Australian *Monomorium*: Systematics and species delimitation with a focus on the *M. rothsteini* complex

Kathryn Sparks
School of Biological Sciences
The University of Adelaide
Australia

A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Sciences at the University of Adelaide

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ABSTRACT

*Monomorium* is a speciose genus of myrmicine ants that are found in all major continents including a significant Australian radiation. The systematics of the group is, however, problematic. At the generic level, *Monomorium* represents a polyphyletic assemblage of lineages within the Solenopsidini and requires systematic reassessment of the major clades. At the species level there is taxonomic disagreement about what constitutes a species and how much morphological variation a species can contain. This thesis presents the first molecular study of the Australasian *Monomorium* and presents a systematic framework which is used to test the monophyly of the Australian species groups and explore species diversity across the major clades. In addition, an investigation of the putative *M. rothsteini* species complex is presented as well as taxonomic descriptions of the 23 species identified as part of that study.

An investigation of the relationships among the Australian species of *Monomorium* is presented in Chapter 2. Molecular sequence data from the mitochondrial cytochrome oxidase subunit 1 (*COI*) and the two nuclear markers wingless (*wg*) and elongation factor subunit 1 alpha, F2 copy (*EF1αF2*) was obtained and used to generate a phylogeny of 22 Australian and 9 extralimital species. The Australian species were recovered in two separate clades. Clade 1 which comprised predominantly those species with 11-segmented antennae (including *M. antipodum* from New Zealand) plus the *M. sordidum/M. rothsteini* radiation represents the Australian component of *Monomorium s.str.* while Clade 2, containing those species with 12-segmented antennae, including species from New Caledonia and New Zealand, represents an independent lineage from *Monomorium s.s.* Subsequently, *Chelaner* was brought out of synonymy to encompass those species resolved in Clade 2 and their morphological allies. A phylogenetic analysis using an expanded dataset of *COI* sequences revealed the paraphyly of four of the Australian species groups and of five species suggesting unrecognized species diversity across the two genera.

Chapter 3 presents an investigation of cryptic species diversity in the *M. rothsteini* species complex. A combination of *COI* sequences, morphology and collection records for 171 samples from across the geographic range of *M. rothsteini* was used in a species delimitation study that provides evidence for 38 separate mitochondrial
lineages. Morphological assessment of the clades revealed a complex and overlapping pattern with most lineages morphologically distinct from their sister lineage, some having complete overlap with one or more lineages and a majority occurring sympatrically with one or more genetically and morphologically distinct lineages. Haplotype networks of the nuclear markers EF1αF2 and wg indicated a rapid and recent speciation event with introgression in both the nuclear and mitochondrial genomes.

Of the 38 lineages identified in Chapter 3, 22 were determined as having sufficient evidence to enable formal description. A taxonomic revision of the *M. rothsteini* complex was undertaken and presented in Chapter 4 in which 18 new species were described and four names were brought out of synonymy. Taxonomic descriptions, images, distribution maps and a key are provided.

The species paraphyly discovered as part of this study in both *Monomorium* and *Cheliner* highlights the limitations of taxonomies based solely on morphological characters in problematic ant groups. This issue and its broader implications for ant research in Australia, as well as potential future directions to resolve the issue are discussed in the final chapter.
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CHAPTER I: General Introduction

1.1 Overview

Ants are ubiquitous and abundant over much of the Earth’s land surfaces. Their morphological and behavioral diversity and species-richness reflect the range of habitats they have come to occupy as well as their rich and varied trophic associations with other plant and animal species (Hölldobler & Wilson 1990, Huxley & Cutler 1991, Lach et al. 2010).

The success of the ants has been largely attributed to their eusocial behaviour and the evolution of the metapleural gland (Hölldobler & Wilson 1990). Living in large colonies with overlap of generations enables specialised caste differentiation and division of labour between reproductives and workers. Worker ants are specialised foragers, brood carers and defenders of the nest. They are able to exploit food resources by communicating via odour trails and rapid recruitment. Nest mates undertake individual tasks that contribute to the overall benefit of the colony but can assemble quickly in large numbers to defend against predators. Species with polymorphic workers take division of labour one step further with some worker morphs having special adaptations for particular tasks such as nest defence and seed milling. The metapleural gland in the posterior mesosoma secretes antibacterial and anti-fungal agents which protect both adults and brood from microorganisms. The success of ants as the only eusocial, soil dwelling predators has been largely attributed to this specialised structure (Hölldobler & Wilson 1990).

Ants belong to the single family Formicidae (Hymenoptera) which contains 16 extant subfamilies although four of these encompass 90% of all described ant species (Bolton 2014), and include the three most widespread and diverse subfamilies: Dolichoderinae, Formicinae and Myrmicinae. With well over 6,000 described species in 138 genera (Bolton 2014, Ward et al. 2014) the Myrmicinae is the largest and most diverse subfamily and spans all major land masses (Agosti et al. 2005). These ants are predominantly generalist omnivores but many species have become specialist predators, fungus cultivators or granivores. Monomorium Mayr is one of the largest myrmicine genera with approximately 350 described species, 61 of these being known from Australia (Heterick 2001, Heterick 2003, Heterick 2006).
A previous taxonomic revision of the Australian species of *Monomorium* (Heterick 2001) highlighted both the intra- and inter-specific morphological diversity in the genus and laid the foundation for this study.

Intra-specific morphological variation in worker ants can take the form of the distinct polymorphism of caste differentiation but can also include more subtle variation in size, colour, sculpture, pilosity and other morphological as well as molecular traits. The morphological differences among species, whether due to recent radiations or through convergence, can be similarly quite subtle. These factors, in combination, can present a challenge to delimiting species. Traditional taxonomic methods based solely on morphological differences may be inadequate for groups where intra-specific variation obscures differences among species. An integrative approach combining morphology with other sources of evidence such as DNA sequences, distributional and ecological information may provide more robust, enduring and rigorous species hypotheses.

1.2 Recent advances in higher level ant systematics

Molecular systematics has recently contributed substantially to understanding the phylogenetic relationships both between and within the major ant lineages (Ward 2014). Refinement of higher level ant relationships can greatly increase understanding of the evolutionary origins of some aspects of ant biology and the rise and dominance of ants in the world’s ecosystems. Phylogenetic analyses have been used to infer the origins of the complex set of behaviours exhibited by the three army ant subfamilies (Brady 2003), and used in combination with fossil evidence to infer a historical biogeography of the Myrmeciinae (Ward & Brady 2003). Analysis of multilocus datasets has brought much clarity to the relationships within and among the ant subfamilies and provided robust support for the monophyly of the formicoid clade, the largest ant lineage, which contains most of the highly diverse and ecologically dominant subfamilies (eg. Formicinae, Dolichoderinae and Myrmicinae) (Ward & Downie 2005, Brady et al. 2006, Moreau et al. 2006, Schultz & Brady 2008, Ward 2011, Brady et al. 2014).

The Myrmicinae, the largest of the formicoid subfamilies, is the most recent group to have undergone a large scale molecular phylogenetic treatment (Ward et al. 2014). This study, which was based on 11 nuclear gene fragments and 251 species from
across the subfamily and beyond, resulted in a systematic reassessment of the group
with numerous generic synonymies. Of most interest was the high level of poly- and
paraphyly among many of the species-rich genera. Within the tribe Solenopsidini four
1841, and *Monomorium* were not monophyletic. The situation for *Solenopsis* was
resolved through synonymy, and *Rogeria* and *Adelomyrmex* are likely to be split along
geographic lines. However, resolving the systematics of *Monomorium* is a more
challenging task as its representative species were distributed across six separate
clades, one in the Crematigastriini. Ward et al. (2014) went a long way towards
improving this situation by resurrecting *Syllophopsis* Santschi, 1915 to restore the
*hildebrandti*- and *fossulatum*-groups to monophyly and *Trichomyrmex* Jerdon, 1851
to encompass the *destructor*- and *scabriceps*-groups, now nested within the
Crematogastrini. Despite these changes *Monomorium* remains polyphyletic and
dispersed across four independent clades (Fig. 1).

