fashion of *perfecting* providing further insight into the occurrence of inaccurate mimicry and mimic evolution more broadly.

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Chapter 3: The evolution of myrmecomorphy in spiders – a molecular phylogenetic approach

Abstract

The convergent evolution of traits is best exemplified in the phenomenon of mimicry. However, the tempo and pace of this process is not well understood. While many mimics are strikingly accurate others bear only a slight resemblance to their model. One hypothesis, the *perfecting* hypothesis, proposes that inaccurate mimics are in a transitory phase toward better mimicry. Here we used myrmecomorphic (ant-mimicking) spiders that vary in their level of mimic accuracy to investigate this concept. Using a relatively novel method of gene subsampling, known as ultraconserved elements (UCEs) this study reconstructed a phylogenetic tree depicting the relationships of myrmecomorphic species within two subfamilies of spiders Castianeirinae (Corinnidae) and Myrmarachninae (Salticidae). Following this, the overall mimicry accuracy and the assessed traits, evaluated using a scoring method (Chapter 2), were mapped onto the phylogenies with the intention of observing the pattern in mimic accuracy and trait evolution. Results indicated that mimic accuracy and traits, such as the constriction of the body, evolves in a step-wise fashion. This supported the prediction of the *perfecting* hypothesis that accuracy, and the traits that contribute to accuracy, are under directional selection and that inaccurate mimics are in the process of developing more accurate mimicry.

Introduction

Mimicry, a paradigm of convergent evolution (Reed *et al*. 2011), is often noted as evidence of the power of natural selection in generating spectacular adaptations (Johnstone 2002; Kazemi *et al*. 2014). It involves the evolution of phenotypic traits in an organism (the *mimic*) that resemble those possessed by another unrelated organism (the *model*) (Edmunds 1974; Endler 1981; Kikuchi and Pfennig 2013). In visual Batesian mimicry a harmless and palatable mimic gains a selective benefit because a third organism (receiver), often a visually-guided predator, avoids the mimic due to misidentifying it with the well-defended, noxious or unpalatable model (Vane-Wright 1980; Gilbert 2004; Ruxton *et al*. 2004; Charlesworth and Charlesworth 2011). It is therefore expected that those mimics that evolve traits more closely resembling the traits of their model will gain the greater fitness benefit as the ability of predators to discriminate between the mimic and its' dangerous or unpalatable model is reduced. There is evidence that traits involved in mimicry are under strong selective pressures to better resemble those characteristics seen in their respective model (e.g. Ceccarelli 2013) suggesting that natural selection is constantly driving the ever-increasing perfection in mimic accuracy (Mappes and Alatalo 1997; Wickler 2013; Taylor *et al*. 2016). Supporting this idea are many

examples of mimics throughout the natural world that have evolved traits that resemble their model so effectively it is extremely difficult to differentiate them from their model. For example, the ant-mimicking spider *Sphecotypus niger* is a remarkable mimic of the ponerine ant, *Pachycondyla villosa* (Oliveira 1986, 1988; Cushing 1997; Leister and Miller 2014). However, the extent of mimics that have evolved only a vague similarity to their model is widespread, with some accounts suggesting that inaccurate mimicry may be the rule rather than the exception (Mokkonen and Lindstedt 2016). Thus, inaccurate mimicry may be an important phenomenon from which we can gain better insight into mimicry evolution and potential limits to natural selection (Kikuchi and Pfennig 2013).

A prime example of Batesian mimicry can be found in ant-mimicking (myrmecomorphic) spiders (Cushing 1997, 2012). Myrmecomorphy is very common in spiders, being the most common form of mimicry in this order (Araneae) (Reiskind 1971; Cushing 1997; Pekár and Jarab 2011a). In spiders alone, both accurate and inaccurate myrmecomorphy has evolved independently in 16 families (Pekár 2014). The evolution of accurate myrmecomorphy in spiders involves radical changes to the spider body plan. Myrmecomorphic traits such as the elongation of the body (Durkee *et al*. 2011; Nelson 2012; Ceccarelli 2013; Candiani and Bonaldo 2017), extension of the pedicel (Cloudsley-Thompson 1995; Edmunds 2006; Durkee *et al*. 2011; Ceccarelli 2013), constrictions to the body (Edmunds 2006; Deeleman-Reinhold 2001; Pekár 2014; Candiani and Bonaldo 2017), colour changes (Cloudsley-Thompson 1995; Durkee *et al*. 2011; Pekár 2014), patches of hair (or setae) (Edmunds 2006) and the thinning of the legs (Cloudsley-Thompson 1995; Deeleman-Reinhold 2001; Durkee *et al*. 2011; Pekár 2014) are integrated to produce a convincing ant-like illusion. However, in inaccurate mimics only a portion of these traits are present (Edmunds 2006; Pekár and Jarab 2011a).

Many hypotheses have been suggested to explain the occurrence of inaccurate mimics (see Chapter 1 for review of these hypotheses) (Kikuchi and Pfennig 2013). One prediction proposes that inaccurate mimics have evolved only the traits that are relevant to the predators' perception (i.e. salient traits or because of limitations to the predators' perception and discriminative capabilities) and the evolution of any further traits would provide no selective benefit to the mimic (Kikuchi and Pfennig 2010; Jackson and Nelson 2012; Cuthill 2014; Kazemi *et al*. 2014; Taylor *et al*. 2016; Jamie 2017; Sherratt and Peet-Paré 2017). While these hypotheses assume that inaccurate mimics are at an evolutionary stable state, it is however conceivable that the evolution of accurate mimicry may involve a process of incremental trait evolution, requiring many mutational or recombination steps. Inaccurate mimics may therefore be viewed as species undergoing more, or less, constant directional selection slowly improving their resemblance to their model (termed the *perfecting* hypothesis) (Edmunds 2000; Edmunds 2006). Due to the phylogenetic distance between spiders and ants the progression of evolving derived ant-like traits in a spider, which can only occur on the basis of

precursor structures, is likely to limit the appearance of new ant-like traits. As a consequence of this phylogenetic burden there are likely constraints on evolving from the basic spider body plan state to one resembling an ant, making more evolutionary steps necessary.

Testing the perfecting hypothesis requires molecular tools to follow the evolution of deceptive traits along a reconstructed phylogeny (Mokkonen and Lindstedt 2016). Phylogenetic studies investigating myrmecomorphy are however limited in number. The results of a generic- and family-level phylogenetic study of myrmecomorphic spiders conducted by Pekár (2014) indicated that on the family-level phylogeny the presence of myrmecomorphy are more recent evolutionary events and is confined mostly to derived cursorial families. The phylogeny of two spider families (Corinnidae and Salticidae) used in this study revealed that the ancestral state for Corrinidae was inaccurate myrmecomorphy while in Salticidae the ancestral state was non-mimetic (Pekár 2014). While these results support the prediction that the evolution of myrmecomorphy has transitioned from inaccurate to accurate, some estimates indicated a potential reversal in evolutionary direction, from accurate to inaccurate. Pekár (2014) concluded that while these findings support the notion that inaccurate myrmecomorphy in spiders is ancestral to accurate the author suggests a species-level phylogeny is required to substantiate this conclusion. In another molecular phylogenetic study, using the myrmecomorphic spider genus *Myrmarachne* (Salticidae), Ceccarelli and Crozier (2007) demonstrated that rather than co-speciating with their ant models *Myrmarachne* spp. have converged towards various sympatric ant species and concluded that this genus of spiders is under strong selective pressure to radiate and evolve closer phenotypic resemblance to their ant models.

To investigate the idea that inaccurate myrmecomorphs are at an intermediate phase in their eventual evolution toward accurate mimicry a phylogenetic study incorporating morphological data of traits known to contribute to ant-likeness in spiders, as well as an assessment of the level of mimic accuracy, is required. In comparative evolutionary biology the incorporation of morphological traits with phylogenies can provide inferences about how animals evolve, adapt to their environment, and how body-plan evolution result in convergence (Revell 2013; Giribet 2015). Analysing genomic data in conjunction with morphological data (i.e. phylogenetic comparative approaches) provides the most promising assessment of the evolution of a given trait (Giribet 2015) and over the last 25 years this approach has become central to studies in evolutionary biology (Freckleton *et al*. 2002; Losos 2011; Revell 2013).

An important decision in the age of genome-scale DNA sequencing is the choice of molecular marker for a phylogenetic study (Collins and Hrbek 2015). Spider phylogenetics has traditionally been performed using a selection of up to 15 markers (including those markers that have been humorously

termed the "usual suspects": COI, 12S, 16S, 18S, 28S, H3) (Dimitrov *et al*. 2017). However, very few of these markers have proven effective at the species level and often produce contradictory results (Hamilton *et al*. 2016). Advances in sequencing technologies (e.g. next-generation sequencing) and molecular methods have resulted in rapid and massive sequencing increasing the scale and scope of many research questions (Grover *et al*. 2012). As whole genome sequencing is unnecessarily complex and often unwarranted, ecologists and evolutionary biologists typically focus on a narrower subset of the genome. These *reduced representation* methods include a mix of transcriptomic, restriction enzyme-based and targeted enrichment approaches. These methods are less expensive than whole genome sequencing and facilitate the collection of large numbers of loci from large numbers of specimens (Faircloth 2017). One method of *reduced representation* that has grown rapidly in popularity is the targeted enrichment of conserved or ultraconserved genomic elements (*sensu* Faircloth *et al*. 2012). Synthetic oligonucleotide 'baits', that are complementary to identified highly conserved genomic regions, are designed. The genomic library is then hybridised to the oligonucleotide baits and subsequently the hybridised bait and the library structure is drawn out. The bait sequences are then removed and the remaining pool of enriched, targeted DNA is sequenced using massively paralleled sequencing (Faircloth 2017). Ultraconserved elements (UCEs) can also be utilised as anchors to retrieve the DNA sequences that flank the core UCE region. While the highly conserved UCE core region, thought to be regulatory genes and/or enhance gene expression, are informative at deep evolutionary time scales the flanking regions show increased genetic variability (or phylogenetic informativeness) as the distance from the core region increases (Faircloth *et al*. 2012). These flanking regions are suited to shallow evolutionary time scales and are useful for species delimitation and species-level phylogenetics providing biologically relevant information equal to or exceeding that of the protein-coding markers that are traditionally used (Blaimer *et al*. 2015; Gilbert *et al*. 2015).

There are several other reasons that make UCEs ideal markers for molecular systematics. The UCE protocol, unlike transcriptomes, requires only DNA and can be performed using relatively low starting concentrations of DNA allowing the method to be extended to small-bodied taxa and even "standard" museum specimens (i.e. with no special preservation of DNA material) with various levels of DNA degradation (e.g. McCormack *et al*. 2015; Blaimer *et al*. 2016; Ruane and Austin 2017; Hedin *et al*. 2018a,b). Another attractive feature of this method is the fact that the targeted regions are highly conserved and shared among distantly related taxa meaning that once a bait set is designed it is capable of capturing unpreceded amounts of genomic data from non-model taxa, overcoming the need for prior genomic information (Faircloth *et al*. 2012; Bossert and Danforth 2018). This allows

for the generation of genome-scale data for non-model organisms in a cost-and-time-effective manner (Glenn and Faircloth 2016).

The first protocol and probeset for UCEs was designed by Faircloth *et al*. (2012) for amniotes but has since been successfully applied in a variety of organisms including mammals (McCormack *et al*. 2012), birds (McCormack *et al*. 2013; Smith *et al*. 2014), reptiles (Crawford *et al*. 2012; Crawford *et al*. 2015; Ruane and Austin 2017), fish (Gilbert *et al*. 2015; Chakrabarty *et al*. 2017), and arthropod taxa such as Hemiptera, Diptera, Coleoptera, Lepidoptera, and Hymenoptera (Blaimer *et al*. 2015, 2016; Faircloth *et al*. 2015; Faircloth 2017; Branstetter *et al*. 2016a,b, 2017a,b). More recently a probeset has been designed to target sequences from any species in the arachnid tree of life (Starrett *et al*. 2017). The arachnid-specific UCEs have been successfully utilised in phylogenetic studies of arachnids showing utility and phylogenetic informativeness for ancient divergences between orders (>400 MYA) to more congeneric divergences (<10 MYA) (Starrett *et al*. 2017, Hedin *et al*. 2018a,b; Derkarabetian *et al*. 2018). This thesis will extend the use of UCEs in arachnids by implementing this method to obtain gene sequence data and build a phylogeny for myrmecomorphic spiders (Araneae).

The use of phylogenies has assumed a central role in evolutionary biology over the recent decades (Losos 2011; Revell 2012). One important component when studying the evolutionary history of traits is to reconstruct past phenotypes possessed by ancestral species based on the traits present in their extant descendants (Revell 2013). By mapping the trait values (established in Chapter 2) into the phylogeny, ancestral state estimations can be established allowing for inferences to be formed on the patterns of trait evolution and mimic accuracy in myrmecomorphic spiders. If the *perfecting* hypothesis is relevant, and accurate mimicry evolves via a gradual, step-wise process, then it is expected that we would observe moderately accurate species diverging from inaccurate ancestors and accurate species diverging from a moderately accurate ancestor. A highly accurate ancestor diverging from an inaccurate ancestor may suggest that gradual steps are not necessary to evolve accurate mimicry and that the *perfecting* hypothesis is not relevant. Similarly, the mapping of certain traits may also indicate this pattern of step-wise evolution. Ultimately, this approach will provide further insight into the patterns of trait evolution in myrmecomorphic spiders and whether the *perfecting* hypothesis can explain the widespread occurrence of inaccurate mimics.

Methods

Specimen collection

Specimens used in the phylogenetic analysis include those utilised in the mimic quality assessment table (MQAT) in Chapter 2 (Table S1). Outgroups for Castianeirinae consisted of representatives of the subfamily Corinninae and Phrurolithinae (Wheeler *et al.* 2016). Outgroups for Myrmarachninae included members of the subfamily Sitticinae (Maddison 2015).

Molecular data collection

Extraction of genomic DNA was performed using two different approaches. DNA from Australian specimens (n = 39), including those collected in the field or obtained from the Queensland Museum and prior studies, were extracted using either leg/s or whole individuals utilising the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocol.

An alternative extraction protocol, adapted from Tin *et al*. (2014), was adopted for all specimens acquired from the Harvard University Museum of Comparative Zoology (n = 12) to ensure a viable quantity of DNA was extracted from these preserved specimens (stored in 70-95% ethanol). Prior to extractions, samples were washed in Molecular Biology Grade Water (Mediatech, Inc.) to remove any ethanol from the storage process, dried, and then placed into tubes with 200 µl of extraction buffer. Samples were then incubated in a water bath at 58 ^{o}C for 24 hrs for tissue lysis to occur. Following tissue lysis, bead purification was performed to separate the beads attached to the genomic and mitochondrial DNA from proteins and other contaminants.

Quantification of extracted DNA was performed using both high sensitivity and broad range assays on a Qubit fluorometer (Life Technologies, Inc.). Following this, gel electrophoresis was conducted on a 1% agarose gel to view DNA distribution and assess the quality to determine the appropriate amount of sonication time.

Sonication

Sonication of 500 ng of DNA, in a sample volume of 130 µl of AE buffer, was performed to fragment samples collected in the field and/or stored in ≥ 95% ethanol using a Covaris S220 sonicator for 80 s with a Peak Incidence Power of 105.0, Duty Factor of 5%, and 200 cycles per burst. From the resulting fragmented DNA, 4 µl was run out on a 1.2% agarose gel to confirm sonication success and view the distribution of DNA fragments. The target size range for the sonicated DNA fragments was between 300-500 bp, but not exceeding 1000 bp. The remaining samples, where potential partial DNA degradation was expected (i.e. preserved specimens stored in < 95% ethanol and museum specimens) were not sonicated.

Library Preparation (End repair/A-tailing, Adapter Ligation & Library Amplification)

Library preparation included some modification to the protocol of Starrett *et al*. (2017) and the UCE website (ultraconserved.org). The KAPA Hyper Prep Kit (Kapa Biosystems) was utilised for preparation of libraries using up to 250 ng DNA (i.e., half reaction of manufacturer's protocol) as starting material. Serapure SPRI beads (Rohland and Reich 2012; Glenn *et al*. 2016) were used in all clean-up steps. For those samples with a total concentration < 250 ng DNA, all DNA was used in library preparation.

Following end-repair and A-tailing, libraries were ligated to universal adapters using varying concentrations depending on the volume of input DNA. Samples with low input DNA and/or from specimens stored in < 95% ethanol were ligated using 5 μM universal stubby Y-yoke adapters, while high input specimens were ligated using 10 μM universal stubby Y-yoke adapters (baddna.uga.org). Another bead clean-up step was performed following adapter ligation. The concentration of samples was again measured using a Qubit fluorometer. The adapter-ligated library was then amplified on a Mastercycler EP gradient thermocycler (Eppendorf, Hamburg, Germany) in a 50-μL total reaction volume consisting of 15 μL of adapter-ligated DNA, 5 μM of each of the Illumina TruSeq dual-indexed primers (i5 and i7) with dual-indexed 8-bp indexes (Glenn *et al*. 2016), and 2x KAPA HiFi HotStart ReadyMix. Amplification conditions involved an initial denaturation step at 98 ℃ for 45 s, 16 cycles of denaturation at 98 ℃ for 15 s, primer annealing at 60 ℃ for 30 s, extension at 72 ℃ for 60 s and then a final extension of 72 ℃ for 60 s. Bead clean-up was again performed post-amplification and quantified using a Qubit fluorometer. Each column was pooled in 1000 ng total pools by combining equimolar amounts of the amplified libraries consisting of eight samples each, i.e. 125 ng per sample.

Target Enrichment of Illumina Libraries/Hybridisation

Pooled libraries then underwent target enrichment using the myBaits custom kit (Arbor Biosciences, Ann Arbor, MI) following the protocol detailed in the Hybridization Capture for Targeted NGS manual v 4.01 (www.arborbiosci.com/mybaits-manual) using the arachnid bait set, Arachnida 1.1K version 1 kit (Arbor Biosciences, Ann Arbor, MI; Faircloth 2017). Hybridisation was performed at 60 ^{o}C for 24 hours to allow baits to encounter and hybridise with the targeted library molecules. Libraries, consisting of the bait-target hybrids, were then bound to streptavidin-coated magnetic beads (Dynabeads MyOne C1, Invitrogen) and washed with warm buffer, following the Arbor Biosciences protocol, to remove non-target DNA.

Post-hybridisation Amplification using Illumina Libraries

Post-hybridisation, the pooled samples were amplified in a 50 µl reaction consisting of 15 µl of hybridized pools, 5 µl dH_20, 5 µM of each of the Illumina P5/P7 primers, and 2X Kapa HiFi HotStart ReadyMix. Amplification involved a thermal profile comprising an initial denaturation step at 98 °C for 45 s, followed by 16 cycles of denaturation at 98 °C for 15 s, primer annealing at 60 °C for 30 s, extension at 72 °C for 60 s, then a final extension of 72 °C for 5 minutes. An additional clean-up was then conducted, and libraries were again quantified using a Qubit fluorometer. Equimolar mixes were prepared for sequencing using an Illumina HiSeq 2500 (Bauer Core Facility at Harvard University) using 125 bp PE reads.

Read processing, contig assembly and matrix creation

Processing of raw demultiplexed read data was performed using the PHYLUCE pipeline (Faircloth 2015). The Illumiprocessor wrapper (Faircloth 2013), using default settings, was implemented to remove adapters and for quality control trimming. Read assemblies were created with Velvet 1.21 (Zerbino and Birney 2008) at default settings. Probes were matched to contigs from all samples using minimum coverage and minimum identity values of 65. The UCE loci was then aligned using MAAFT (Katoh and Standley 2013) and trimmed using GBLOCKS (Castresana 2000; Talavera and Castresana 2007) with custom blocks settings (b1 = 0.5, b2 = 0.5, b3 = 6, b4 = 6) applied in the PHYLUCE pipeline. Individual UCE alignments were then imported into Geneious 11.1.5 (http://www. geneious.com, Kearse *et al*. 2012) and manually inspected to remove any potential non-homologous sequences and obvious alignment errors. Two data sets were made, one containing the Myrmarachninae subfamily (including outgroup), the other containing the Castianeirinae subfamily (including outgroup). For each of the datasets a matrix was created with 50% taxon coverage.

Phylogenetic analysis

UCE alignments were imported into Geneious 11.1.5 (http://www. geneious.com, Kearse *et al*. 2012) for maximum likelihood analysis. Maximum likelihood trees were estimated using RAxML v8.2.11 (Stamatakis 2014), implementing the rapid bootstrap algorithm (Stamatakis *et al*. 2008), 200 bootstrap replicates and the GTRGAMMA model. Maximum likelihood analyses were conducted on the 50% taxon coverage data sets. The two phylogenies (one for each subfamily: Myrmarachninae and Castianeirinae) were then exported as newick files for the mapping of traits and mimic accuracy using the R platform.

Mapping of mimic accuracy and myrmecomorphic traits

To reconstruct the evolutionary history of mimic accuracy and trait evolution in these myrmecomorphic spiders the scores for mimic accuracy, as well as the scores for the traits utilised in the MQAT, were mapped onto the phylogenies. Mimic accuracy and traits evaluated in the MQAT were mapped in R (R Development Core Team 2018) using the packages 'ape' (Analysis of Phylogenetics and Evolution) (Paradis *et al*. 2004), 'phytools' (Revell 2012), and 'geiger' (Harmon *et al*. 2008). To analyse the mimic accuracy data, 'geiger' was used to calculate the likelihood fit of a constant Brownian motion to alternatives models (i.e. Ornstein-Uhlenbeck and Early Burst models) in an Akaike's Information Criterion (AIC) framework (Harmon *et al*. 2008). The likelihood fit for both the discrete (binary) trait data and the continuous trait data were calculated using various models for trait evolution, i.e. equal rates, all rates different, symmetrical, and self-written models OrderedBF (OrdBF) and Ordered FW (OrdFW), with the best fit model utilised to stochastically map the trait data onto a single phylogeny for visualisation using the sim.map in the 'phytools' package (Revell 2012). The stochastic mapping of traits is a technique that allows sampling trait histories in proportion to their probability (Revell 2013). Models were compared by applying AIC. Due to the small sample size the corrected AIC (AICc) was utilised (Anderson *et al*. 2000). The best fit model was determined by ranking the selected models according to their Akaike weights (AICcw) (Anderson *et al*. 2000). The AICcw represent the probability that a particular model, relative to a set of models, is best suited to the given data, with the highest AICcw indicating the best fit model for the dataset (Burnham and Anderson 2002).

Results

During the process of quality control, it became apparent that numerous samples had become contaminated as they did not fit into the phylogeny consistent with prior studies (e.g. Edwards and Benjamin 2009; Wheeler *et al*. 2016; Pekár *et al*. 2017). This is likely due to this author's inexperience with the UCE method and potential error in following protocol for these samples. Consequently, 11 samples (all Castianeirinae) have been excluded from further analyses. Due to the contamination of many Castianeirinae samples, particularly numerous species assessed by the MQAT to be at the higher end of the accuracy spectrum, significant information has been lost for any comparative analyses. Thus, only the mimic accuracy analysis of Castianeirinae will be presented with the results mainly focusing on the analyses of the Myrmarachninae.

Sequencing results (UCEs)

Sequencing results per sample, including the number of UCE loci in the final matrix, number of post-QC reads, and number of contigs for both the Castianeirinae and Myrmarachninae are presented in Tables S3 and S4 respectively. Matrix statistics are presented in Table 1. The number of recovered UCE loci varied between 32 and 644 (average = 416.7). On average there were 1 548 510 raw reads (post-QC) per sample. Assemblies produced an average of 306 457 contigs per sample.

Table 1. Matrix statistics including the number of loci, total length of sequence, mean length per loci, and the minimum and maximum lengths of loci. PI, parsimony-informative sites. Values include the outgroups.

		50% taxon coverage					
	N	Loci	Length	Mean length	Min-Max length	PI	% PI
Castianeirinae	21	79	12 317	155.91	125-644	1 790	14.5
Myrmarachninae	32	698	145 752	208.81	32-574	28 314	19.4
Mean		**388.5**	**79 035**	**182.36**		**15 947**	16.95

Phylogeny

The RAxML trees, with bootstrap values, for the subfamilies Castianeirinae and Myrmarachninae are presented in Figures S13 and S14, respectively. The Castianeirinae phylogeny is congruent with that established by Wheeler *et al*. (2016) in their extensive work on Araneae phylogenetics. The closer relationships within the phylogeny of this study between the genera *Castianeira* and *Serendib* and the genera *Nyssus* and *Corinnomma* were consistent with those depicted by Wheeler *et al*. (2016). The phylogeny of the Myrmarachninae has not been evaluated using molecular methods but Edwards and Benjamin (2009) conducted a phylogenetic analysis of this subfamily using morphological characters. The molecular phylogeny built in this study recovered topologies that are mostly congruent with the morphological phylogeny, with one exception. The strict consensus parsimonious analysis of Myrmarachninae using morphological data places *Myrmarachne formicaria* in a clade containing *Myrmapana parallela* and more distantly related to *Myrmaplata plataleoides*. However, the molecular phylogeny created here places *Myrmarachne formicaria* more closely related to *Myrmaplata plataleoides* than to *Myrmapana parallela*. The phylogenetic relationships between numerous Australian members of the genus *Myrmarachne* have been established (Pekár *et al*. 2017). The close phylogenetic relationships shared by *Myrmarachne macleayana* and *Myrmarachne bicolor*, those shared by *Myrmarachne smaragdina*, *Myrmarachne helensmithae*, and *Myrmarachne macaulayi*, as well as those between *Myrmarachne luctuosa* and *Myrmarachne erythrocephala* reported by Pekár *et al*. (2017a) are supported by this current study.

Mapping mimic accuracy

The likelihood fit of both the Castianeirinae and Myrmarachninae phylogenetic data with the mimic accuracy scores was analysed using Brownian motion ('random walk'), Ornstein-Uhlenbeck (constrained evolution) and Early Burst (early adaptive radiation) models (Table 2).

Table 2. Corrected Akaike information criterion weight (AICcw) values indicating the relative likelihood fit of the mimic accuracy data using Brownian motion (BM), Ornstein-Uhlenbeck (OU) and Early Burst (EB) models in the subfamilies Castianeirinae and Myrmarachninae. The favoured model (highest value) is in bold.

	BM model	OU model	EB model
Castaineirinae Mimic Accuracy	**0.6278481**	0.2130175	0.1591344
Myrmarachninae Mimic Accuracy	**0.582255**	0.2461335	0.1716116

Figure 1 shows the accuracy mapping for Castianeirinae using maximum likelihood, assuming Brownian motion within the ace function (Schluter *et al*. 1997). The outgroup for the Castianeirinae

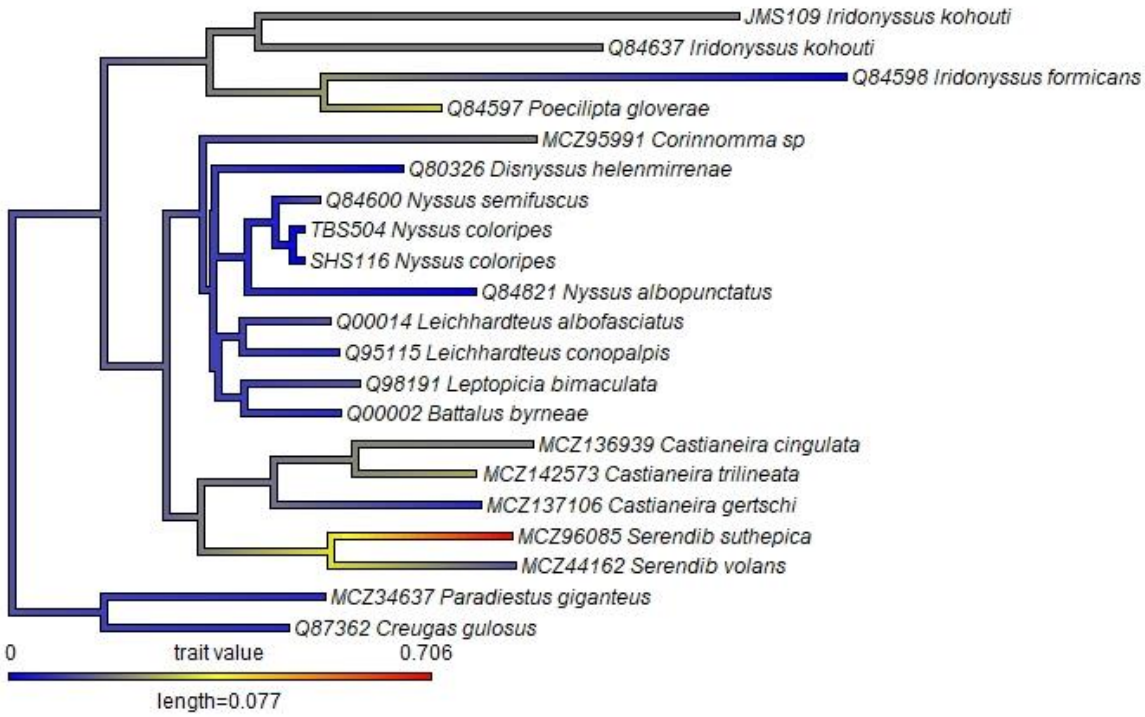

Figure 1. Mimic accuracy mapped onto the reconstructed phylogeny for the Castianeirinae subfamily. Heat map indicates the level of mimic accuracy from inaccurate mimic (blue) through intermediate (yellow) to highly accurate (red). The horizontal bar functions simultaneously as a legend and a scale. The mapped colours translate the heat colours (blue through red) into the posterior probability (computed as the relative frequency across stochastic maps) of the level of accuracy being in the condition of highly accurate. The length of the bar also provides a scale for the branch lengths of the tree (i.e. nucleotide substitutions per site) (Revell 2013). Bootstrap values are displayed in the RAxML tree in Fig. S13.

are placed at the base of the phylogeny. The lower clade containing the genera *Castianeira* and *Serendib* consist of mimics that range in accuracy from poor to moderate, with only one highly accurate species present, *Serendib suthepica*. The species in the remaining phylogeny range from inaccurate to moderately accurate.

The Brownian motion model was also the best fit model for the Myrmarachninae phylogenetic data, shown in Figure 2. The outgroup consisted of the seven species at the base of the phylogeny. The lower clade consisting of the genera *Judalana, Rhombonotus, Ligonipes,* and *Damoetas* were made of species that were inaccurate to moderate-high accurate mimics. The uppermost clade consisting of the *Myrmapeni* and *Myrmapana* genera were moderate to moderate-high in their level of mimic accuracy. The clade below the uppermost clade, ranging from *Myrmarachne formicaria* down to *Myrmarachne macleayana*, consisted of mimics that have evolved a moderate to high level of mimic accuracy. The remaining clade with *Myrmarachne erythrocephala* and *Myrmarachne luctuosa* were considered moderate mimics.

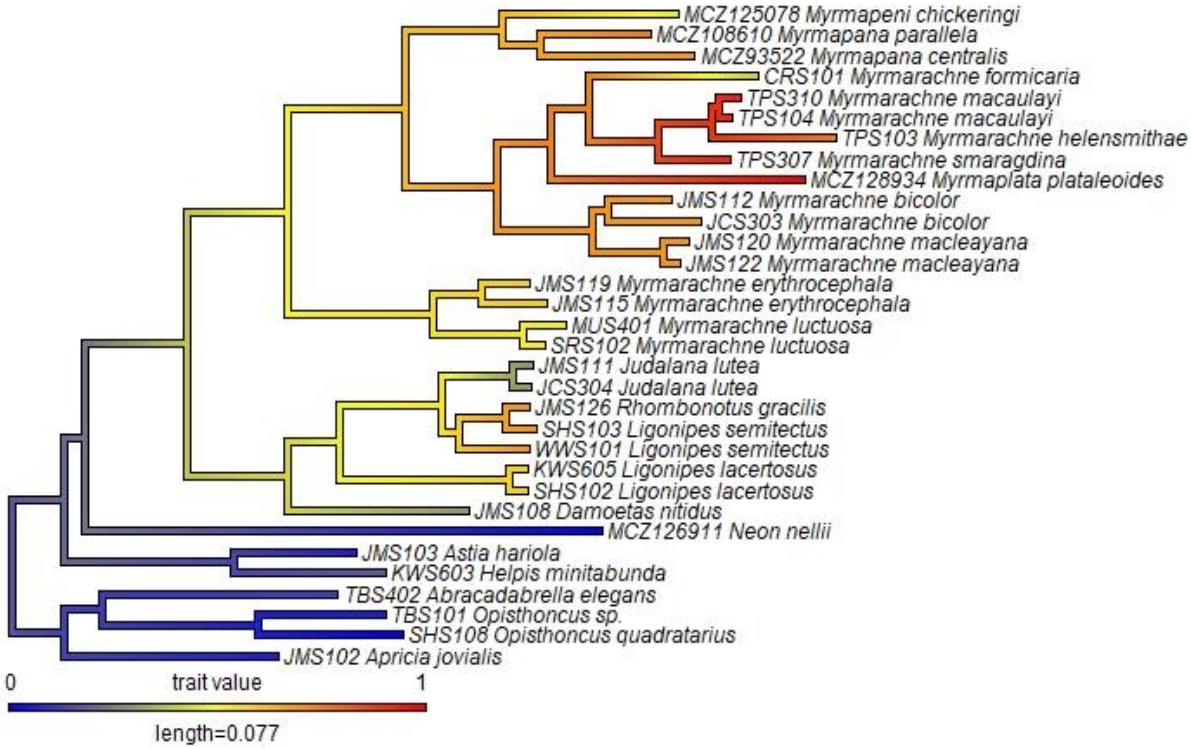

Figure 2. Mimic accuracy mapped onto the Myrmarachninae phylogeny. Heat map indicates the level of mimic accuracy from inaccurate mimic (blue) through intermediate (yellow) to highly accurate (red). The horizontal bar functions simultaneously as a legend and a scale. The mapped colours translate the heat colours (blue through red) into the posterior probability (computed as the relative frequency across stochastic maps) of the level of accuracy being in the condition of highly accurate. The length of the bar also provides a scale for the branch lengths of the tree (i.e. nucleotide substitutions per site) (Revell 2013). Bootstrap values are displayed in the RAxML tree in Fig. S14.