Advances in higher level ant systematics have paved the way for well-informed
generic and species-level studies. Among the Myrmicinae, *Crematogaster* Lund
(1831) (Blaimer 2010, 2012d, 2012a, b, c), *Pheidole* Westwood (1839) (Moreau
(Garcia & Fisher 2012a, b, 2014b, a), and *Stenamma* Westwood (1839) (Branstetter
2009, 2012, 2013) have all undergone recent revisions. However knowledge of
species-level diversity remains poor. The ants are considered hyperdiverse with over
12,000 described species (Agosti et al. 2005) and an unknown number yet to be
discovered and described. The ubiquity and abundance of ants in complex
ecosystems makes them frequent and ideal subjects for ecological research
(Andersen 2008), for biomonitoring and as indicators of biodiversity (Alonso & Agosti
Nakamura et al. 2007). However, as species are the fundamental biological units of
measurement for comparison in these studies, a reliance on species-level
identification or at least the ability to distinguish morphospecies is essential.
Consequently, the vast number of undescribed species of ants and the lack of
species-level identification tools is often seen as an impediment to this research
(Andersen et al. 2002). Ward (2007) summarized the state of alpha-level taxonomy in
the ants and highlighted the significant amount of work yet to be done on speciose
and widely distributed genera such as *Camponotus, Pheidole, Solenopsis* and *Nylanderia*. However, even for genera that have been subject to large scale revisions, significant work remains to be done. New species are being discovered through the use of DNA sequencing (Schlick-Steiner et al. 2006a, Schlick-Steiner et al. 2006b, Shoemaker et al. 2006) and the same technology, coupled with morphological and biogeographical information, is being used to uphold (Knaden et al. 2005) or synonymise species (Steiner et al. 2006a). More comprehensive surveys in poorly studied regions are also required and will no doubt significantly increase estimates of ant species-level diversity.

**Figure 1.** Phylogram of the Solenopsidini showing the position of five independent *Monomorium* clades (adapted from Ward et al. 2014).

### 1.3 Taxonomic history of the Australian *Monomorium*.

*Monomorium* was first described by Mayr in 1855 and had accumulated 10 subgenera by the early 20th century (reviewed in Bolton, 1987). Ettershank (1966)
raised *Anillomyrma* Emery, 1913 to generic rank and *Chelaner* Emery, 1914 to encompass 37 of the known Australian species together with a further 10 from New Zealand, New Caledonia and New Guinea that all possessed 12-segmented antennae. The remaining 13 Australian species with 11-segmented antennae remained within *Monomorium sensu stricto*. Bolton (1987), within the context of an Afrotropical revision of the Solenopsidini, reviewed the characters on which Ettershank had based the elevation of *Chelaner* and concluded that they were variable within the two genera and consequently synonymised *Chelaner* under *Monomorium*.

The Australian *Monomorium* did not receive further systematic attention until Heterick’s (2001) monograph in which 41 new species were described and 16 synonymies proposed, bringing the total number of species to 59 (Heterick 2001). This number has subsequently increased to 61 following the description of two new species (Heterick 2003). Heterick (2001) divided the Australian species into seven species groups (Table 1) based on “several shared structural characters” but did not clearly outline what the characters were or provide diagnoses for, or a key, to the groups. However, a preliminary cladistic analysis of morphology provided some support for the species groupings. In particular the *monomorium*-group, representing five of the 17 species was recovered as monophyletic as were the *bicorne*- and *kilianii*-groups. In comparison, the *rubriceps*-group was not supported as a natural group, with species distributed throughout the tree.

An alternative view of *Monomorium* species richness in Australia has been proposed (Andersen 2007) (Table 1), in which many of Heterick’s species are referred to as species groups within broader radiations, and the number of species estimated to be around 500, a view that is supported, at least in part, by mitochondrial DNA (Andersen et al. 2013a). Andersen (2000) also provides a key to nine species groups from monsoonal northern Australia that differ markedly from Heterick’s (2001) seven species groups.
Table 1. Australian species groups of *Monomorium* (modified from Heterick 2001). Species highlighted in blue are those that were formerly placed in the genus *Chelaner*. Species that are in boxes are possible/likely species complexes based on levels of intra-specific morphological variation (Heterick 2001, Andersen 2007).

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<th>bicorne-group (1)</th>
<th>falcatum-group (2)</th>
<th>insolescens-group (3)</th>
<th>killanii-group (4)</th>
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<td>anthracinum Heterick</td>
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<td>xantheklemma Heterick</td>
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1.4 The *Monomorium rothsteini* Forel, 1902 species complex

One of the species most likely to be a species-complex is *M. “rothsteini”* (indicated hereon by inverted commas around the name) Species in this complex are distributed over most of mainland Australia, with a predominantly Eyrean and Toressian distribution, which extends as far south as the Victorian mallee and as far north as the Tiwi Islands (Andersen et al. 2004). Across this geographic range, distinct variants can be identified. Heterick (2001) describes the forms *doddi, leda* and *humilior*, found in the tropics and sub-tropics, as large bodied with an orange or fulvous head, orange mesosoma and brown metasoma. In contrast *subapterum bogischi*, with a more widespread distribution, is smaller with a brown or crimson head capsule, and paler mesonotum. This form also has distinctive bicoloured queens. The form *subapterum*, which has an apparently north and south-western distribution, is highlighted as one of the more obviously distinct forms, described as gracile in appearance with a flattened clypeal margin and lacking the median clypeal carina that is obvious in other forms (Heterick 2001). A similar form to *subapterum* occurs in the Tanami desert but differs significantly in colour. These variations apparently grade into one another along geographical clines with lighter and smaller forms in the south grading into darker and larger forms in the north. Heterick (2001) also observed marked size and colour variation among nest mates. It is on this evidence that he concluded that *M. rothsteini* was a single, highly variable species. In comparison, Andersen (2007) estimates *M. “rothsteini”* is likely to be a species complex with at least 50 species.

Due to the relative abundance of the *M. “rothsteini”* complex throughout the arid and tropical zones, it is frequently encountered in ecological studies and field observations have been numerous. Species in this complex are true seed harvesters, over 90% of the diet being seed or plant material (Briese & Macauley 1977), with a small proportion of the diet consisting of invertebrate protein (Davison 1982, Andersen et al. 2000). One population has been shown to harvest seeds from 10 different plant species in nine genera at a site in western NSW (Davison 1982) and another population 27 plant species at a tropical savannah site (Andersen et al. 2000). This seed diversity reflects the seasonal availability of seed species, as well as size, chemical composition, and presence or absence of a hard seed coat (Davison 1982). Peak foraging in Davison’s (1982) study was in summer with activity...
outside the nest ceasing completely during winter, while in the northern tropics the species forages year round (Andersen 1991, Andersen et al. 2000). Seeds may be collected up to 40 m from the nest and stored in nest chambers or granaries before being fed to larvae (Briese 1982). In a laboratory colony workers were not observed eating any seeds directly but fed on oral secretions from the larvae (Davison 1982).