The mapping of mimic accuracy in the Myrmarachninae reveals that the moderately accurate species have diverged from inaccurate ancestors and accurate species have diverged from a moderately accurate ancestor indicating that mimic improvement follows a gradual step-wise trajectory.

Trait mapping

The evaluation of trait evolution in Castianeirinae is inconclusive given the loss of numerous samples of higher accuracy specimens due to contamination during processing. Consequently, the phylogeny does not contain the range of species (or traits) needed to infer the evolution of the traits used by the MQAT. Therefore, no trait analysis was performed for the Castianeirinae

Each trait type for the Myrmarachninae was assessed to determine which model/s fit the data best including: Equal rates (ER); All Rates Different (ARD); Symmetric Rates (SYM); Ordered states model - allowing back and forth transitions (OrdBF); and Ordered states model - allowing only forward transitions (OrdFW). The corrected Akaike information criterion weight (AICcw) results for likelihood fit for the Myrmarachninae subfamily is presented in Table 3.

The Myrmarachninae analyses indicate that some of the traits were ancestral traits having evolved in a common ancestor to all the species in the phylogeny. These traits included elongated prosoma (Fig. S15), elongated opisthosoma (Fig. S16), elongated pedicel (Fig. S17) and improved colouration (Fig. S18). As these traits were not scored in a graded way, as were the constrictions, they are less informative when investigating whether the traits involved with myrmecomorphy evolve via a gradual process.

The lateral prosomal constriction using the OrdBF model is presented in Figure 3. This trait was absent in the outgroup with the ancestral state estimation indicating the common ancestor to all the myrmecomorphic species had evolved the slight lateral prosomal constriction trait. The lower clade of myrmecomorphs consisted of species that had a slight constriction, except for *Ligonipes semitectus* and *Rhombonotus gracilis* which had the moderate constriction, and the loss of the lateral prosomal constriction trait in *Damoetas nitidus*. The uppermost clade consisted of the genera *Myrmapeni* and *Myrmapana* that had the slight lateral prosomal constriction with a moderate constriction in *Myrmapana parallela*. The middle clade evolved from a common ancestor that is estimated to have had the moderate lateral prosomal constriction. This clade consisted of species with the lateral prosomal constriction ranging from moderate to extreme.

Table 3. Corrected Akaike information criterion weight (AICcw) for the likelihood fit of the Myrmarachninae phylogenetic data with the selected models: Equal rates (ER); All Rates Different (ARD); Symmetric Rates (SYM); Ordered states model - allowing back and forth transitions (OrdBF); and Ordered states model - allowing only forward transitions (OrdFW). The ER and ARD models were used when there were only two trait states in the data.

Trait	ER model	SYM model	ARD model	OrdBF model	OrdFW model	Trait states
Thin Legs	0.494	N/A	0.506	N/A	N/A	2
Elongated prosoma	0.112	N/A	0.888	N/A	N/A	2
Elongated opisthosoma	0.754	N/A	0.246	N/A	N/A	2
Elongated pedicel	0.534	N/A	0.466	N/A	N/A	2
Improved colouration	0.742	N/A	0.258	N/A	N/A	2
Lateral Prosomal Constriction	0.035	0.007	0	0.949	0.008	4
Dorsal Prosomal Constriction	0.386	0.005	0	0.608	0.001	4
Lateral Opisthosomal Constriction	0.176	0.068	0	0.755	0.001	4
Dorsal Opisthosomal Constriction	0.159	0.068	0	0.772	0	4

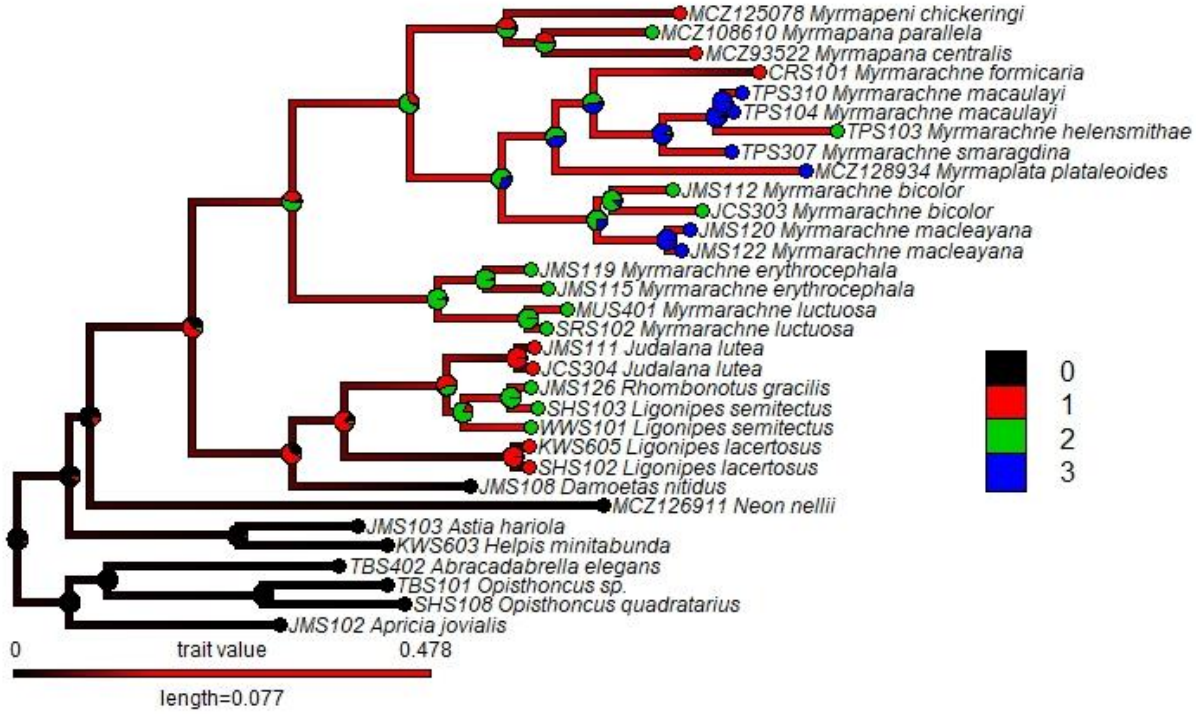

Figure 3. Mapping of the lateral prosomal constriction trait for the Myrmarachninae using the OrdBF model. Tip node colours indicate the binary scoring of the trait (shown in the legend). Pie charts indicate the likelihood of the trait being absent/present, and if present to what degree, determined by ancestral state estimation, where black indicates no constriction, red indicates a slight constriction, green indicates a moderate constriction, and blue indicates an extreme constriction. The mapped colours of the branches translate the heat colours (black through red) into the continuous score for the lateral prosomal constriction. The length of the bar provides a scale for the scoring of the lateral prosomal constriction trait.

The AICcw results indicated that the OrdBF model was the best fit when mapping the dorsal prosomal constriction trait indicating that the constriction evolved incrementally (Fig. 4). However, the AICcw count for the ER model was also a potential mode of evolution for this trait (Fig. 5). The OrdBF model predicted that the dorsal prosomal constriction trait evolved independently on three occasions and was lost on two separate occasions, while the ER model predicted four independent evolution events and two losses of the constriction trait. This trait was mostly absent in the species in the basal lineages of the phylogeny but was a predominant feature in the more recently diverged species of the phylogeny where it has evolved into extreme dorsal prosomal constrictions.

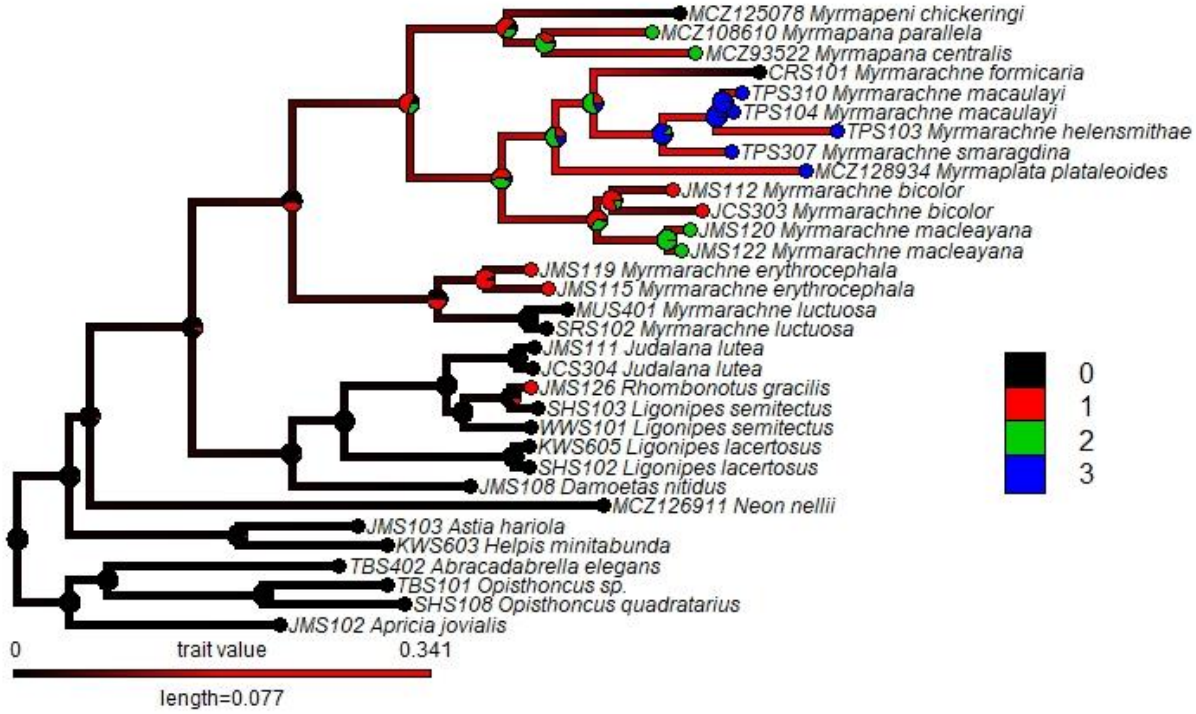

Figure 4. Mapping of the dorsal prosomal constriction trait for the Myrmarachninae using the OrdBF model. Tip node colours indicate the binary scoring of the trait (shown in the legend). Pie charts indicate the likelihood of the trait being absent/present, and if present to what degree, determined by ancestral state estimation, where black indicates no constriction, red indicates a slight constriction, green indicates a moderate constriction, and blue indicates an extreme constriction. The mapped colours of the branches translate the heat colours (black through red) into the continuous score for the dorsal prosomal constriction. The length of the bar provides a scale for the scoring of the dorsal prosomal constriction trait.

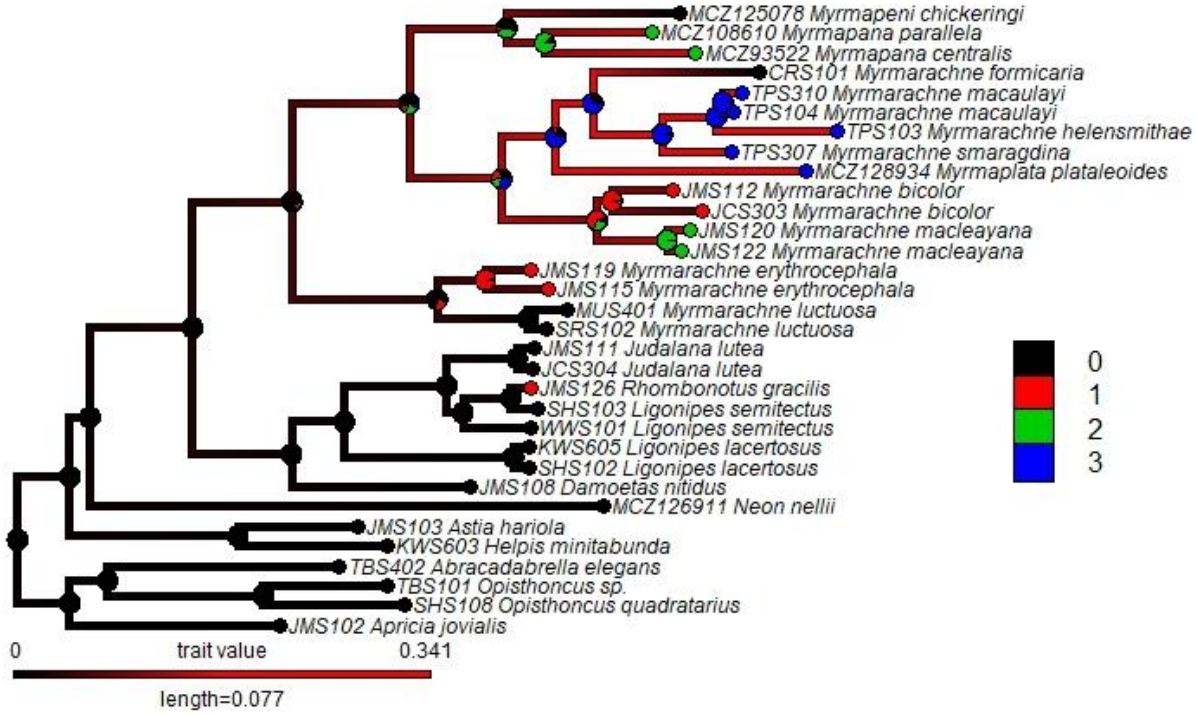

Figure 5. Mapping of the dorsal prosomal constriction trait for the Myrmarachninae using the ER model. Tip node colours indicate the binary scoring of the trait (shown in the legend). Pie charts indicate the likelihood of the trait being

absent/present, and if present to what degree, determined by ancestral state estimation, where black indicates no constriction, red indicates a slight constriction, green indicates a moderate constriction, and blue indicates an extreme constriction. The mapped colours of the branches translate the heat colours (black through red) into the continuous score for the dorsal prosomal constriction. The length of the bar provides a scale for the scoring of the dorsal prosomal constriction trait.

The lateral opisthosomal constriction mapped using the OrdBF model (Fig. 6) showed that a slight constriction is likely to have already been present in the ancestor to all the myrmecomorphs included in the phylogeny. According to this model, all species had retained a slight constriction or further evolved the degree of lateral prosomal constriction, the exception being *Judalana lutea* that has lost this trait.

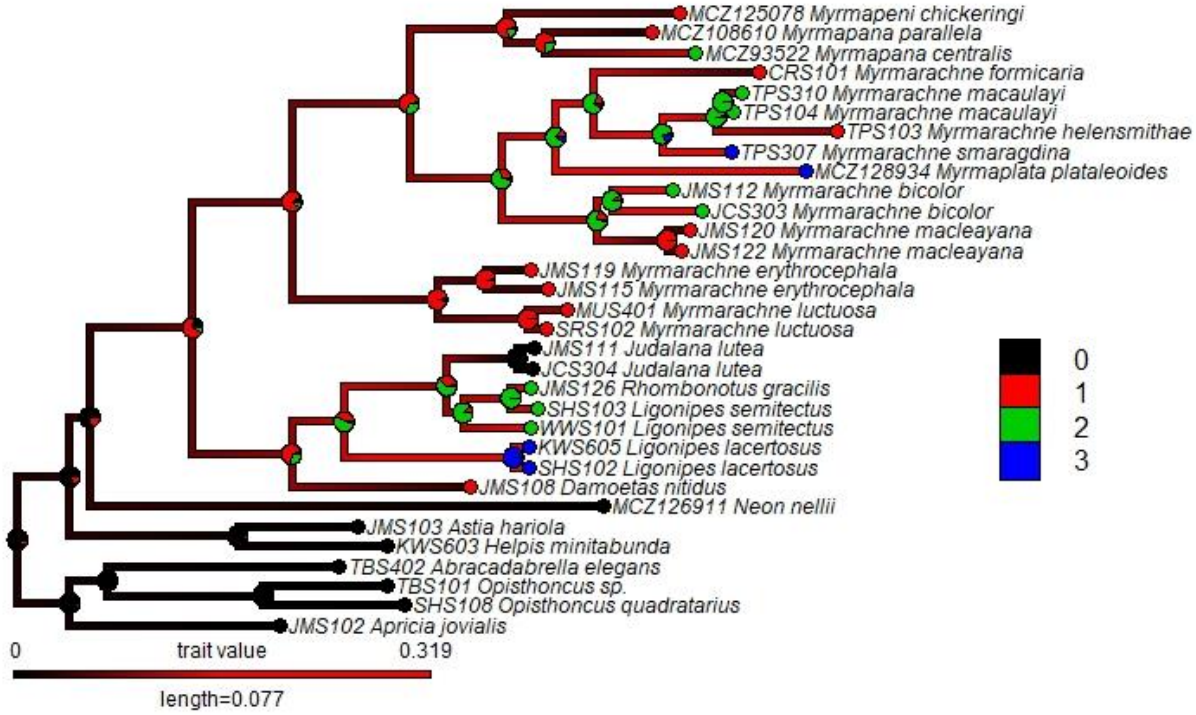

Figure 6. Mapping of the lateral opisthosomal constriction trait for the Myrmarachninae using the OrdBF model. Tip node colours indicate the binary scoring of the trait (shown in the legend). Pie charts indicate the likelihood of the trait being absent/present, and if present to what degree, determined by ancestral state estimation, where black indicates no constriction, red indicates a slight constriction, green indicates a moderate constriction, and blue indicates an extreme constriction. The mapped colours of the branches translate the heat colours (black through red) into the continuous score for the lateral opisthosomal constriction. The length of the bar provides a scale for the scoring of the lateral opisthosomal constriction trait.

The mapping of the dorsal opisthosomal constriction trait with the OrdBF model suggested that there may have been a slight constriction in the common ancestor to all the myrmecomorphs or that it may have evolved on two independent occasions. While the moderate dorsal opisthosomal constriction had occurred in the majority of species in the phylogeny, an extreme dorsal opisthosomal constriction

was present in four of the species, *Ligonipes semitectus*, *Myrmaplata plataleoides*, *Myrmarachne macaulayi*, and *Myrmapana parallela*. The loss of the dorsal opisthosomal constriction appeared to have occurred in three of the species, *Myrmarachne formicaria*, *Judalana lutea*, and *Damoetas nitidus.*

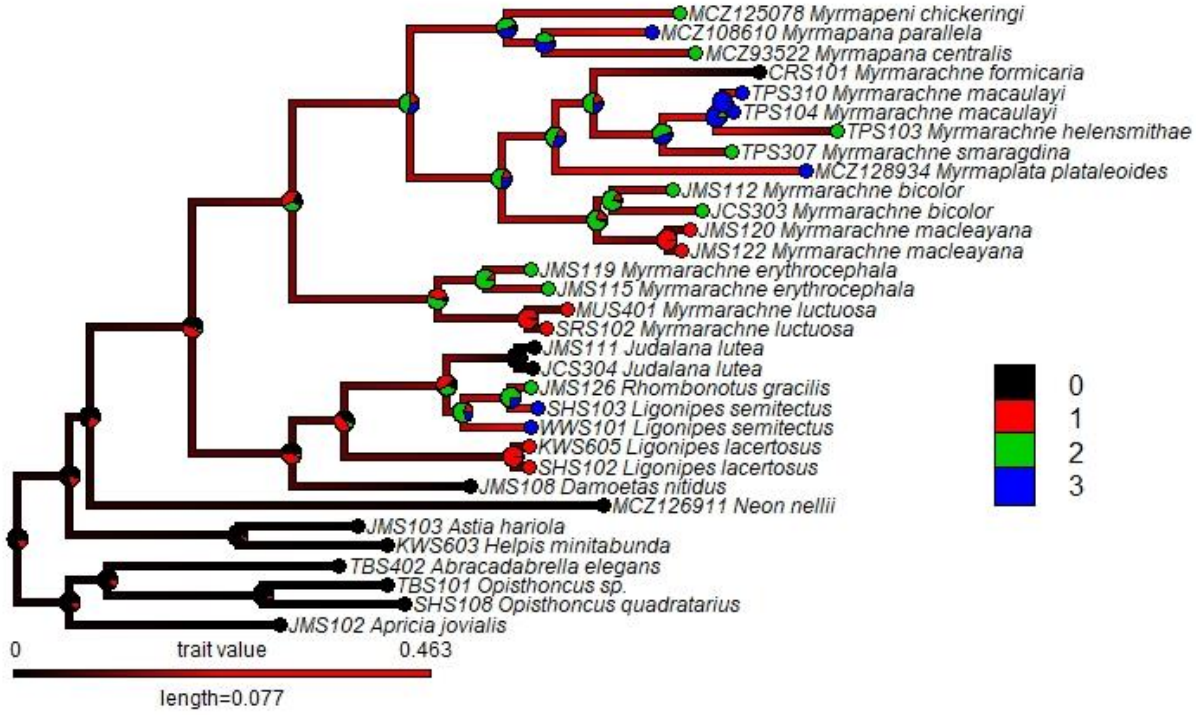

Figure 7. Mapping of the dorsal opisthosomal constriction trait for the Myrmarachninae using the OrdBF model. Tip node colours indicate the binary scoring of the trait (shown in the legend). Pie charts indicate the likelihood of the trait being absent/present, and if present to what degree, determined by ancestral state estimation, where black indicates no constriction, red indicates a slight constriction, green indicates a moderate constriction, and blue indicates an extreme constriction. The mapped colours of the branches translate the heat colours (black through red) into the continuous score for the dorsal opisthosomal constriction. The length of the bar provides a scale for the scoring of the dorsal opisthosomal constriction trait.

Discussion

The *perfecting* hypothesis predicts that inaccurate mimics are in a transitionary or intermediate phase with selection in the process of driving an ever-increasing perfection in mimic accuracy that will eventually result in accurate mimicry (Edmunds 2000; Edmunds 2006). This is contrary to the assumption of all the currently proposed hypotheses, that is that inaccurate mimics are maintained via certain mechanisms (e.g. predator indifference or limitations to predatory perception) (Kikuchi and Pfennig 2013). These hypotheses assume that inaccurate mimics are at an evolutionary stable state where any further improvement to mimic accuracy provides little, to no benefit, or even reduces

their fitness (Taylor *et al*. 2016). In this chapter, the evolution of inaccurate mimicry was considered a dynamic process following an ongoing trajectory toward mimic accuracy.

To investigate whether the predictions of the *perfecting* hypothesis can explain inaccurate mimicry, mimic accuracy and trait scoring quantified using the MQAT (Chapter 2) were mapped onto the phylogenies built using the UCE method. This combined approach allowed for the observation of distinct patterns in the evolution of myrmecomorphic accuracy and the accumulation of the multiple traits known to facilitate ant-likeness in spiders. The results of the analyses provided some evidence of a pattern of evolution that indicates mimic accuracy is improved through an incremental process.

The phylogenetic analysis of the UCEs in this study produced a strongly supported phylogeny for each of the targeted subfamilies: Castianeirinae and Myrmarachninae. The Castianeirinae phylogeny was consistent with the phylogeny of Wheeler *et al*. (2016) presented in their extensive phylogenetic work on the spider tree of life. Previous hypotheses regarding the phylogeny of Myrmarachninae have rested primarily on morphologic characters (Edwards and Benjamin 2009). The Myrmarachninae phylogeny built in this study is consistent with the morphological assessment of this group with only a discrepancy in the placement of *Myrmarachne formicaria* (the only European species). The strong support provided by our molecular phylogeny using UCEs indicates that its placement in the molecular phylogeny is potentially the correct placement for this species. The position of the Australian *Myrmarachne* is consistent with the molecular phylogenetic work of Pekár *et al*. (2017a).

The level of accuracy in these myrmecomorphic spiders were assessed via the accumulation of traits thought to contribute to ant-like resemblance. In the Myrmarachninae, accuracy in some of the lineages has improved over the phylogenetic history of the group. The highly accurate myrmecomorphs such as *Myrmarachne plataleoides*, *Myrmarachne macaulayi* and *Myrmarachne smaragdina* have diverged from a moderately accurate ancestor, suggesting that there has been a process of perfection in these lineages. The same pattern is also observed in *Rhombonotus gracilis*, *Ligonipes semitectus*, and the genus *Myrmapana*. Contrary to this, some species appear to have shown no improvement in accuracy since their last divergence event, which may suggest they have attained an equilibrium state, or that the right mutations have not occurred creating the variation essential for natural selection to act upon. However, there are also instances of a reduction in mimic accuracy in cases such as *Damoetas nitidus*, *Judalana lutea*, *Myrmarachne formicaria*, and *Myrmapeni chickeringi*. This reduction in accuracy may indicate an ecological or behavioural change.

The mapping of mimic accuracy also reveals that a lower level of mimic accuracy is observed in the clade that diverged first in this phylogeny (i.e. the clade containing the genera *Damoetas*, *Ligonipes*, *Rhombonotus*, and *Judalana*). The lack of mimic accuracy improvement may indicate a phylogenetic constraint in this clade or differences in their ecology or behaviours, such as foraging. The events that followed the divergence of this clade lead to higher levels of mimicry such as those seen in *Myrmaplata plataleoides*, *Myrmarachne smaragdina*, *Myrmarachne macaulayi*, and *Myrmarachne helensmithae*. The traits that influence this higher level of accuracy in these species are the constrictive modifications to the prosoma and opisthosoma. While most of the myrmecomorphic species used in this study have many traits in common, the species in the clade containing the inaccurate mimics have relatively few occurrences of constrictions greater than a slight constriction, with the exception of the *Ligonipes* genera. This may suggest that there is a constraint in these spiders preventing the evolution of moderate to extreme morphological modifications.

To further investigate the evolutionary change in the traits of myrmecomorphic spiders the assessment of the degree of change in the constrictions of the prosoma and the opisthosoma were mapped for the Myrmarachninae. The mapping of the constriction traits onto the phylogeny indicates that constrictions evolve via a step-wise process from a slight constriction to a moderate constriction and then into an extreme constriction. For example, ancestral state estimations as well as the pattern of extant species possessing the lateral constrictions of the prosoma show this trait to evolve from a slight constriction leading to moderate constrictions, and moderate constrictions then evolving into extreme ones. This pattern would suggest that the perfection of the lateral constriction of the prosoma takes incremental steps to reach the extreme constrictions of highly accurate myrmecomorphic species. This same pattern is also observed in the dorsal opisthosomal constriction, the lateral opisthosomal constriction and the dorsal prosomal constriction (OrdBF model). However, there is an instance in the dorsal prosomal constriction (ER model) that indicates the transition to an extreme constriction without a moderately constricted ancestor. This may indicate that more species are needed to fully elucidate the momentum of the evolution of this trait. Alternatively, it may support the idea that this trait may evolve via one large mutational step in some species. It is important to consider that the range of traits required for accurate myrmecomorphy may not be equally labile, or flexible to modification, due to their function or underlying genetic limitations. This could also mean that some traits may show stronger convergence than others (Arbuckle *et al*. 2014).

While the progression toward accurate mimicry makes intuitive sense as species that increase ant-likeness are likely to benefit from increased survivability, and hence fitness (Ruxton *et al*. 2004; Kazemi *et al*. 2014; Bosque *et al*. 2018), some species have shown no improvement since the last

divergence based on the ancestral state estimations, with a few species showing a loss of mimic accuracy. Therefore, it may be that while some species of myrmecomorphic spiders have progressively evolved accurate mimicry from an inaccurate form, others may be prevented from acquiring some of the traits due to a constraint or trade-off (Holen and Johnstone 2004; Pekár and Jarab 2011b; Penney *et al*. 2012; Morris and Reader 2016) or one of the factors, proposed in the alternative non-mutually exclusive hypotheses, may be currently reducing or removing selective pressure to improve. Given the putative costs of ant-mimicry to a spider (e.g. increased costs of movement, developmental costs and reduced fecundity) some traits may only be beneficial under certain conditions or circumstances. The loss of accuracy is perplexing but may be explained by a change in ecological conditions (Azmeh *et al*. 1998; Sherratt 2002) (perhaps the loss of a predator) or an evolutionary reversal away from mimicry (perhaps due to mimetic breakdown due to the loss of the model) (Brower 1960; Kikuchi and Pfennig 2013). Pekár (2014) noted that accurate mimicry involves complex adaptations and that reversed evolution from this state is unlikely. However, reversal may be possible for inaccurate mimics that have not acquired all the traits necessary for accurate mimicry (Pekár 2014).

The disparities between accurate and inaccurate mimics may reflect general and specialist mimics or be a consequence of varying selection pressures imposed by a range of predators, as well as environmental and mutational stochasticity (Maruyama and Parker 2017). Distinguishing between specialist and generalist mimics requires knowledge of the model ant and the specific traits being mimicked. For example, some of the traits used in this study may not be relevant to accurately imitate a specific ant model (e.g. the body shape of an ant in the genus *Opisthopsis* is visually quite different to that seen in an ant in the genus *Oecophylla*). Additionally, including ecologically relevant data on the model species to better identify the differences in selective pressures may provide further insight into whether certain environmental characteristics favour inaccurate mimicry or accurate mimicry. While myrmecomorphs converge on ant-like appearance, the different ant species that are mimicked occupy different niches and microhabitats. This could result in certain myrmecomorphs being limited to acquiring certain traits that are conducive to the environment in which the ants occupy, as different niches inhabited by the ant models may require a different range of traits (Arbuckle *et al*. 2014). For example, while evolving a highly accurate level of mimicry may impede the spiders' ability to move fast (Pekár *et al*. 2011). This would not be a concern if the ants with which the mimic associates have an arboreal lifestyle where the mimic need only drop from the branch or leaf to escape a potential predator. Alternatively, if the ant being mimicked occupies grassland or dirt patches then the ability to escape may override the need for better mimicry.

In summation, the MQAT proved useful in assessing overall accuracy as well as differences amongst traits. The application of this assessment with the UCE built phylogeny provided evidence that there is perfecting in mimic accuracy in some lineages and in some of the traits known to influence mimic accuracy, such as the constrictions of the prosoma and the opisthosoma. With the contamination of samples during the molecular processing, and the subsequent loss of many of the Castianeirinae myrmecomorphs from this study, the patterns seen in the Myrmarachninae cannot be further supported in another distantly related and independently evolved myrmecomorphic group and should thus be the focus of further study.

It is important to note that inaccurate mimicry is a complex phenomenon that is unlikely to be explained by one single hypothesis in any specific mimicry system. The phylogenetic patterns observed here predict that many of the Myrmarachninae species used in this study have improved their level of accuracy in a step-wise, gradual process. However, evidence of *perfecting* in this system is not to the exclusion of the alternative hypotheses. Given these accurate species evolved from inaccurate ancestors other influences must have allowed their persistence until an ecological change or gene mutation occurred resulting in an improvement to their level of mimic accuracy. This is not to argue that all inaccurate mimic species will inevitably evolve to a highly accurate mimetic form, and therefore, future studies of the *perfecting* hypothesis should consider the influence of other factors predicted by the alternative non-mutually exclusive hypotheses. Also, given the fact that a few of the Myrmarachninae species have not improved their ant-likeness it would be informative to compare the ecology (e.g., movement patterns, activity cycles, commensalism with ants, microhabitats, etc.) between those species that have reached a higher level of accuracy with those species that have not, as ecological factors will strongly affect selection pressures. Future studies on myrmecomorphic spiders promise to reveal many aspects surrounding the nature of complex phenotypic change as well as the evolutionary and genetic forces that shape mimetic species and more broadly trait evolution in general. Ultimately, this idea could be further extended into more distantly related taxa, such as myrmecomorphic beetles and other arthropods, and even into other systems with known inaccurate mimicry such as hoverflies (Gilbert 2004) and kingsnakes (Savage and Slowinski 1992; Brodie and Brodie 2004). The results of this thesis questions the way we think about mimic evolution and reinforces the necessity to examine the rates of phenotypic change in mimetic species in a phylogenetic context.

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Supplementary Material

Table S1. Table of spiders used in the mimic quality assessment table (MQAT), including the collection date and location and the gender of the specimen.