*Monomorium “rothsteini”* nests in the ground and maintains obvious nest middens consisting of seed husks, arthropod fragments and small pebbles (Davison 1982). Nest densities have been observed at 3 per 1000m$^2$ in the tropics (Andersen et al. 2000) and up to 14 per 500m$^2$ in a temperate study (Briese 1982). At least one *M. “rothsteini”* population has been observed to produce nests by fission where one large colony gives rise to several smaller ones, which can result in a high localised density of nests (Briese 1982). Colony size can be up to 58,000 workers in large colonies with multiple entrances (Davison 1982).

*Monomorium “rothsteini”* workers are generally small, slow moving and non-aggressive. The species’ range overlaps with more aggressive and dominant food competitors such as species of *Iridomyrmex* and yet it remains a very successful element of a highly diverse fauna. The reason for the success of these ants may lie in unique aspects of their venom chemistry. Application of *M. “rothsteini”* venom alkaloids to food stations has been shown to deter other species of ants (Andersen et al. 1991). This may explain the ability of *M. “rothsteini”* to monopolise food resources in the presence of more aggressive ants and other species of *Monomorium* (Andersen 1992).

Venom chemistry may also have some taxonomic importance, particularly at higher classification levels. Studies on the venom chemistry of *Monomorium, Solenopsis* and *Megalomyrmex* Forel (1885) have revealed similarities that reflect the close relatedness of these three genera (Jones et al. 1991, Jones et al. 1996). Further, the venom of two Australian *Monomorium* species contain an alkaloid with long carbon side-chains, a feature of the venom chemistry that has not been found in other *Monomorium* species from the USA, Europe and South Africa (Andersen et al. 1991). Venom chemistry may also help to identify cryptic diversity. A comparison of venom alkaloids between populations of the New Zealand species *M. antarcticum* has indicated there may be four or more “species” comprising a species-complex (Jones...
et al. 1988b), although these data are yet to be supported by further taxonomic research. Conspicuous differences in venom chemistry discovered between queens and workers of the same species may indicate that venom plays a different role among castes; for example, protecting the eggs from fungal infections in queens versus defense in workers (Jones et al. 1991, Jones et al. 1996), but this may limit its utility as taxonomic character.

Venom chemistry is yet to be proven a useful character in species level taxonomy. However, differences discovered in the venom chemistry between populations of *M. rothsteini* (Jones et al. 2009) lends support to the species-complex hypothesis and may form part of the suite of diverse properties that could assist in delimiting these species.

### 1.5 Species concepts, species delimitation and integrative taxonomy

Ernst Mayr has often been described as the forerunner of modern species concepts, and his *Systematics and the Origin of Species* (Mayr 1942) was a watershed publication in the history of the field (de Queiroz 2005, Hey 2006). Although Mayr's ideas about species concepts evolved throughout his career (reviewed in Hey 2006) he is generally credited with defining species in terms of reproductive isolation, commonly referred to as the "biological species concept". Despite the significant impact and enduring influence of Mayr's biological species concept, a diverse assemblage of alternative concepts has amassed since its publication. The diversity in these ideas reflects the range of biological fields of the authors and each concept probably has some level of applicability in the field for which it was designed. However, if a true understanding of the nature of species is to be achieved then a concept that is more unified and more generally applicable is required.

In reviewing species concepts de Queiroz (2005, 2007) argues that all species concepts have a common element and that the differences are actually secondary operational criteria that confuse the species concept issue with species delimitation. The common element in all concepts is that species arise by evolution, that is, they are separately evolving metapopulation lineages (de Queiroz 2007). The defining elements on which the species concepts are based are not cast aside in this definition but are re-employed as "secondary species criteria". As a lineage diverges it attains one or more of these secondary criteria (e.g. reproductive isolation or
genotypic monophyly). With further divergence lineages may attain additional criteria (e.g. distinct ecologies or morphological diagnosability), increasing the ease with which species can be delimited. The point is that a lineage does not necessarily have to have taken on any particular criteria to be considered a species. It is the “cut-off” point that remains subjective. Is a species considered to be distinct when it has attained morphological diagnosability or genetic monophyly or reproductive isolation? This decision will inevitably be a matter of scientific opinion. However, de Queiroz (2007) proposes that the secondary criteria be removed from the species concept debate altogether and instead be used, in this way, as lines of evidence in delimiting species.

There has been a general acceptance of the concept that species are separate evolutionary lineages and increased interest in the problematic area of species delimitation. With the advancement of DNA technology (Shaffer & Thomson 2007, Vogler & Monaghan 2007), empirical methods (Sites & Marshall 2003, 2004, Knowles & Carstens 2007), GIS and ecological modelling (Raxworthy et al. 2007, Rissler & Apodaca 2007) there is a growing number of lines of evidence that can be used to support species hypotheses (Dayrat 2005). The use of DNA sequences in delimiting species has had the greatest impact and generated the most controversy over the past decade. Using single gene sequences, namely that of the mitochondrial gene (mtDNA) cytochrome c oxidase I (COI), was proposed as a novel and rapid method for discovering and identifying species (Hebert et al. 2003, Hebert & Gregory 2005) and assessing biodiversity (Smith et al. 2005). Termed “DNA barcoding”, this use of DNA sequences has been touted as the solution to the apparent global crisis in alpha (species) level taxonomy (Hebert & Gregory 2005) and the inherent difficulties in morphological taxonomy; for example phenotypic plasticity, recognition of cryptic taxa, and inadequacies of keys based on morphology (Hebert & Gregory 2005).

Despite numerous criticisms of DNA barcoding (Will & Rubinoff 2004, Ebach & Holdrege 2005a, b, Will et al. 2005, Wheeler 2007), with many studies based on the inadequacy of using single datasets and fixed divergence thresholds, it is clear that DNA sequence data is a powerful addition to the taxonomy toolbox. For example some recent ant studies that have used sequence data to inform taxonomy include:

There is now growing interest in and support for integrative taxonomy (DeSalle et al. 2005) or iterative taxonomy (Yeates et al. 2011). Integrative taxonomy is not a new concept. Taxonomists have been using multiple lines of evidence *sensu* de Queiroz (2007) (e.g. host records, distribution in time and space) to compliment studies of morphology long before molecular sequences became conventional. Although, it is in the context of integrative taxonomy that the use of DNA sequences has the most widely accepted application. There are a growing number of studies using varied molecular datasets (allozymes, nuclear and mitochondrial DNA, microsatellites or single nucleotide polymorphisms) in combination with other data sources, including morphology, to delineate species and discover cryptic speciation. These multidisciplinary studies employ such diverse datasets as fungal symbionts in agricultural ants (Schultz et al. 2002), inter-specific aggression tests (Steiner et al. 2004), morphometrics (Steiner et al. 2006a, Steiner et al. 2006b) and cuticular hydrocarbons (Lucas et al. 2002) to formulate robust species hypotheses

1.6 Aims of this project

The aim of this study is to progress the systematic resolution of the Australian species of *Monomorium*. Specifically:

1. To develop a molecular phylogenetic framework for the Australian *Monomorium*, to test the monophyly of the Australian species-groups as defined by Heterick (2001).

2. To explore the evidence for unrecognized species diversity.

3. To test the species-complex hypothesis for *M. rothsteini*.

4. To describe any new species arising from the outcomes of the above aims.

1.7 Conclusions

The evolution and higher level systematics of ants has attracted much scientific attention in recent years. Large scale ant phylogenies have greatly improved the understanding of most high-level (subfamily) relationships but there remain
significant knowledge gaps in species-level diversity with many hyper-diverse genera yet to be fully revised.