Unique I.D.	Subfamily	Genus/Species	Collection date	Location; GPS co-ordinate	Sex
16734	Castianeirinae	*Aetius nocturnus*	1/12/2007	Phetchabun, Thailand; 16° 39.572´ N; 101° 8.194´ E	male
Q00002	Castianeirinae	*Battalus byrneae*	Unknown	Church Hill, Tasmania	female
136939	Castianeirinae	*Castianeira cingulata*	25/07 - 1/08/2008	Massachusetts, United States; 42° 16´ 8.4´´ N; 70° 55´ 20.1´´ W	male
137106	Castianeirinae	*Castianeira gertschi*	25/09 - 2/10/2007	Massachusetts, United States; 42° 20´ 28.2´´ N; 70° 53´ 46´´ W	female
126957	Castianeirinae	*Castianeira longipalpa*	17/07/2009	Massachusetts, United States; Lat: 41.304387°; Long: -70.260663°	female
142573	Castianeirinae	*Castianeira trilineata*	8/10/2006	Massachusetts, United States; Lat: 41.265778°; Long: -70.177858°	female
Q00013	Castianeirinae	*Copa kabana*	Unknown	Provided by Queensland Museum, Australia	male
95991	Castianeirinae	*Corinnomma sp.*	25/05/2009	Petchaburi, Thailand; 12° 49.302´ N; 99° 22.263´ E	male
Q80326	Castianeirinae	*Disnyssus helenmirrenae*	3/06/2006	Provided by Queensland Museum, Australia	male
Q84598	Castianeirinae	*Iridonyssus formicans*	2008	Provided by Queensland Museum, Australia	male
JMS109	Castianeirinae	*Iridonyssus kohouti*	7/11/2017	New South Wales, Australia; Lat: -33.773383; Long: 151.115996 (+/- 5m)	male
Q84637	Castianeirinae	*Iridonyssus kohouti*	16/1/2008 - 7/02/2008	Provided by Queensland Museum, Australia	female
Q00014	Castianeirinae	*Leichhardteus albofasciatus*	Unknown	Provided by Queensland Museum, Australia	male
Q95115	Castianeirinae	*Leichhardteus conopalpis*	21/11/08 - 16/06/09	Provided by Queensland Museum, Australia	male
Q98191	Castianeirinae	*Leptopicia bimaculata*	Sep-13	Provided by Queensland Museum, Australia	male
79128	Castianeirinae	*Mazax pax*	1998	Granada, Nicaragua	female
28153	Castianeirinae	*Mazax spinosa*	30/11/1998	Granada, Nicaragua	female
67875	Castianeirinae	*Myrmecotypus rettenmeyeri*	5/05/1964	Canal Zone, Panama; Lat: 9.154722°; Long: -79.848056° (+/- 3646m)	female
Q44095	Castianeirinae	*Nucastia supunnoides*	12/02/1997 - 28/02/1997	Provided by Queensland Museum, Australia	male
Q84821	Castianeirinae	*Nyssus albopunctatus*	Cannot read label	Provided by Queensland Museum, Australia	female
Q00016	Castianeirinae	*Nyssus avidus*	Jul - Aug 2017	Cape York, Australia	female
Q50886	Castianeirinae	*Nyssus luteofinis*	3-11/02/1999	Provided by Queensland Museum, Australia	male
Q00017	Castianeirinae	*Nyssus paradoxus*	Unknown	Provided by Queensland Museum, Australia	female
SHS116	Castianeirinae	*Nysuss coloripes*	14/03/2018	Queensland, Australia; Lat: -27.87675; Long: 153.155394444 (+/- 5m)	male
TBS504	Castianeirinae	*Nysuss coloripes*	13/03/2018	Queensland, Australia; Lat: -27.897525; Long: 153.179747222222 (+/- 5m)	male
Q84600	Castianeirinae	*Nysuss semifuscus*	16/1/2008 - 7/02/2008	Provided by Queensland Museum, Australia	male
Q84597	Castianeirinae	*Poecilipta gloverae*	16/1/2008 - 7/02/2008	Provided by Queensland Museum, Australia	female
96085	Castianeirinae	*Serendib suthepica*	23/10/2006	Phetchabun, Thailand; 16° 42.47´ N; 101° 35.26´ E	male
44162	Castianeirinae	*Serendib volans*	26/06/2007	Nakhon Ratchasima, Thailand; 14° 28.524´ N; 101° 22.928´ E	female
28119	Castianeirinae	*Sphecotypus niger*	3/07/1991	Puntarenas, Costa Rica	female
Q87362	Corinninae	*Creugas gulosus*	18-20/12/2008	Provided by Queensland Museum, Australia	female
34637	Corinninae	*Paradiestus gigantea*	13-15/01/1995	Santa Catarina, Brazil	female
JMS108	Myrmarachninae	*Damoetas nitidus*	30/04/2017	New South Wales, Australia; Lat: -33.62804167; Long: 150.7675333 (+/- 10m)	Subadult female
JCS304	Myrmarachninae	*Judalana lutea*	2/08/2018	Queensland, Australia; Lat: -19.3311424; Long: 146.758453 (+/- 10m)	male
JMS111	Myrmarachninae	*Judalana lutea*	21/06/2017	Queensland, Australia; Lat: -23.40175556; Long: 150.4920361 (+/- 10m)	female
KWS605	Myrmarachninae	*Ligonipes lacertosus*	12/07/2017	New South Wales, Australia; Lat: -33.707405556; Long: 151.1770944 (+/- 5m)	female
SHS102	Myrmarachninae	*Ligonipes lacertosus*	14/03/2018	Queensland, Australia; Lat: -27.87675; Long: 153.155394444 (+/- 5m)	female
SHS103	Myrmarachninae	*Ligonipes semitectus*	14/03/2018	Queensland, Australia; Lat: -27.87675; Long: 153.155394444 (+/- 5m)	female
WWS101	Myrmarachninae	*Ligonipes semitectus*	17/03/2018	New South Wales, Australia; Lat: -32.154258333; Long: 152.32200556 (+/- 5m)	female
93522	Myrmarachninae	*Myrmapana centralis*	27/08/1982	Puntarenas, Costa Rica	female
108610	Myrmarachninae	*Myrmapana parallela*	6/09/2011	Región Autónoma del Atlántico Sur, Nicaragua; Lat: 12.67009°; Long: -83.71576° (+/- 100m)	male
125078	Myrmarachninae	*Myrmapeni chickeringi*	6/10/2011	Región Autónoma del Atlántico Sur, Nicaragua; Lat: 12.67321°; Long: -83.7093° (+/- 100m)	male
128934	Myrmarachninae	*Myrmaplata plataleoides*	4-10/03/2007	Sakon Nakhon, Thailand; 17° 7.34´ N; 104° .788´ E	male
JCS303	Myrmarachninae	*Myrmarachne bicolor*	2/08/2018	Queensland, Australia; Lat: -19.33114242; Long: 146.758453 (+/- 10m)	female
JMS112	Myrmarachninae	*Myrmarachne bicolor*	18/06/2017	Queensland, Australia; Lat: -27.39862222; Long: 152.6181194 (+/- 5m)	female
JMS115	Myrmarachninae	*Myrmarachne erythrocephala*	16/05/2017	New South Wales, Australia; Lat: -33.77179444; Long: 151.1152028 (+/- 30m)	female
JMS119	Myrmarachninae	*Myrmarachne erythrocephala*	30/07/2017	New South Wales, Australia; Lat: -33.89245556; Long: 151.2374722 (+/- 5m)	female
CRS101	Myrmarachninae	*Myrmarachne formicaria*	Unknown	Provided by Masaryk University, Brno, Czech Republic	female
TPS103	Myrmarachninae	*Myrmarachne helensmithae*	2/07/2018	Queensland, Australia; Lat: -19.310696 / Long: 146.764057 (+/- 10m)	female
MUS401	Myrmarachninae	*Myrmarachne luctuosa*	15/01/2018	New South Wales, Australia; Lat: -33.773567 / Long: 151.115519 (+/- 10m)	male
SRS102	Myrmarachninae	*Myrmarachne luctuosa*	17/03/2018	New South Wales, Australia; Lat: -29.08769167; Long: 153.0021972 (+/- 5m)	male
TPS310	Myrmarachninae	*Myrmarachne macaulayi*	2/08/2018	Queensland, Australia; Lat: -19.309169; Long: 146.765339 (+/- 10m)	Juvenile/unknown
TPS104	Myrmarachninae	*Myrmarachne macaulayi*	2/07/2018	Queensland, Australia; Lat: -19.310696 / Long: 146.764057 (+/- 10m)	female
JMS120	Myrmarachninae	*Myrmarachne macleayana*	22/06/2017	Queensland, Australia; Lat: -23.89678889; Long: 151.2624056 (+/- 100m)	Subadult female
JMS122	Myrmarachninae	*Myrmarachne macleayana*	21/06/2017	Queensland, Australia; Lat: -23.39944722; Long: 150.5049139 (+/- 5m)	female
TPS307	Myrmarachninae	*Myrmarachne smaragdina*	2/08/2018	Queensland, Australia; Lat: -19.309169; Long: 146.765339 (+/- 10m)	female
JMS126	Myrmarachninae	*Rhombonotus gracilis*	22/06/2017	Queensland, Australia; Lat: -23.89678889; Long: 151.2624056 (+/- 100m)	Juvenile/unknown
TBS402	Salticinae	*Abracadabrella elegans*	13/03/2018	Queensland, Australia; Lat: -27.9028138889; Long: 153.180663889 (+/- 5m)	male
JMS102	Salticinae	*Apricia jovialis*	5/02/2017	New South Wales, Australia; Lat: -33.769456; Long: 151.11334 (+/- 100m)	female
JMS103	Salticinae	*Astia hariola*	14/04/2017	New South Wales, Australia; Lat: -33.06791667; Long: 151.3827056 (+/- 5m)	female
KWS603	Salticinae	*Helpis minitabunda*	12/07/2017	New South Wales, Australia; Lat: -33.70740556; Long: 151.1770944 (+/- 5m)	female
126911	Salticinae	*Neon nellii*	27/08/2006	Massachusetts, United States; Lat: 41.302813°; Long: -70.257054°	female
SHS108	Salticinae	*Opisthoncus quadratarius*	14/03/2018	Queensland, Australia; Lat: -27.87675; Long: 153.155394444 (+/- 5m)	female
TBS101	Salticinae	*Opisthoncus sp.*	3/12/2018	Queensland, Australia; Lat: -27.9036111111111; Long: 153.182402777778 (+/- 5m)	female

Table S2. The data used to compare the dorsal and lateral shape analysis with the MQAT scores assessing all traits, as well as traits only effecting the dorsal shape and traits only effecting the lateral shape (both binary and continuous; n = 11).

Specimen type	Subfamily	Species	Dorsal Shape Values	Lateral Shape Values	Binary (All traits)	Binary (dorsal traits)	Binary (lateral traits)	Continuous (All traits)	Continuous (dorsal traits)	Continuous (lateral traits)
non-mimic	Salticinae	*Apricia jovialis*	-0.9944	-0.9485	0.0588	0.1111	0.1111	0.1574	0.1464	0.1464
non-mimic	Salticinae	*Astia hariola*	-0.9700	-1.1653	0.0588	0.1111	0.1111	0.1412	0.1092	0.1092
non-mimic	Castianeirinae	*Nyssus coloripes*	-0.5161	-0.9147	0	0	0	0.1298	0.1021	0.1021
mimetic spider	Myrmarachninae	*Damoetas nitidus*	-0.5016	-0.6576	0.2941	0.3333	0.4444	0.2338	0.2013	0.2071
mimetic spider	Myrmarachninae	*Judalana lutea*	-0.4055	-0.6821	0.2941	0.3333	0.4444	0.1993	0.1955	0.2045
mimetic spider	Myrmarachninae	*Myrmarachne luctuosa*	-0.1158	-0.4534	0.5294	0.4444	0.6667	0.3306	0.2487	0.2811
mimetic spider	Myrmarachninae	*Myrmarachne macleayana*	-0.1037	-0.4672	0.7059	0.6667	0.7778	0.3371	0.2603	0.2727
mimetic spider	Myrmarachninae	*Rhombonotus gracilis*	-0.0650	-0.3331	0.7059	0.6667	0.7778	0.3075	0.2100	0.2277
mimetic spider	Castianeirinae	*Iridonyssus kohouti*	-0.0060	-0.6460	0.1765	0.2222	0.3333	0.1510	0.1361	0.1387
mimetic spider	Myrmarachninae	*Myrmarachne bicolor*	0.0677	-0.6293	0.7059	0.6667	0.7778	0.3154	0.2782	0.2955
mimetic spider	Myrmarachninae	*Myrmarachne erythrocephala*	0.1273	-0.4147	0.5882	0.6667	0.6667	0.3064	0.2486	0.2329

Table S3. Sequencing results for the subfamily Castianeirinae showing the number of UCE loci in the final matrix, and the number of reads (post quality control) and contigs per sample.

Unique I.D.	Species	UCE loci in final matrix	Number of reads (post-QC)	Number of contigs
Q00002	*Battalus byrneae*	623	4 397 318	504 483
MCZ136939	*Castianeira cingulata*	198	327 938	22 978
MCZ137106	*Castianeira gertschi*	210	495 598	34 426
MCZ142573	*Castianeira trilineata*	197	326 029	21 544
MCZ95991	*Corinnomma* sp.	221	585 220	41 473
Q87362	*Creugas gulosus*	522	3 329 157	377 430
Q80326	*Disnyssus helenmirrenae*	515	7 153 474	644 975
Q84598	*Iridonyssus formicans*	305	245 111	79 707
JMS109	*Iridonyssus kohouti*	427	1 605 640	389 091
Q84637	*Iridonyssus kohouti*	256	505 506	121 538
Q00014	*Leichhardteus albofasciatus*	535	5 647 431	1 108 369
Q95115	*Leichhardteus conopalpis*	404	243 125	44 681
Q98191	*Leptopicia bimaculata*	634	1 720 163	294 272
Q84821	*Nyssus albopunctatus*	222	82 150	18 235
SHS116	*Nysuss coloripes*	644	1 523 026	522 518
TBS504	*Nysuss coloripes*	616	1 473 736	451 556
Q84600	*Nysuss semifuscus*	583	823 486	174 831
MCZ34637	*Paradiestus giganteus*	125	422 282	13 081
Q84597	*Poecilipta gloverae*	272	1 026 758	230 312
MCZ96085	*Serendib suthepica*	402	253 230	55 080
MCZ44162	*Serendib volans*	482	466 539	64 579

Table S4. Sequencing results for the subfamily Myrmarachninae showing the number of UCE loci in the final matrix, and the number of reads (post quality control) and contigs per sample.

Unique I.D.	Species	UCE loci in final matrix	Number of reads (post-QC)	Number of contigs
TBS402	*Abracadabrella elegans*	480	2 290 876	694 624
JMS102	*Apricia jovialis*	500	1 428 549	415 548
JMS103	*Astia hariola*	464	2 379 630	136 009
JMS108	*Damoetas nitidus*	520	1 459 030	370 295
KWS603	*Helpis minitabunda*	455	2 870 911	861 143
JMS111	*Judalana lutea*	465	1 222 324	364 409
JCS304	*Judalana lutea*	513	1 217 119	327 080
KWS605	*Ligonipes lacertosus*	524	1 278 857	347 216
SHS102	*Ligonipes lacertosus*	520	1 235 896	450 870
WWS101	*Ligonipes semitectus*	489	824 669	210 006
SHS103	*Ligonipes semitectus*	428	1 105 005	273 504
MCZ93522	*Myrmapana centralis*	195	212 159	25 647
MCZ108610	*Myrmapana parallela*	412	1 315 071	83 491
MCZ125078	*Myrmapeni chickeringi*	346	419 726	44 520
MCZ128934	*Myrmaplata plataleoides*	38	99 054	7 576
JMS112	*Myrmarachne bicolor*	550	2 979 372	543 657
JCS303	*Myrmarachne bicolor*	538	1 206 513	271 373
JMS119	*Myrmarachne erythrocephala*	555	1 154 182	314 280
JMS115	*Myrmarachne erythrocephala*	32	1 330 311	18 838
CRS101	*Myrmarachne formicaria*	349	1 106 034	176 467
TPS103	*Myrmarachne helensmithae*	240	1 149 597	297 437
SRS102	*Myrmarachne luctuosa*	574	731 121	197 245
MUS401	*Myrmarachne luctuosa*	441	3 243 697	780 267
TPS310	*Myrmarachne macaulayi*	502	1 459 861	392 311
TPS104	*Myrmarachne macaulayi*	534	1 260 449	326 338
JMS122	*Myrmarachne macleayana*	512	2 441 875	111 515
JMS120	*Myrmarachne macleayana*	497	2 874 948	688 364
TPS307	*Myrmarachne smaragdina*	523	2 148 993	437 563
MCZ126911	*Neon nellii*	66	66 678	5 916
SHS108	*Opisthoncus quadratarius*	503	2 243 745	702 749
TBS101	*Opisthoncus* sp.	429	1 423 914	383 764
JMS126	*Rhombonotus gracilis*	497	3 237 922	767 037

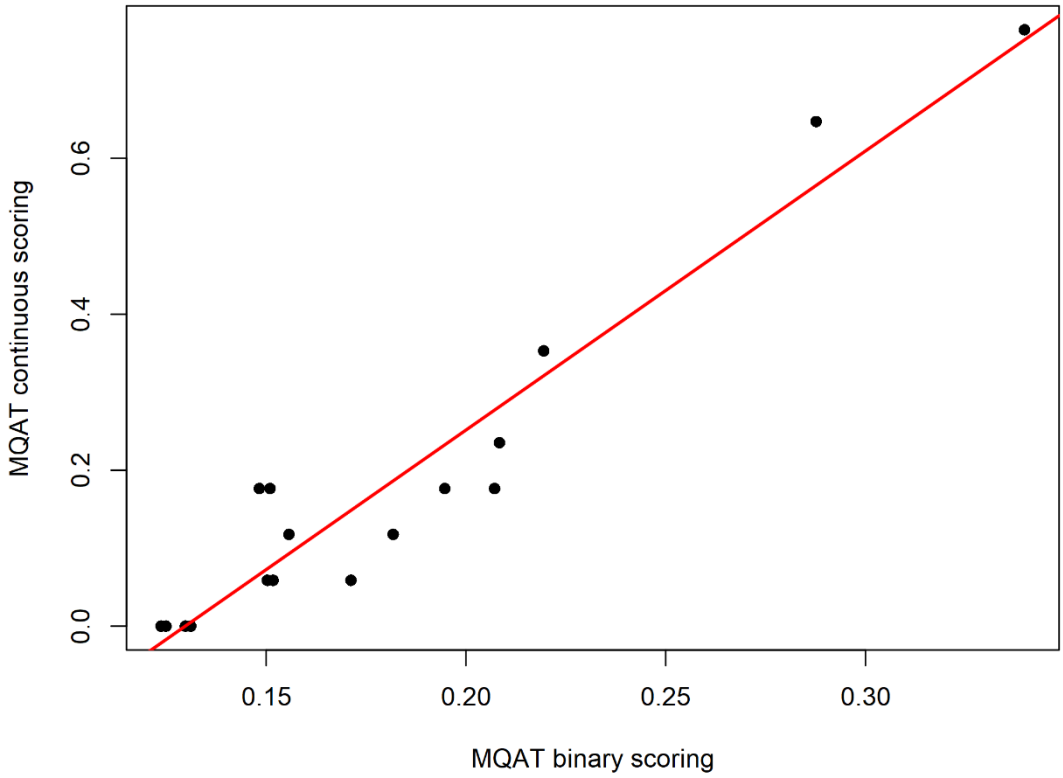

Figure S1. Linear regression of the relationship between the binary and continuous assessments of the mimic assessment quality table (MQAT) in the Castianeirinae subfamily (*n* = 17), with the exclusion of specimens where data is incomplete.

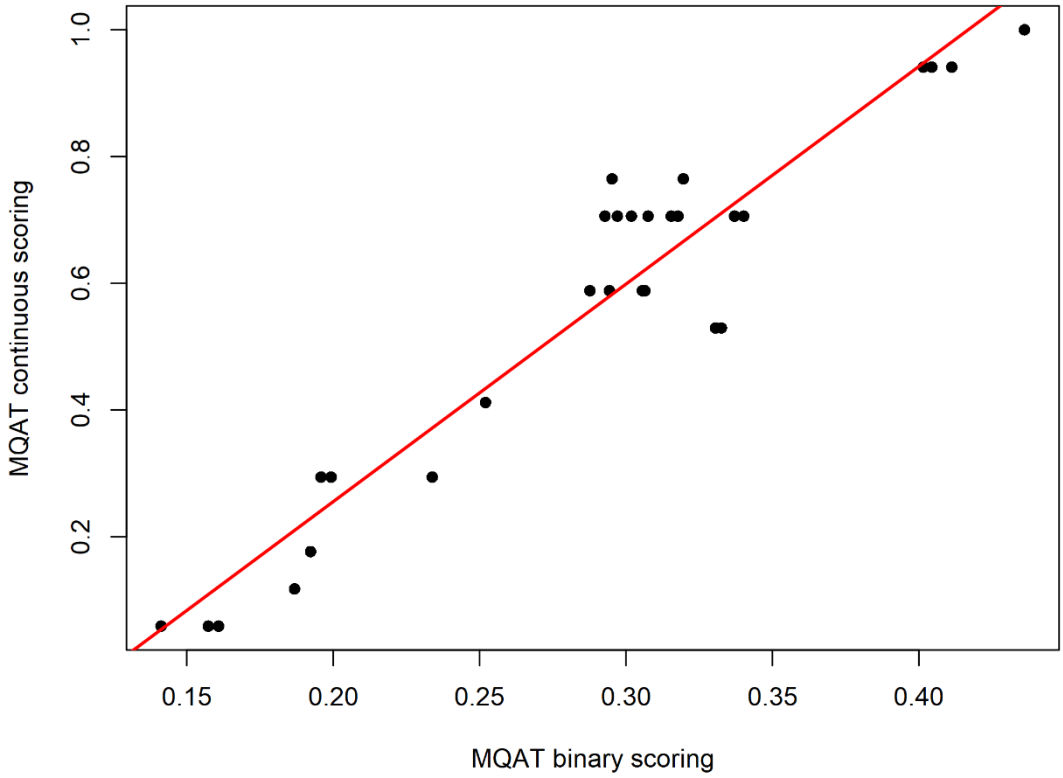

Figure S2. Linear regression of the relationship between the binary and continuous assessments of the mimic assessment quality table (MQAT) in the Myrmarachninae subfamily (*n* = 29), with the exclusion of specimens where data is incomplete.

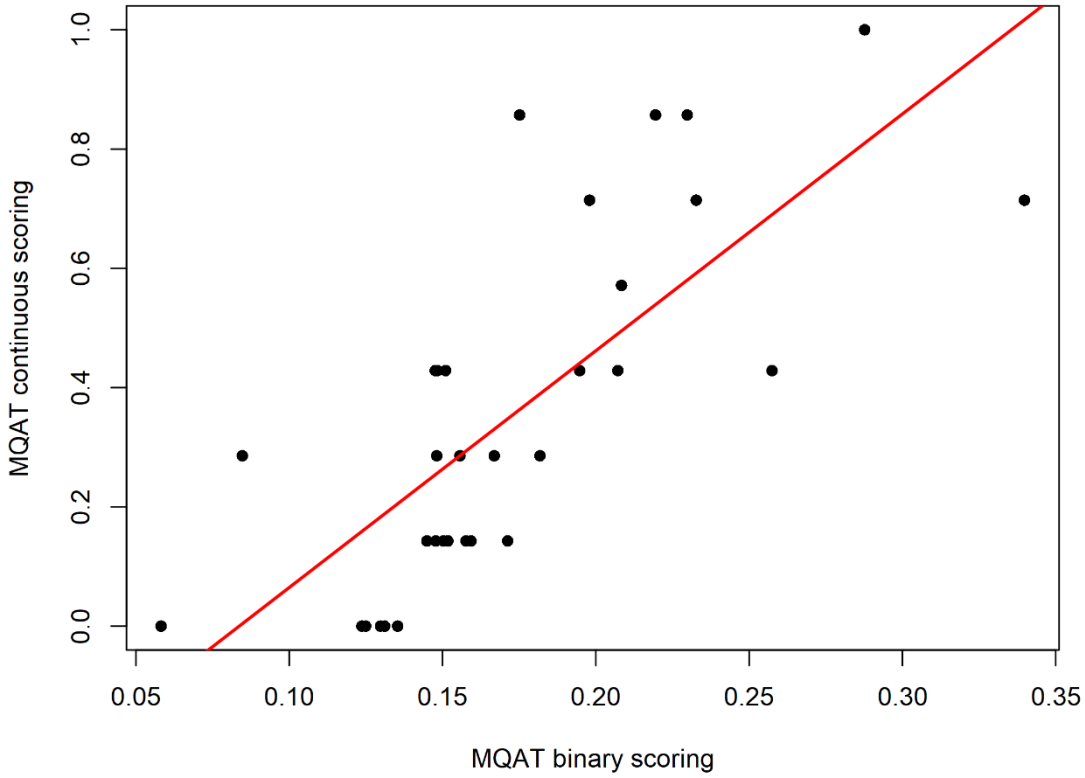

Figure S3. Linear regression of the relationship between the binary and continuous assessments of the mimic assessment quality table (MQAT) in the Castianeirinae subfamily (*n* = 32), with constrictions of the prosoma and opisthosoma scored strictly absence/presence, i.e. not graded 1-3.

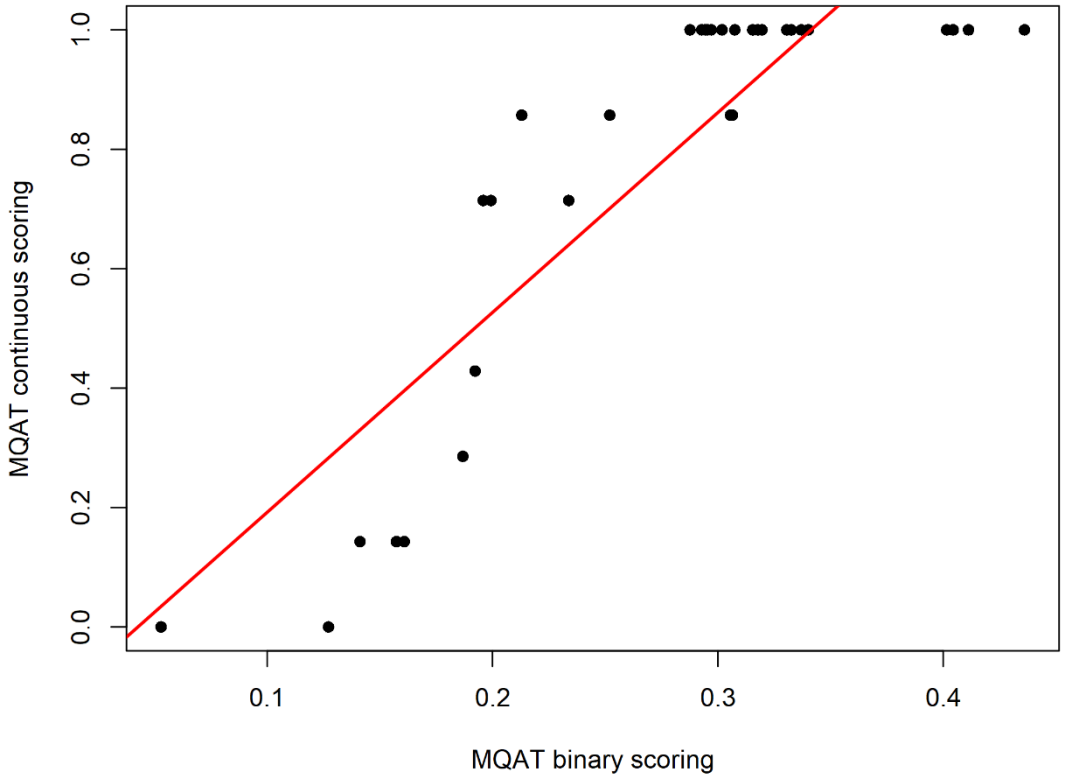

Figure S4. Linear regression of the relationship between the binary and continuous assessments of the mimic assessment quality table (MQAT) in the Myrmarachninae subfamily (*n* = 32), with constrictions of the prosoma and opisthosoma scored strictly absence/presence, i.e. not graded 1-3.

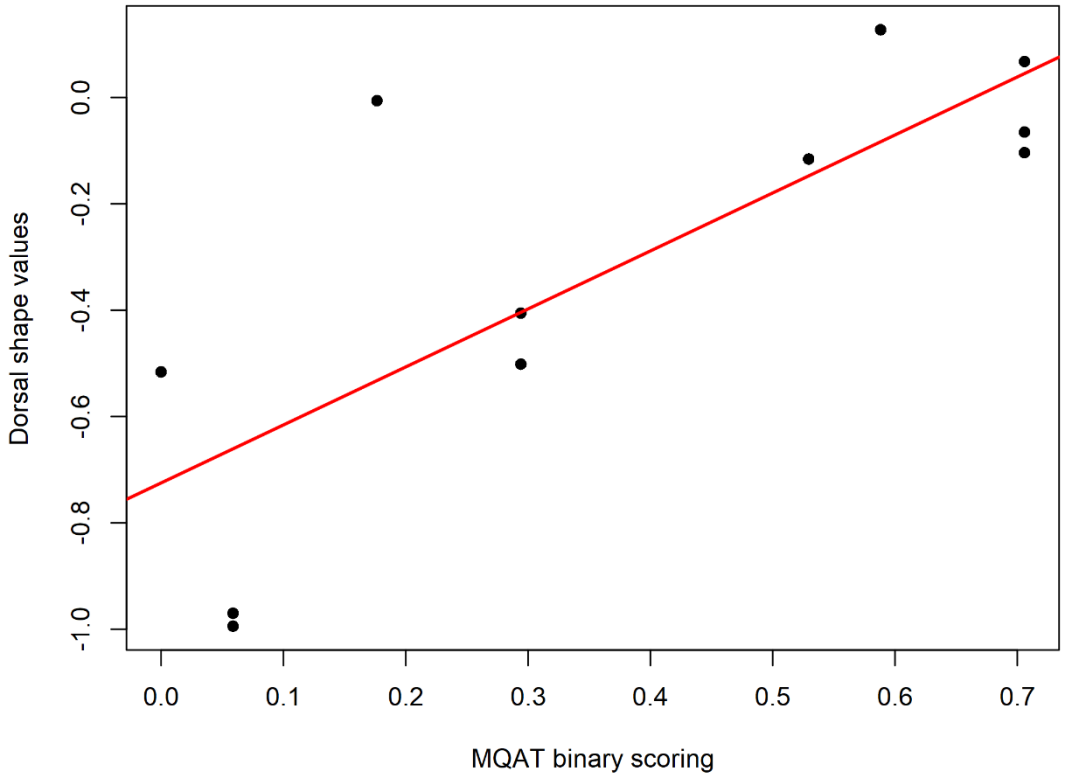

Figure S5. Linear regression of the MQAT binary scores and the Dorsal Shape values (n = 11).

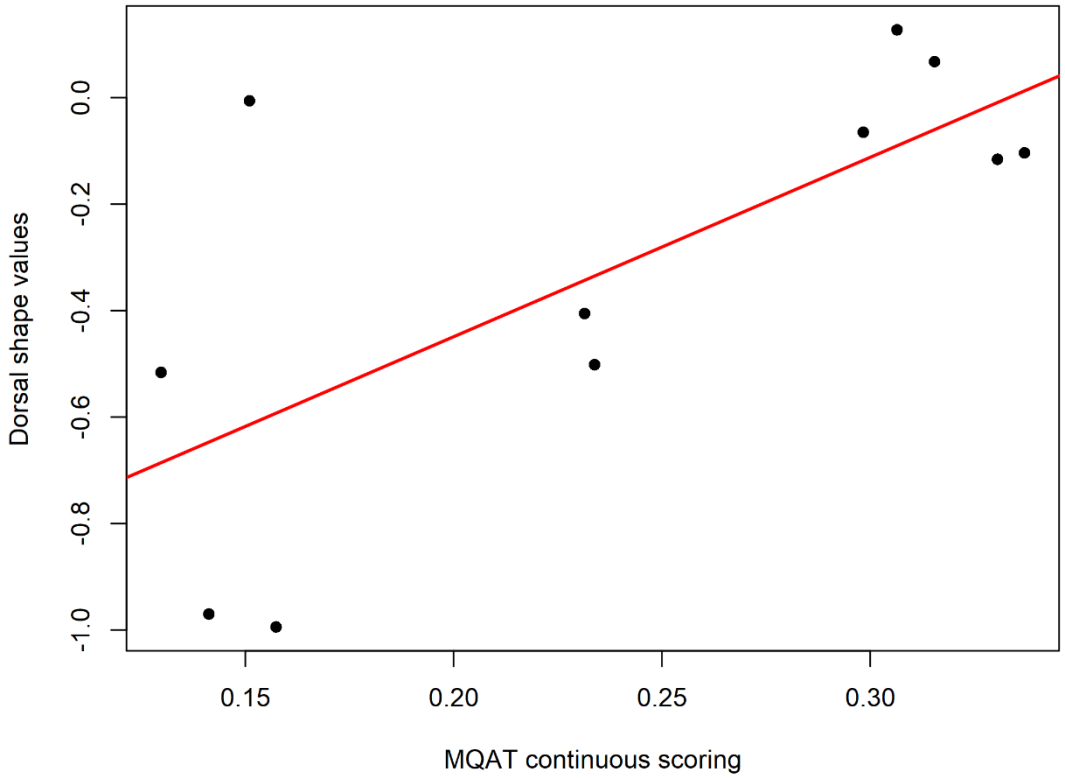

Figure S6. Linear regression of the MQAT continuous scores and the Dorsal Shape values (n = 11).

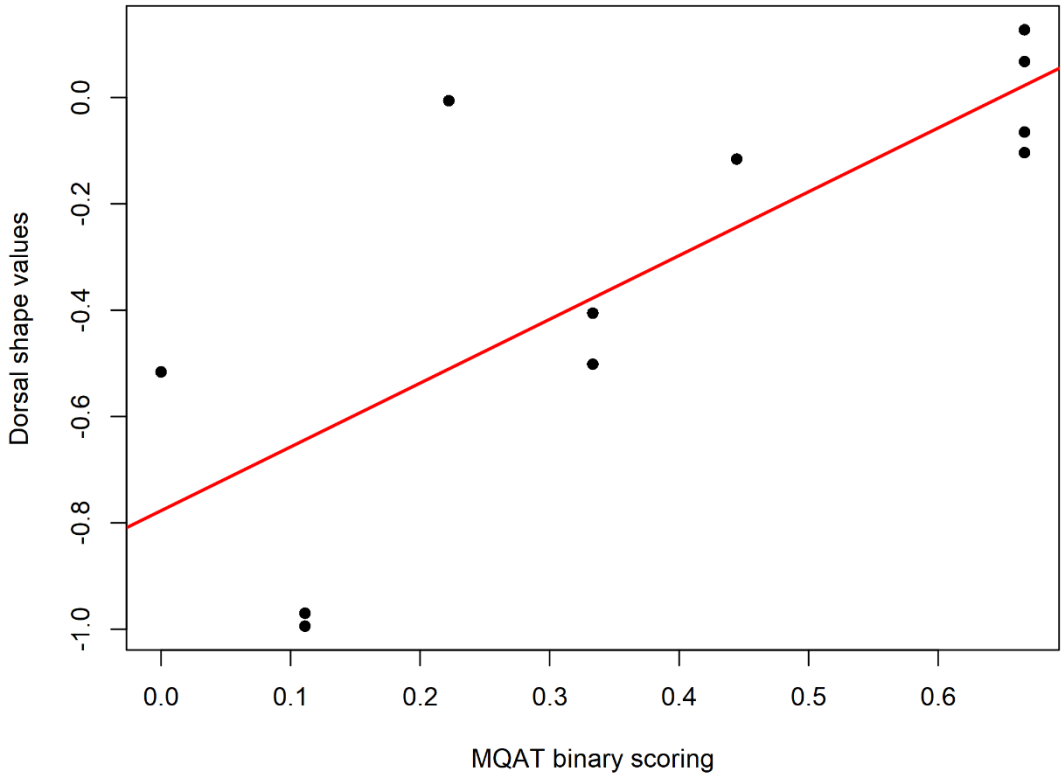

Figure S7. Linear regression of the MQAT binary scores (excluding traits not effecting dorsal shape) and the Dorsal Shape values (n = 11).