Historically, ant species' descriptions have been based on worker morphology. Delimiting species based solely on morphology has proved problematic for some groups where clear differences among species are concealed by marked intra-specific variation. An integrative approach to taxonomy that couples morphology with other sources of information, lending further evidence to species hypotheses, can provide resolution where morphology alone cannot.

The revision of the Australian species of Monomorium by Heterick (2001) has provided a broad foundation on which further work can be based. Several probable species complexes were highlighted in his revision and it is likely, based on levels of intra-specific morphological variation, that more will be identified. This study goes at least part way towards addressing the problematic species level taxonomy of one challenging group of myrmicine ants, the M. “rothsteini” complex.

1.8 Structure of this thesis

Two of the three results chapters of this thesis (Chapters 3 and 4) consist of two published papers while Chapter 2 is written as a paper ready for submission. The list of references for Chapters 3 and 4 are in those chapters as part of the inserted publications. A single list of cited reference for the remaining chapters is provided in a separate section at the end of the thesis.
CHAPTER II: Molecular phylogenetics of Australian *Monomorium* ants (Hymenoptera: Formicidae): para- and polyphyly and the resurrection of *Chelaner*.

KATHRYN S. SPARKS¹, ALAN N. ANDERSEN² and ANDREW D. AUSTIN³

¹South Australian Museum, North Terrace, Adelaide SA 5000, and Australian Centre for Evolutionary Biology and Biodiversity, and School of Earth & Environmental Sciences, The University of Adelaide, 5005, Australia, email: kate.sparks@samuseum.sa.gov.au

²CSIRO Ecosystem Sciences, Tropical Ecosystems Research Centre, PMB 44 Winnellie, NT 0822, Australia, email: Alan.Andersen@csiro.au

³Australian Centre for Evolutionary Biology and Biodiversity, and School of Earth & Environmental Sciences, The University of Adelaide, 5005, Australia, email: andy.austin@adelaide.edu.au

*Author for correspondence

2.1 Abstract

*Monomorium* is a cosmopolitan genus of myrmicine ants (tribe Solenopsidini) that has undergone numerous systematic changes and at present represents several paraphyletic lineages. The taxonomy at the species level is also problematic as simplified and overlapping morphology is thought to obscure species boundaries. This study uses molecular phylogenetic analysis of two nuclear genes and one mitochondrial gene to investigate the systematics of the Australian species of *Monomorium*. Under both Bayesian Inference and Maximum Likelihood the separation of the Australian *Monomorium* into two major paraphyletic clades is supported. One clade comprises the small species with 11-segmented antennae, along with the *M. rothsteini* and *M. sordidum* radiations. It also includes three African species (*M. floricolor*, *M. junodi*, and *M. pharaonis*) we included in our analysis, and corresponds to the ‘core’ *Monomorium* identified in a recent phylogenetic analysis of the genus. The second clade includes all other non-cryptobiotic Australian species with 12-segmented antennae, along with the New Zealand species *M. antarcticum
and *M. smithii*, and two undescribed species from New Caledonia. Our analyses do
not recover a monophyletic relationship between two species of cryptobiotic
*Syllophopsis*. Two undescribed Australian cryptobiotic species allied to *Monomorium*
are not recovered as monophyletic with species of either *Syllophopsis* or
*Anillomyrma*. COI analysis indicates that several currently recognized Australian
species of *Monomorium* (*M. fieldi, M. laeve, M. sydneyense, M. stictonotum* and *M.
leae*) are paraphyletic. The New Zealand *M. antipodum* is recovered as a valid
species, and has a close molecular relationship with a sample collected from eastern
Australia. We resurrect the genus *Chelaner* Emery to encompass those species
within the second major Australian clade, and outline morphological characters to
separate *Chelaner* from *Monomorium* in Australia. Based on the phylogeny, we
hypothesise that *Chelaner* is an older radiation with a Gondwannan origin, while
*Monomorium* represents a more recent incursion into Australia via Asia.

2.2 Introduction

*Monomorium* Mayr, 1855 is a large genus of Myrmicine ants with a distribution that
covers all major continents. The genus was first described in 1855 and by the early
20th century had accumulated 10 subgenera erected by various authors (reviewed in
Bolton, 1987). One of these was *Chelaner* Emery 1914, which Ettershank (1966)
raised to generic level to encompassed 37 of the known Australian species together
with a further 10 from New Zealand, New Caledonia and New Guinea. The other 13
Australian species remained within *Monomorium* sensu stricto. After reviewing the
characters on which Ettershank had defined *Chelaner*, namely the palpal formula and
propodeal spiracle, both of which were variable in the two genera, Bolton (1987)
synonymised *Chelaner* with *Monomorium*. In his review of the Afrotropical
*Monomorium*, (Bolton 1987) did not use any subgeneric groupings, preferring to use
a species-group classification for the Afrotropical fauna. He also outlined three
possible species-groups for the Australian fauna, expressing the realisation that there
was much work to be done on the genus in the Australasian region.

No further taxonomic work was undertaken on the genus in Australia until Heterick’s
(2001, 2003) revision of the fauna which brought the total number of species to 61.
Like Bolton (1987), Heterick (2001) used seven species-groups to associate
morphologically similar taxa, but it is unclear what characters these groups are based
on. Subsequent work on what Heterick (2001) described as morphologically variable species has shown them to represent highly diverse species complexes, and Andersen (2007) estimates the true number of Australian species to be around 500.

A recent global reassessment of myrmicine systematics based on a molecular phylogenetic study has partially resolved some generic-level issues associated with *Monomorium* (Ward et al. 2014). *Trichomyrmex* Mayr, 1865 was resurrected to accommodate members of the *destructor*-group, and belongs to a different tribe (Crematogastrini). *Syllophopsis* Santschi, 1915 was also re-established to include those species belonging to the *hildebrandti*- and *fossulatum*-groups. Those species of *Monomorium* that were not assigned to other genera remain in four polyphyletic clades. New Zealand’s *M. antarcticum*, the only species of ‘Chelaner’ included in the analysis, formed a clade with *M.* (previously *Nothidris*) *denticulatum*, along with species from the austral genera *Austromorium* (Australia) and *Oxyepoecus* (South America). In contrast, *M.* nr. *fieldi*, the only Australian species included in the analysis, belonged to a clade that included Asian and African species. Some taxa previously referred to *Chelaner* (the *rothsteini* and *sordidum* groups) are believed to be related to *M.* *fieldi* and allies (including Asian and African species) rather than to the Gondwanan groups of typical *Chelaner* (Heterick 2001; Andersen 2007). There is therefore a pressing need for a broader phylogenetic assessment of the Australian *Monomorium* fauna, with more comprehensive sampling of these clades, to establish a more stable systematics.