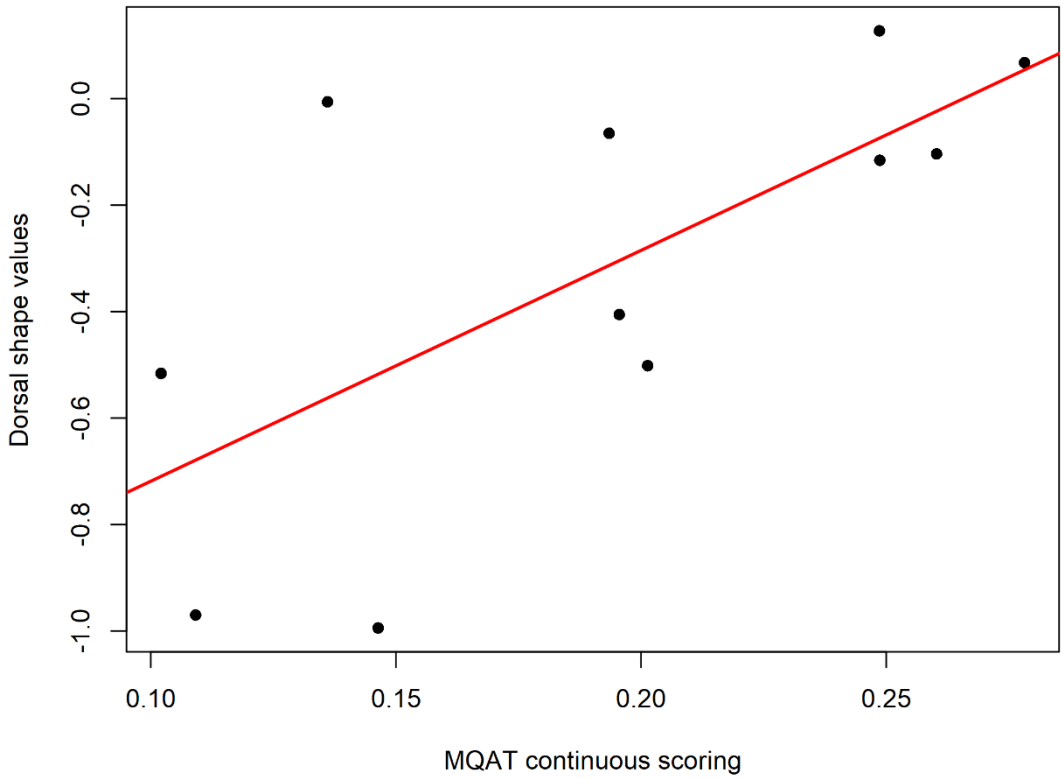

Figure S8. Linear regression of the MQAT continuous scores (excluding traits not effecting dorsal shape) and the Dorsal Shape values (n = 11).

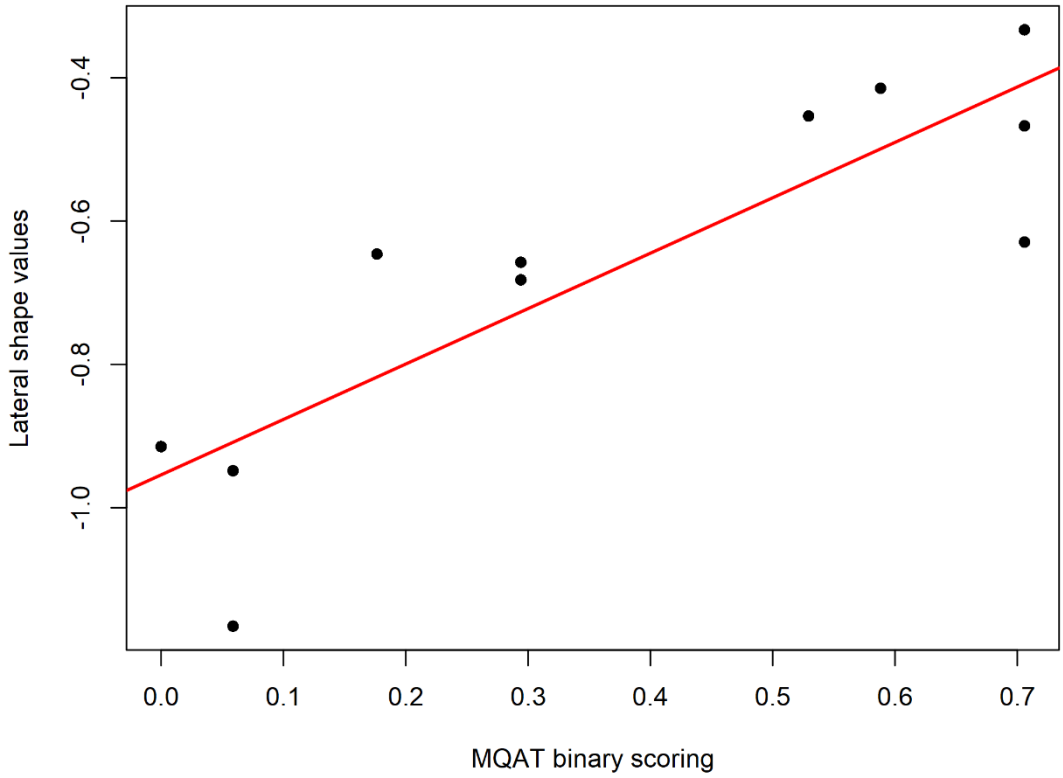

Figure S9. Linear regression of the MQAT binary scores and the Lateral Shape values (*n* = 11).

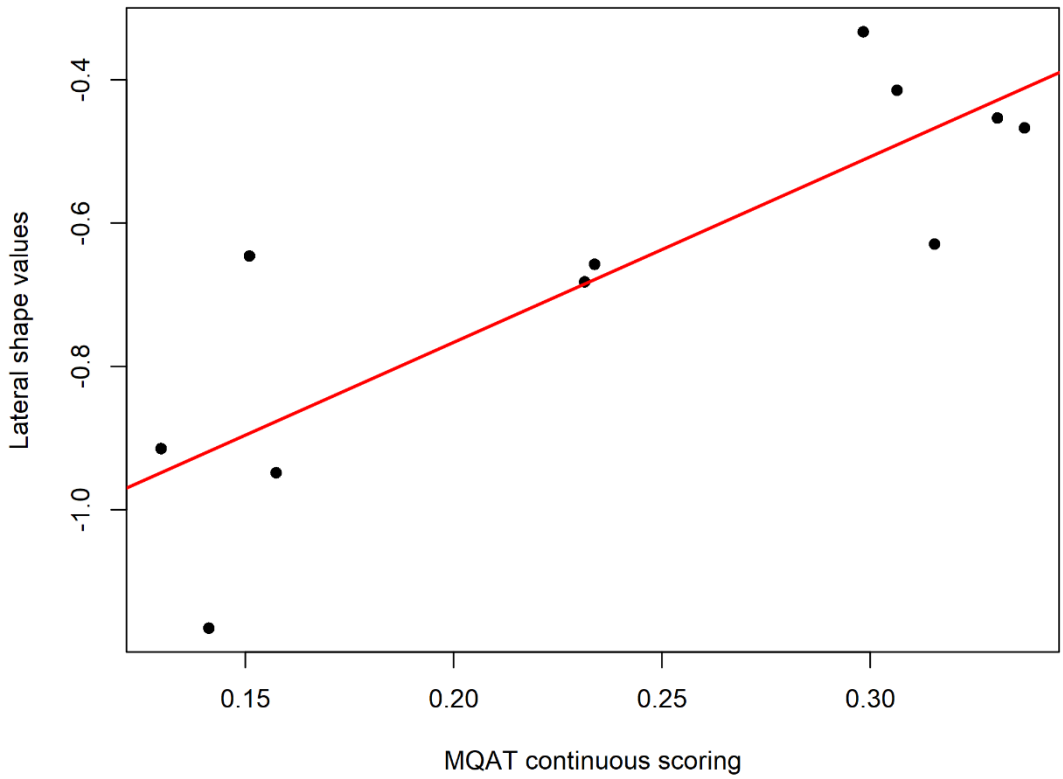

Figure S10. Linear regression of the MQAT continuous scores and the Lateral Shape values (*n* = 11).

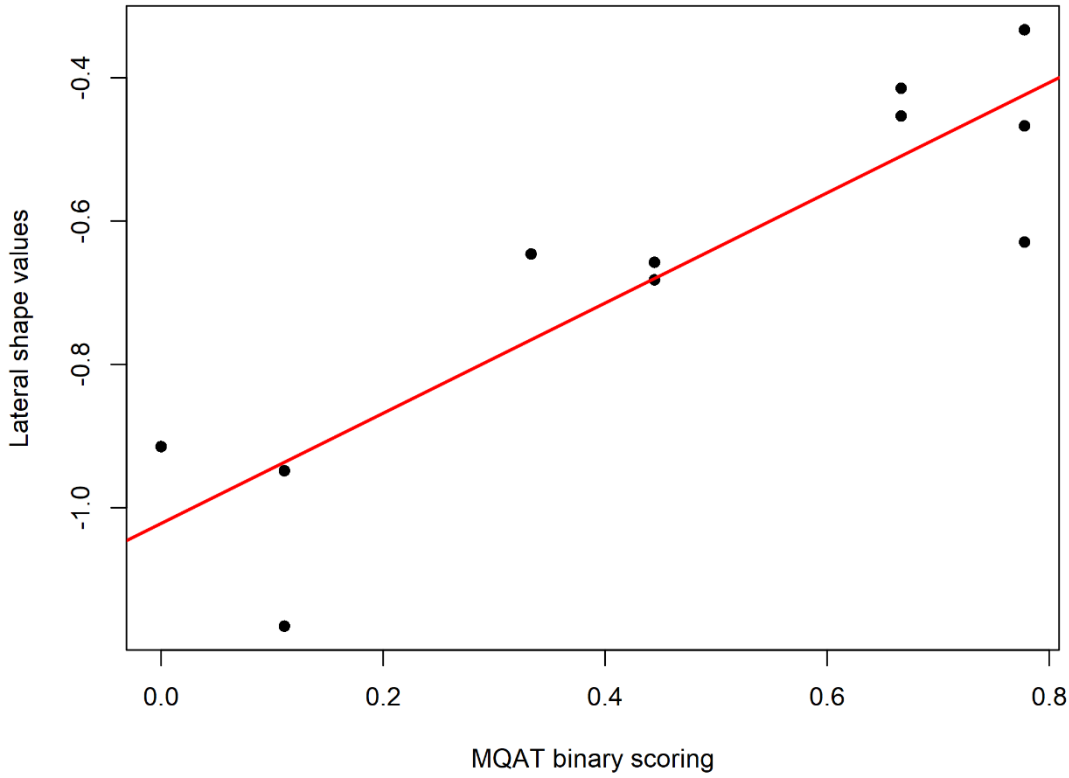

Figure S11. Linear regression of the MQAT binary scores (excluding traits not effecting lateral shape) and the Lateral Shape values (n = 11).

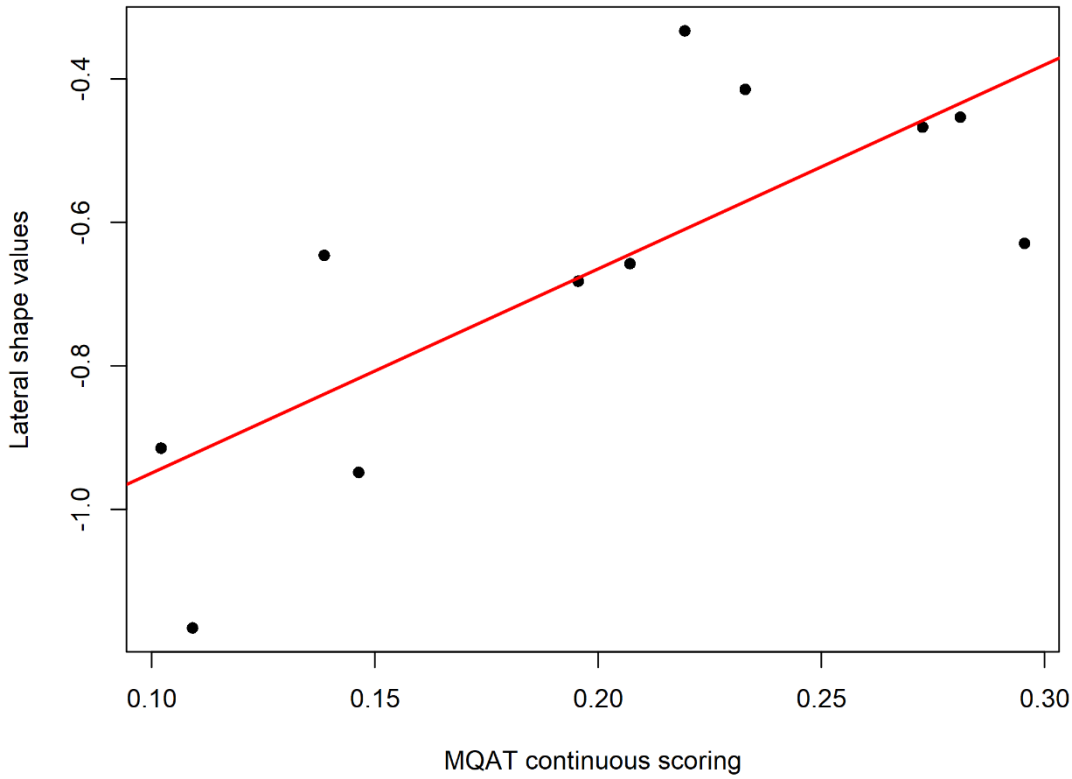

Figure S12. Linear regression of the MQAT continuous scores (excluding traits not effecting lateral) and the Lateral Shape values (n = 11).

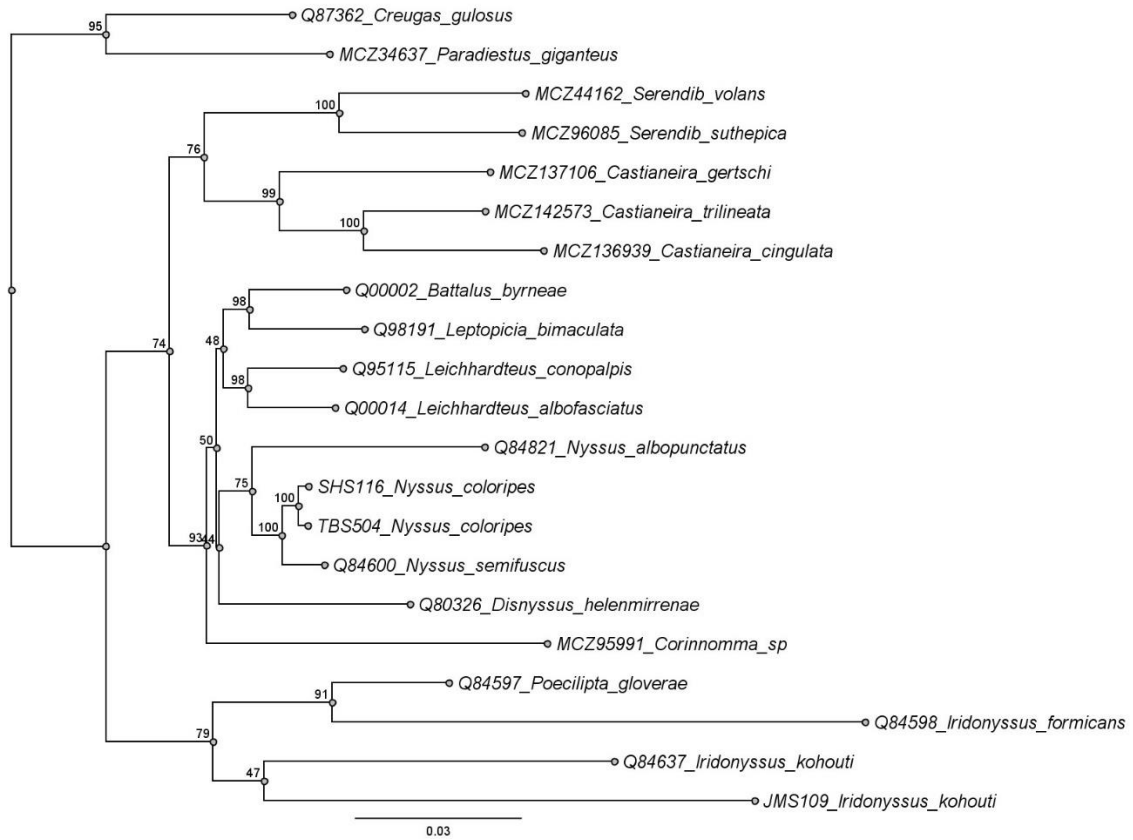

Figure S13. The RAxML tree (with bootstrap values) for the subfamily Castianeirinae (n = 19), with outgroup at the top of the phylogeny (n = 2). Scale bar indicates the number of nucleotide substitutions per site.

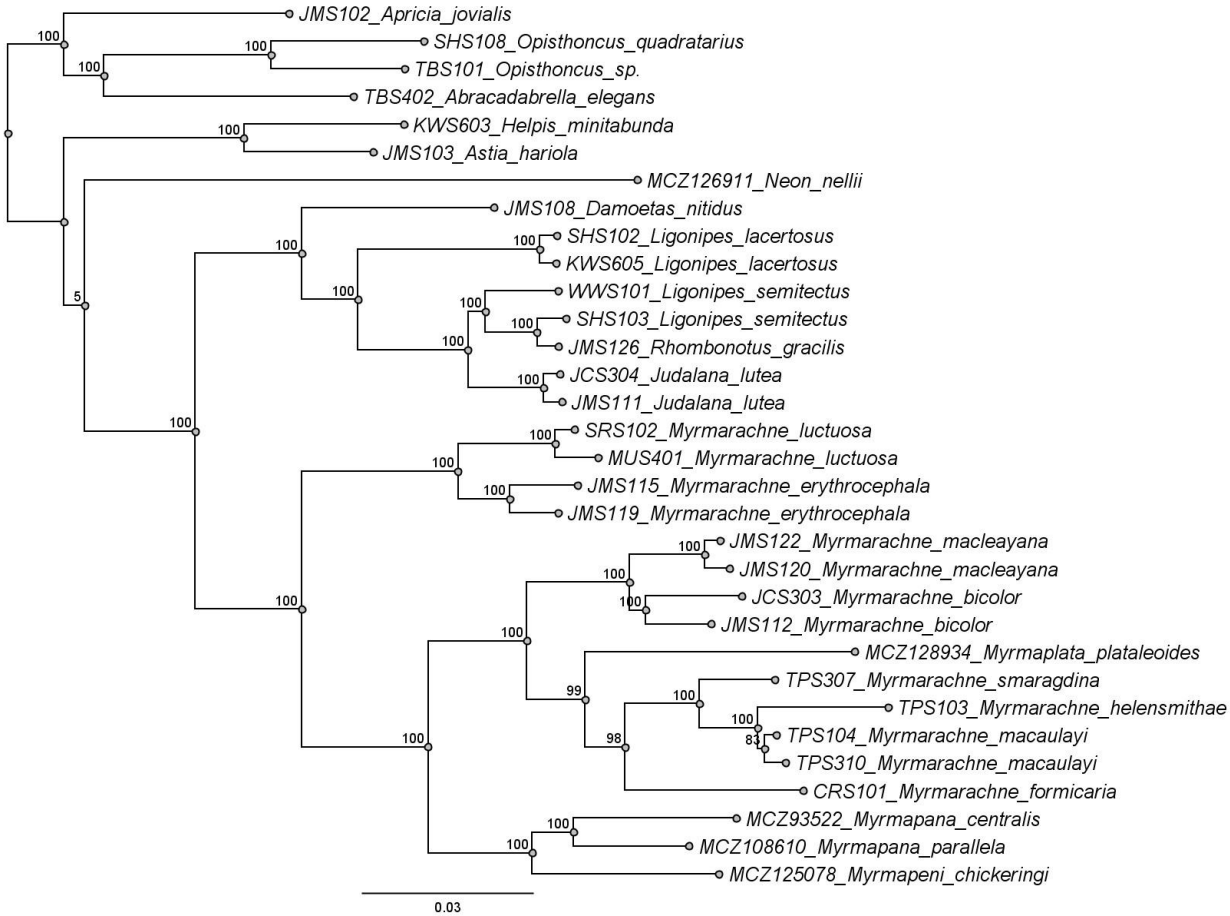

Figure S14. The RAxML tree (with bootstrap values) for the subfamily Myrmarachninae (n = 25), with outgroup at the top of the phylogeny (n = 7). Scale bar indicates the number of nucleotide substitutions per site.

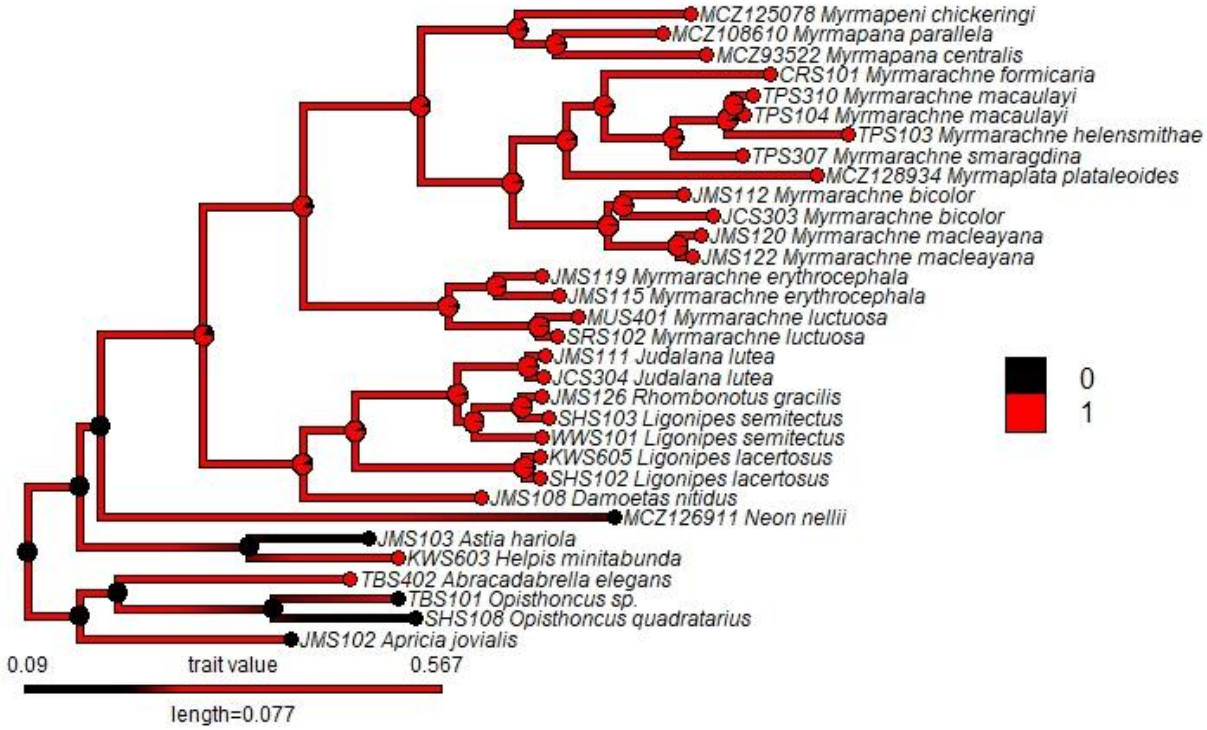

Figure S15. Mapping of the elongated prosoma trait for the Myrmarachninae using the ARD model. Tip node colours indicate the binary (absence/presence) of the trait. Pie charts indicate the likelihood of the trait being absent/present, determined by ancestral state estimation. Scale has a duel function: length indicates the number of nucleotide substitutions per site (i.e. branch lengths) and the heat mapping shows the continuous scoring of the trait.

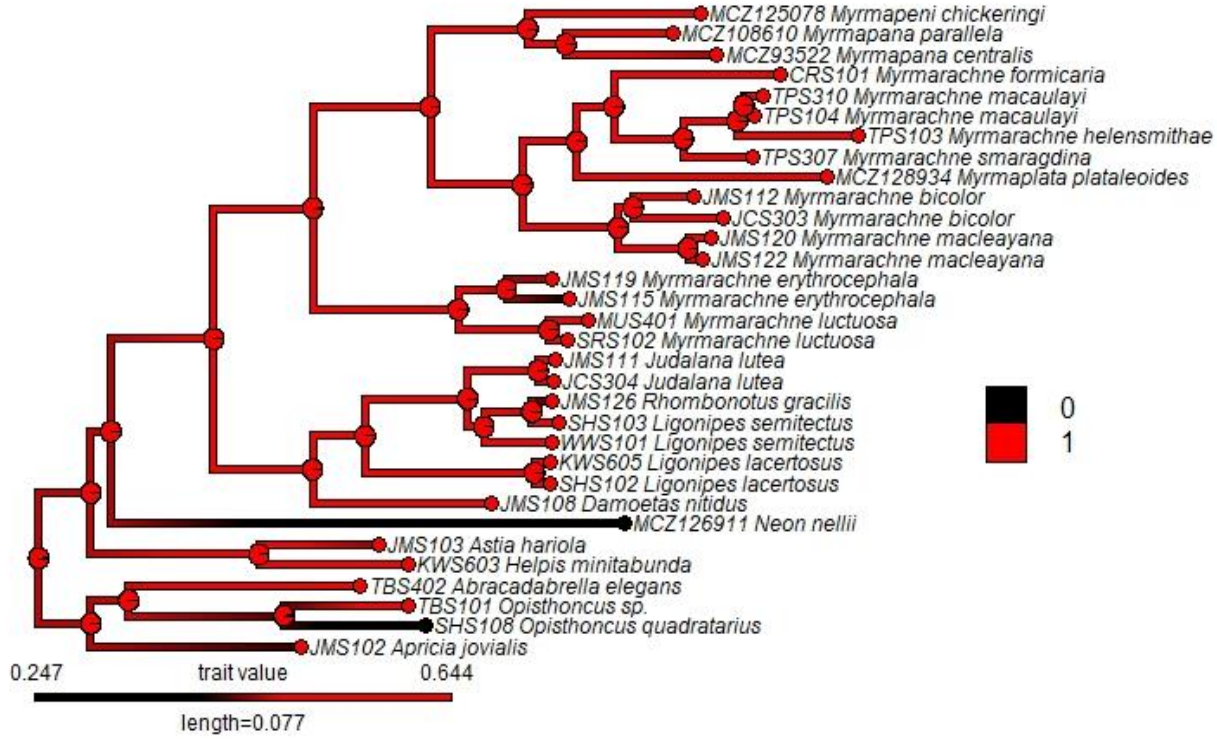

Figure S16. Mapping of the elongated opisthosoma trait for the Myrmarachninae using the ER model. Tip node colours indicate the binary (absence/presence) of the trait. Pie charts indicate the likelihood of the trait being absent/present, determined by ancestral state estimation. Scale has a duel function: length indicates the number of nucleotide substitutions per site (i.e. branch lengths) and the heat mapping shows the continuous scoring of the trait.

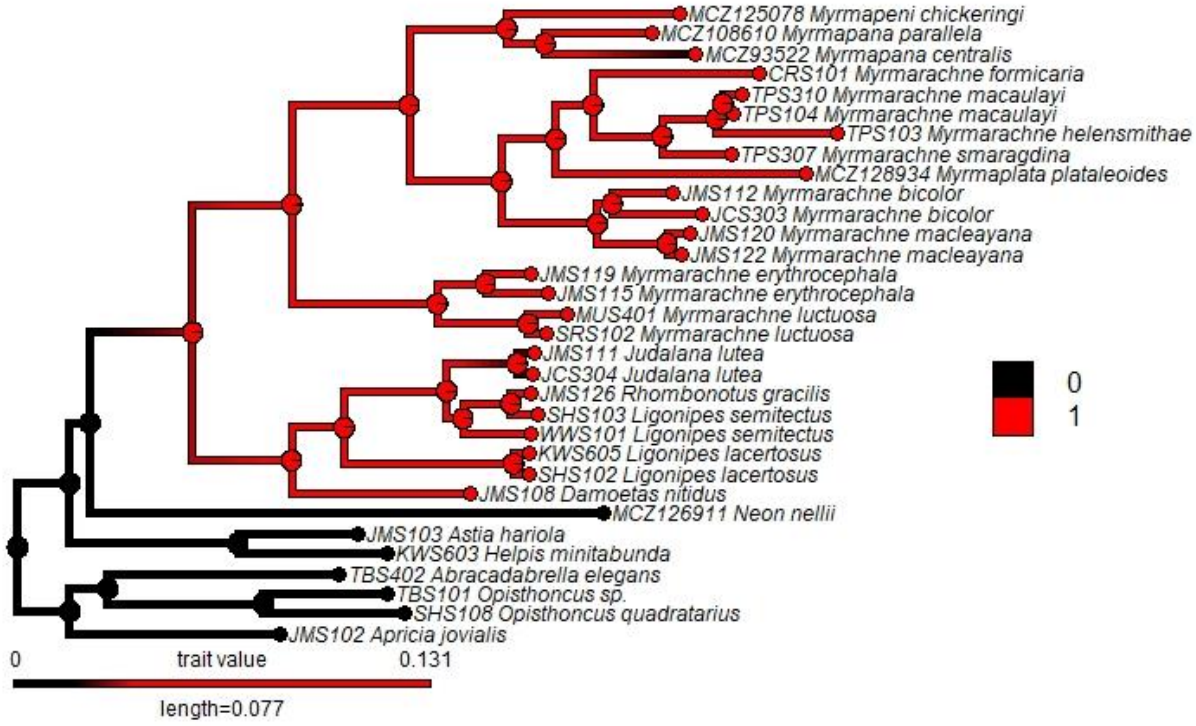

Figure S17. Mapping of the elongated pedicel trait for the Myrmarachninae using the ER model. Tip node colours indicate the binary (absence/presence) of the trait. Pie charts indicate the likelihood of the trait being absent/present, determined by ancestral state estimation. Scale has a duel function: length indicates the number of nucleotide substitutions per site (i.e. branch lengths) and the heat mapping shows the continuous scoring of the trait.

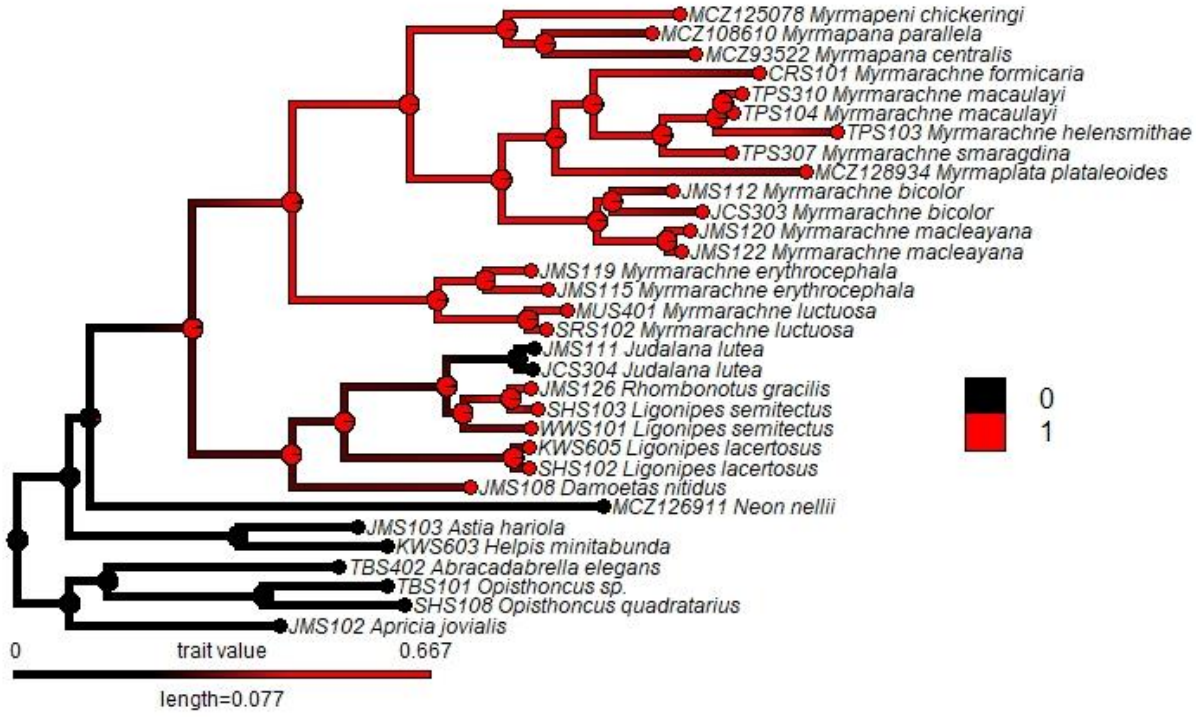

Figure S18. Mapping of the improved colouration trait for the Myrmarachninae using the ER model. Tip node colours indicate the binary (absence/presence) of the trait. Pie charts indicate the likelihood of the trait being absent/present, determined by ancestral state estimation. Scale has a duel function: length indicates the number of nucleotide substitutions per site (i.e. branch lengths) and the heat mapping shows the continuous scoring of the trait.