Our aim in this study is to present such an assessment by reconstructing a phylogeny of the Australian species. We examine relationships among major Australian lineages, and test the monophyly of the morphologically based species-groups, with a particular focus on resolving the status of *Chelaner*. We also resolve the phylogenetic relationships of two Australian cryptobiotic species that are apparently allied to *Monomorium*, and explore genetic variation in species showing high morphological variability. Finally, we assess the phylogenetic validity of the species groups recognized by Heterick (2001).
2.3 Materials and Methods

2.3.1 Species selection and specimen collection.

An attempt was made to source as many representatives of Australian *Monomorium* species as possible, but many taxa are rare in collections and seldom encountered in the field. Suitable specimens were available for sequencing from 21 of the 61 species recognised by Heterick (2001). Where possible, multiple specimens that spanned the morphological diversity of a species were used. *Monomorium fieldi* Forel, 1910b, *M. leae* Forel, 1913, *M. laeve* Mayr 1876, and *M. sydneyense* Forel, 1902 are all very small, widespread, generalist species that exhibit variation in colour and sculpture. Included under these names were samples that represented some of the morphological variants attributed to these species. These taxa are here referred to as *M. fieldi* (donisthorpei-form), *M. fieldi* (nigrius-form), *M. fieldi* (sp. A), *M. fieldi* (sp. 18), *M. leae* (dark form), *M. leae* (light form), *M. leae* (flavipes-form), *M. laeve* (sp. 23), *M. laeve* (sp. 24), *M. laeve* (sp. 33), and *M. sydneyense* (carinatum-form). In addition, two undescribed taxa of blind, cryptobiotic ants apparently allied to *Monomorium* were included. Their de-pigmented integument, combined with a complete lack of eyes, enlarged fore coxae, short broad fore femora and tibiae, and the absence of the anteroventral process on the petiole present a superficial resemblance to *Anillomyrma* Emery, 1913 (Bolton 1987, Eguchi et al. 2010). However, these ants possess many characters that set them apart from *Anillomyrma* including, but not limited to, 12-segmented antennae, six mandibular teeth and denticles, and a short anterior petiolar peduncle. These two taxa also bear a strong resemblance to species of *Syllophopsis*, but there are no species in this genus that have a complete lack of eyes (Heterick 2006). To test the relationship of these taxa to *Anillomyrma* and *Syllophopsis*, sequences of the latter two were obtained from Genbank and included in our analyses.

Extralimital species from Africa (*M. floricolor* Jerdon, 1851, *M. junodi* Forel, 1910b and *M. pharaonis* L., 1758), New Caledonia (*Monomorium* sp. undescribed), Sri Lanka (*M. latinode* Mayr, 1872) and New Zealand (*M. antarcticum* (Smith 1858), *M. antipodum* Forel, 1901 and *M. smithii* Forel, 1892) were also included to inform broader biogeographic relationships between Australian species and those outside the Australian continent. *Monomorium antipodum* is difficult to separate from *M. fieldi*
and its identification in New Zealand has been the subject of some debate (Gunawardana 2005, Don 2007). This species has not been recorded from Australia (Atlas of Living Australia website) but specimens collected in Queensland have been tentatively assigned to this species. We included a specimen of *M. antipodum* from New Zealand and one identified as *M. c.f. antipodum* from Australia to test the validity of the name in relation to *M. fieldi* and to determine the status of the species in Australia.

The outgroup species were chosen to represent lineages of decreasing relatedness to *Monomorium*, and included two genera from the tribe Solenopsidini (*Myrmicaria* Saunders, 1842 and *Solenopsis* Westwood, 1841), three from outside the Solenopsidini (*Cardiocondyla* Emery, 1869, *Stereomyrmex* Emery, 1901 and *Trichomyrmex* Mayr, 1865) and one from Myrmicini (*Myrmica* Latreille, 1804).

Specimens were either collected in the field over the period 2003-2011 by the authors, or donated by other institutions and researchers as ethanol preserved specimens. A full account of specimens, their region of origin and Genbank accession numbers is listed in Table 1.

2.3.2 Molecular protocols and sequence analysis

DNA was extracted from whole ants, or from three legs from the right side of larger specimens using the Puregene DNA Purification Kit (Gentra Systems Inc.). Amplification of the mitochondrial protein coding gene *cytochrome oxidase I* (COI) were obtained by polymerase chain reaction (PCR) using the primers LCOI490: ‘5-GGTCAACAAATCATAAAGATATTGG-3’ and HCO1298: ‘5-TAAACTTCAGGGTGACCAAAAAATCA-3’ (Folmer et al. 1994) and Jerry ‘5-CAACATTTTGTAGTTTGG-3’ (Simon et al. 1994)/Ben ‘5-GCTACTACATAATAKGTATCATG-3’ (Moreau et al. 2006). Amplification of a fragment of the wingless gene was carried out for a subset of samples using primers Wg578F 5’-TGCAACNGTGAACCTGGATGC-3’ (Ward & Downie 2005) and Wg1032R 5’-ACYTGCAGCACCARTGGCG-3’ (Abouheif & Wray 2002) and for *EF1αF2* using the primers F2-557F 5’-GAACGTGAACCTGGATGC-3’ and F2-1118R 5’-TTACCTGAAGGGGAAGACGRAG-3’ (Brady et al. 2006). PCR amplifications were carried out in 25µL containing 13.5µL water, 2.5µL PCR buffer, 2µL dNTP, 3µL MgCl2, 1µL of each primer (5µM), 0.1µL AmpliTaq Gold DNA
Polymerase (Applied Biosystems Inc.) and 2μL extracted DNA. All reactions were initially denatured at 95°C for 9 min followed by 40 cycles of 95°C for 30 s, an annealing temperature of 47°C for 30 s and an extension temperature of 72°C for 60 s. This was followed by a further extension for 6 min at 72°C.

For a small subset of samples the Finnzymes Phire® Animal Tissue Direct PCR Kit was used for DNA extraction and PCR amplification using the dilution protocol and the 3-step PCR protocol with annealing temperatures between 49°C and 59°C. PCR products were visualised on an agarose gel and purified with a PCR Clean-up DNA purification kit (MoBio Laboratories Inc., Solana Beach, CA). Sequencing was undertaken using the ABI prism Big Dye Terminator Cycle sequencing kit (PE Applied Biosystems, Foster City, CA) and sequencing was carried out on an ABI 3730 DNA analyser.

Forward and reverse sequences were trimmed, assembled and aligned by eye using Bioedit 7.0.9 (Hall 1990). Translation of the DNA sequences to proteins was carried out in MEGA v. 5 (Kumar et al. 2008) and checked for the presence of nuclear paralogues. The COI dataset was partitioned by codon, and Modeltest (Posada & Crandall 1998) was used to estimate the best model of sequence evolution and the selected model (GTR+I+G) was used in the Bayesian Inference (BI) analysis. As complete sequence data were only available for a subset of samples for each gene phylogenetic analysis was performed on six different datasets using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The datasets were as follows: EF1αF2+wg (27 samples), COI+EF1αF2 (27 samples), COI+wg (39 samples), a combined 3-gene analysis with missing data (47 samples) and without missing data (22 samples) and a COI only analysis (85 samples). The two-gene analyses were performed for 6 million generations, the three-gene and COI only analysed for 8 million, each sampling every 100 generations. TRACER 1.4 (available from http://beast.bio.ed.ac.uk/Tracer) was used to check for chain convergence and the first 25% of trees were discarded as burn-in.

Maximum likelihood (ML) trees were generated for all five datasets using the program RAxXML accessed through the Vital-IT Unit of the Swiss Institute of Bioinformatics (http://phylobench.vital-it.ch/raxmlbb/) and the results compared with the Bayesian analyses.
2.4 Results

2.4.1 Monomorium phylogenetics

The analyses strongly supported the monophyly of the Solenopsidini clade (Ward et al. 2014) for all analyses (Fig 1-4, S2) except the COI+EF1αF2 (Fig. S1). Within the Solenopsidini, two separate Monomorium clades were resolved with strong support in all analyses (Fig. 1-4) except for the COI only analysis (Fig. 4) which had moderate BI support but poor ML support for Clade 2 (PP 0.78, BS 0.25) (Fig. 4). Clade 1 contained the small, Australian and New Zealand species that have 11-segmented antennae, along with *M. rothsteini* forel, 1902 and *M. sordidum* Forel, 1902, which have 12-segmented antennae. It also includes the three African species, *M. floricolor*, *M. pharaonis*, and *M. junodi*. This clade corresponds to the ‘core’ *Monomorium sensu* Ward et al. (2014).

Clade 2 comprises the remaining Australian *Monomorium* species with 12-segmented antennae as well as *M. antarcticum* and *M. smithi* from New Zealand and the two undescribed species from New Caledonia. The undescribed, de-pigmented and blind taxa of uncertain placement (*Monomorium* spp. (CJB30906 and QM31277)), formed a sister relationship with Clade 2 with strong support in three analyses (3-genes (Fig. 1), 3-genes (Fig. 2) and $EF1\alpha F2+wg$ (Fig. 3)). None of the analyses supported a monophyletic relationship between these taxa and either of the two *Syllophopsis* species or the clade containing the two *Anillomyrma* species.

*Monomorium latinode* fell outside both of these clades but formed a sister relationship with Clade 1 with strong support in two analyses (3-genes (Fig. 2) and COI+EF1αF2 (Fig S1)).

The monophyly of *Syllophopsis* was not supported by any analyses. *Syllophopsis fisheri* was resolved as sister to Clade 1 + *M. latinode*, although support for this relationship varied between the two analyses that included *S. fisheri* (3-gene: PP 51, BS 50 (Fig. 1) and $EF1\alpha F2+wg$: PP 100, BS 58 (Fig. 3)). *Syllophopsis seychellensis* was resolved in a separate clade to *S. fisheri* as sister to Clade 2, also with variable support values (Fig 1-4, S1-2).
The position of *M. decuria* Heterick, 2001 is unclear. The COI analysis places this species in a weakly supported sister relationship with *Monomorium* Clade 1 + *Myrmicaria* whereas the COI+wg analysis places it as a strongly supported member of *Monomorium* Clade 2. *Monomorium decuria* is unique within the Australian *Monomorium* as it is the only species to have 10-segmented antennae, but is otherwise extremely similar morphologically to *M. falcatum* (Heterick 2001, Andersen 2007), which has 12-segmented antennae.

2.4.2 Australian Monomorium species-groups

To further examine the relationships among the Australian species of *Monomorium* a phylogenetic analysis of the COI lineages only was carried out for a much larger dataset that included significantly more species, and, where available, more representatives of each species.
Figure 1. Bayesian tree of the concatenated COI, EF1αF2 and wg data for all samples. Solid circles denote notes with ≥95 Bayesian posterior probability and ≥70 ML bootstrapping support. Unique specimen codes follow the taxon names.
The *monomorium*-group of Heterick (2001) was well supported, forming a monophyletic lineage within Clade 1 (Fig. 4). However, the *rubriceps*-, *longinode*-, and *kilianii*- groups of Heterick (2001) form para- or polyphyletic assemblages. The *rubriceps*-group was split into seven separate lineages (although only two of these had strong support), and members of the *kilianii*-group and the *longinode*-group each bore closer relationships to members of other species groups. *Monomorium tambourinense* in the *kilianii*-group was resolved as more closely related to *M. insolescens* although with moderate support (PP 0.65) and *M. bifidum* in the *longinode*-group was resolved as a sister taxon to *M. legulus*, a member of the *rubriceps*-group, also with moderated support (PP 0.75). Little could be said about the monophyly of the *insolescens*-group, which contains a single species, or the *falcatum*-groups, which is represented here by only one species (*M. decuria*). The two New Zealand species, *M. antarcticum* and *M. smithii*, form a well-supported monophyletic clade, but the relationship among the two species from New Caledonia and the remaining species in Clade 2 is poorly resolved.
Figure 2. Bayesian tree of the 3-gene analysis (COI, EF1αF2 and wg) with complete data for all taxa. Solid circles denote notes with ≥95 Bayesian posterior probability and ≥70 ML bootstrapping support. Other nodes of interest that do not fall into this support category are indicated separately. Unique specimen codes follow the taxon names.
2.4.3 Monomorium species complexes

Within Clade 1, COI analysis indicated that the *M. rothsteini* and *M. sordidum* species complex and *M. sordidum* group which bear close morphological affinities, formed a monophyletic sister clades. However, other ‘species’ in the clade are highly polyphyletic. The ‘carinatum’ form of *M. sydneyense* from northern Australia was monophyletic, but in a different clade to the more typical form from southern Australia (KSS30). Those linages belonging to *M. fieldi* and *M. leave* were even more problematic. The majority of *M. fieldi* samples formed a well-supported clade that was
further divided into five well-supported clades with relatively deep divergences. However, one *M. fieldi* sample (KSS16) was resolved as a member of a clade that included samples of *M. leave* and *M. sydneyense*. *Monomorium laeve* was split into three polyphyletic lineages. The two *M. stictonotum* Heterick, 2001 samples were not resolved as a monophyletic clade. *M. antipodum* from New Zealand and *M. cf. antipodum* from Australia formed a well-supported lineage outside of the *M. fieldi* clades described above.

With a greater number of species represented but fewer duplicates, species paraphyly was less prevalent in Clade 2. *Monomorium leae* was represented by three distinctive morphological types that were also found to be distinctive at a molecular level. In particular, the ‘flavipes’ form was recovered as part of a moderately supported clade (PP 0.88) containing species from four of Heterick’s (2001) species-groups but no other *M. leae* morphotypes.
Figure 4. Baysian tree of the COI only data for all samples. Solid circles denote notes with ≥95 Bayesian posterior probability and ≥70 ML bootstrapping support. The basal node of the two *Monomorium* clades are indicated with an arrow. Unique specimen codes follow the taxon names.
2.4.4 Taxonomic changes

The phylogenetic results here support the monophyly of an Australasian clade of species (Clade 2), hereafter referred to as the ‘Chelaner’ clade. The majority of these species are united by having 12-segmented antennae, a palp formula of 2,3 and a mandibular tooth count of 3-7 (Heterick 2001, Heterick 2003). Members of the *M. monomorium*-group clade overlap morphologically with the ‘Chelaner’ clade in having a mandibular tooth count of 3-4 but having a palp formula of 1,2 or 2,2, and 11-segmented antennae distinguish most species in this clade from the Australasian ‘Chelaner’ clade. However, *M. crinitum* Heterick, 2001, *M. petiolatum* Heterick, 2001, *M. sculpturatum* Clark, 1934, *M. shattucki* Heterick, 2001, and *M. tambourinense* Forel, 1915 from Clade 2 have a palp formula of 2,2 and together with a 12-segmented antenna overlap in both these characters with members of the *M. rothsteini*-complex and *M. sordidum* from Clade 1. Our analyses provide strong molecular support for the placement of the *M. rothsteini*-complex and *M. sordidum* in the *monomorium*-group as proposed by Heterick (2011) on other morphological grounds, and the presence of 12-segmented antennae most likely represents a single character reversal on this branch. Four of the five species listed above were not available for the molecular analysis but bear strong morphological affinities to *M. kilianii* Forel, 1902 (*M. crinitum*, *M. petiolatum* and *M. shattucki*) or *M. leae* (*M. sculpturatum*). The one species available for sequencing, *M. tambourinense*, was strongly supported as a member of the ‘Chelaner’ clade.

*Monomorium decuria* has 10-segmented antennae and is the only Australian *Monomorium* species with this character. As previously mentioned, it is otherwise extremely similar morphologically to *M. falcatum*, which has 12-segmented antennae. Its taxonomic position is somewhat ambiguous as it was not consistently resolved in either the *Monomorium* or the *Chelaner* clades. Our COI+wg analysis provided the strongest support for the placement of this species in the ‘Chelaner’ clade and until further evidence is available we conclude that this species is best placed in this group, as proposed by both Heterick (2001) and Andersen (2007).

*Chelaner* stat. rev. is therefore removed as a junior synonym of *Monomorium* and resurrected as a valid genus-level taxon.
*Chelaner* Emery, stat. rev.


**Diagnosis (worker)**

Clypeus bicarinate, carinae subparallel, converging or diverging, frontal margin overhanging mandibles, with a median clypeal seta. Antennae 12-segmented or 10-segmented (*decuria*) with a three segmented club. Palp formula 2,3 or 2,2, mandibular tooth count 3-7. Propodeum rounded, angulate or dentate; petiole with anterior peduncle.

NB. All species of Australian origin unless indicated otherwise as New Caledonia (NC), New Guinea (NG) or New Zealand (NZ). Dates for authorities are only provided where they have not already been cited above.

Constituent species:

*C. albipes* Heterick, 2001 comb. nov.

*C. antarcticum* Smith F., 1858 stat. rev. (NZ)

*C. anthracinum* Heterick, 2001 comb. nov.

*C. aper* Emery, 1914 stat. rev. (NC)

*C. aper dubium* Emery, 1914 stat. rev. (NC)

*C. bicorne* Forel, 1907 stat. rev.

*C. bifidum* Heterick, 2001 comb. nov.

*C. bihamatum* Heterick, 2001 comb. nov.

*C. brachythrix* Heterick, 2001 comb. nov.
C. burchera Heterick, 2001 comb. nov.
C. capito Heterick, 2001 comb. nov.
C. centrale Forel, 1910a stat. rev.
C. crinitum Heterick, 2001 comb. nov.
C. croceiventre Emery, 1914 stat. rev. (NC)
C. decuria Heterick, 2001 comb. nov.
C. draculai Heterick, 2001 comb. nov.
C. durokoppinense Heterick, 2001 comb. nov.
C. edentatum Emery, 1914 stat. rev. (NG)
C. elegantulum Heterick, 2001 comb. nov.
C. euryodon Heterick, 2001 comb. nov.
C. falcatum McAreavey, 1949 stat. rev.
C. flavonigrum Heterick, 2001 comb. nov.
C. forcipatum Emery, 1914 stat. rev. (NC)
C. gilberti Forel, 1902 stat. rev.
C. kilianii Forel, 1902 stat. rev.
C. lacunosum Heterick, 2001 comb. nov.
C. leae Forel, 1913 stat. rev.
C. legulus Heterick, 2001 comb. nov.
C. longinode Heterick, 2001 comb. nov.
C. longipes Emery, 1914 stat. rev. (NC)
C. macarthuri Heterick, 2001 comb. nov.
C. majori Heterick, 2001 comb. nov.
C. melleum Emery, 1914 stat. rev. (NC)
C. nightcapense Heterick, 2001 comb. nov.
C. nigriceps Heterick, 2001 comb. nov.
C. parantarcticum Heterick, 2001 comb. nov.
C. *petiolatum* Heterick, 2001 comb. nov.
C. *pubescens* Heterick, 2001 comb. nov.
C. *punctulatum* Heterick, 2003 comb. nov.
C. *ravenshoense* Heterick, 2001 comb. nov.
C. *rubriceps* Mayr, 1876 stat. rev.
C. *rufonigrum* Heterick, 2001 comb. nov.
C. *sculpturatum* Clark, 1934 stat. rev.
C. *shattucki* Heterick, 2001 comb. nov.
C. *smithii* Forel, 1892 stat. rev. (NZ)
C. *striatifrons* Heterick, 2001 comb. nov.
C. *sublamellatum* Heterick, 2003 comb. nov.
C. *tricolor* Emery, 1914 stat. rev. (NC)
C. *xantheklemma* Heterick, 2001 comb. nov.

*Monomorium* Mayr, 1855


*Diagnosis (worker, Australasian species)*

Clypeus medially raised and bicarinate, carinae raised and angular or rounded, with a median clypeal seta; palp formula 1,2 or 2,2; number of mandibular teeth 3-4.
Antennae 11- or 12- segmented with a three segmented club. Propodeum rounded or angular; petiole with anterior peduncle.

Constituent Australasian species:

*M. aithoderu* Heterick, 2001
*M. anderseni* Heterick, 2001
M. antipodum Forel, 1901 (NZ)
M. arenarium Heterick, 2001
M. bogischi Wheeler, 1917
M. broschorum Sparks, 2014a
M. capeyork Sparks, 2014a
M. carinatum Heterick, 2001
M. casteneum Heterick, 2001
M. disetigerum Heterick, 2001
M. eremoides Sparks, 2014a
M. eremophilum Heterick, 2001
M. eremum Sparks, 2014a
M. fieldi Forel, 1910b
M. geminum Sparks, 2014a
M. hertogi Sparks, 2014a
M. hoffmanni Sparks, 2014a
M. humiliar Forel, 1910a
M. kidman Sparks, 2014a
M. laeve Mayr, 1876
M. leda Forel, 1915
M. maryannae Sparks, 2014a
M. megalops Heterick, 2001
M. merepah Sparks, 2014a
M. micula Heterick, 2001
M. mitchell Sparks, 2014a
M. nanum Heterick, 2001
M. oodnadatta Sparks, 2014a
M. pilbara Sparks, 2014a
M. rothsteini Forel, 1902
M. silaceum Heterick, 2001
M. sordidum Forel, 1902
M. speculum Sparks, 2014a
M. stagnum Sparks, 2014a
M. stictonotum Heterick, 2001
M. subapterum Wheeler, 1917
M. sydneyense Forel, 1902
M. tenebrosum Sparks, 2014a
M. topend Sparks, 2014a
M. torrens Sparks, 2014a

2.5 Discussion

This study set out to provide a more stable and broadly acceptable taxonomic framework for the Australian species of Monomorium. Our results support most of the taxonomic changes made to the genus by Ward et al. (2014) with the exception of the placement of the hildebrandti- and fossulatum-groups in the same genus, Syllophopsis (S. fisheri and S. seychellense). The EF1αF2+wg analysis resolved the two species S. fisheri and S. seychellensis as sister to the two separate Monomorium clades. The Ward et al. (2014) analysis also did not provide strong support for the monophyly of Syllophopsis and morphological affinity was provided as a secondary line of evidence for their association. Syllophopsis species have characteristics typical of cryptobiotic ants (e.g. very small body size, pale colour, reduced or absent eyes) and their apparent morphological affinity may be a case of convergence associated with living in such habitats. Additional sampling for sequencing, that spans the geographic range of these two species-groups, in addition to careful morphological assessment may be required to fully resolve the relationship among these enigmatic taxa. Our analyses also demonstrate that the blind, cryptobiotic taxa from Australia assigned to Monomorium do not belong to that genus nor to Syllophopsis or Anillomyrma but likely represent a new genus.

A molecular phylogenetic approach has supported the resurrection of Chelaner from synonymy and provided a strong taxonomic framework for the species of what was previously considered Monomorium occuring in Australia, New Zealand, New
Caledonia and New Guinea. *Monomorium s. str.* now contains the speciose radiations of small, generalist species with 11-segmented antennae plus the *M. rothsteini/M. sordidum* radiations with 12-segmented antennae. *Chelaner* now encompasses those species with 12-segmented antennae and a palp formula of 2,3. As so defined this genus is endemic to Australasia with a significant radiation on the Australian continent, with only one species recorded from New Guinea and two from New Zealand, although *C. antarcticum* is likely a species complex (Jones et al. 1988a, Don & Jones 1993). There are six species and one subspecies described from New Caledonia, however many more are known from collections.

An explanation of the morphological and molecular differences between *Monomorium* and *Chelaner* can be inferred from their different biogeographic patterns. The present distribution of *Chelaner* (Australia, New Caledonia, New Guinea and New Zealand) suggests an eastern Gondwanan radiation that began 40-50 Mya (Ward et al. 2014). The phylogenetic analysis of Ward et al. (2014) indicates that it is closely related to the Gondwanan genera in Australia (*Austromorium*) and South America (*‘Nothidris’*). In contrast, the Australian species of *Monomorium* bear close molecular and morphological affinities with a Paleotropical radiation which began 20-25 Mya (Ward et al. 2014), and is a more recent addition to the Australian ant fauna that most likely occurred via the Oriental region.

There is now a growing understanding of the broader systematic relationships among genera in the Solenopsidini and this study has provided a more stable taxonomic basis for a speciose Australian component of the tribe. However, resolving the species-level taxonomy for both *Chelaner* and Australian *Monomorium* remains a mammoth task. Evidence presented here and elsewhere (Andersen et al. 2013a, Sparks et al. 2014b) shows taxa considered to be single variable species are in fact highly diverse species complexes. The species *M. fieldi*, *M. laeve* and *M. sydneyense* are perhaps the most in need of taxonomic reassessment. They have continent-wide distributions, and their small size and simplified morphology makes species delimitation challenging without input from parallel molecular studies. Here we also present molecular evidence for a taxonomic reassessment of *M. stictonotum* and *C. leae* as likely species complexes, and morphological evidence that *M. sordidum*, *C. kiliani*, *C. insolescens* and others (Andersen 2007) are also likely complexes.
Our results support the validity of the New Zealand *M. antipodum* as a separate species from *M. fieldi*. It formed a clade with a similar-looking Australian species, and its affinity with Australian species is supported by analysis of venom chemistry (Don et al. 2001). Such an affinity means that it is likely to have been introduced from Australia, as suggested by Brown (1958). However, further molecular and morphological analysis is required to confirm that it is indeed conspecific with an Australian species.

In conclusion, it is hoped that this study will provide a framework for taxonomic work to be undertaken on *Monomorium* and its relatives in a systematic and progressive manner for what is a dominant and ecologically significant component of the Australian terrestrial ant fauna.

### 2.6 Acknowledgements

We thank Chris Burwell (Queensland Museum), Brian Heterick (Curtin University), Phil Lester (Victoria University), Museum Victoria and the South Australian Museum for the donation of specimens used in this study; Tom Brosch, Claire Pettit and Ellen Schlüns for assistance with field collections and Steve Shattuck and members of the Austin Lab Group who provided valuable advice and assistance. This study was funded by the Commonwealth Environmental Research Facility’s Taxonomic Research and Information Network, the University of Adelaide, the Australian Biological Resources Study (207-58) and the Australian Research Council’s Environmental Futures Network (RN0457921) who provided a PhD scholarship, travel and research costs to K. Sparks.
### Table S1. Genbank accession numbers, unique specimen codes and region of origin for the taxa specimens used in this study

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Figure. S1. Bayesian tree of the concatenated COI and EF1αF2 data. Solid circles denote notes with ≥95 Bayesian posterior probability and ≥70 ML bootstrapping support. The basal node of the two Monomorium clades are indicated with an arrow. Unique specimen codes follow the taxon names.
Figure S2. (previous page). Baysian tree of the concatenated COI and wg data. Solid circles denote notes with ≥95 Bayesian posterior probability and ≥70 ML bootstrapping support. The basal node of the two Monomorium clades are indicated with an arrow. Unique specimen codes follow the taxon names.
CHAPTER III: Navigating the mtDNA road map out of the morphological maze: interpreting morphological variation in the diverse Monomorium rothsteini Forel complex (Hymenoptera: Formicidae).

Kathryn S. Sparks¹, Alan N. Andersen², Stephen C. Donnellan³, and Andrew D. Austin⁴

¹Australian Centre for Evolutionary Biology and Biodiversity, and School of Earth & Environmental Sciences, The University of Adelaide, 5005, Australia, email: kathryn.sparks@adelaide.edu.au

²CSIRO Ecosystem Sciences, OIC, Tropical Ecosystems Research Centre, PMB 44 Winnellie, NT 0822, Australia, email: Alan.Andersen@csiro.au

³South Australian Museum, North Terrace, Adelaide SA 5000, and School of Earth & Environmental Sciences, University of Adelaide SA 5005, Australia, email: Steve.Donnellan@samuseum.sa.gov.au

⁴Australian centre for Evolutionary Biology and Biodiversity, and School of Earth & Environmental Sciences, The University of Adelaide, 5005, Australia, email: andy.austin@adelaide.edu.au
Statement of Authorship

This chapter is a published research article.

Kathryn S. Sparks (candidate)

Corresponding author: Collected specimens in the field, prepared DNA extracts and carried out DNA amplification and sequencing, analysed sequence and morphological data and developed species hypotheses, wrote manuscript and prepared all figures and tables.

Signed

Date 15·2·2015

Alan N. Andersen

Provided specimens for analysis, assisted with some field collections, provided advice of morphological species concepts for the group, provided supervision over the direction of the study and critically reviewed the manuscript. I give consent for Kathryn Sparks (candidate) to include this paper for examination towards the degree of Doctor of Philosophy.

Signed

Date 17 Feb 2015

Stephen C. Donnellan

Provided advice on the direction and methodology for the study, advised on the use of haplotype network analysis and critically reviewed the manuscript. I give consent for Kathryn Sparks (candidate) to include this paper for examination towards the degree of Doctor of Philosophy.

Signed

Date 12 Feb 2015

Andrew D. Austin

Provided supervision over the direction and methodology of the study, assisted with obtaining project funding and critically reviewed the manuscript. I give consent for Kathryn Sparks (candidate) to include this paper for examination towards the degree of Doctor of Philosophy.

Signed

Date 18·2·15

Systematic Entomology, v. 39 (2), pp. 264-278

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It is also available online to authorised users at:

http://dx.doi.org/10.1111/syen.12051
CHAPTER IV: Systematics of the Monomorium rothsteini Forel species complex (Hymenoptera: Formicidae), a problematic ant group in Australia

KATHRYN S. SPARKS¹, ALAN N. ANDERSEN² and ANDREW D. AUSTIN³

¹South Australian Museum, North Terrace, Adelaide SA 5000, and Australian Centre for Evolutionary Biology and Biodiversity, and School of Earth & Environmental Sciences, The University of Adelaide, 5005, Australia, email: kate.sparks@samuseum.sa.gov.au

²CSIRO Ecosystem Sciences, Tropical Ecosystems Research Centre, PMB 44 Winnellie, NT 0822, Australia, email: Alan.Andersen@csiro.au

³Australian Centre for Evolutionary Biology and Biodiversity, and School of Earth & Environmental Sciences, The University of Adelaide, 5005, Australia, email: andy.austin@adelaide.edu.au
Statement of Authorship

This chapter is a published research article.

Kathryn S. Sparks (candidate)

Corresponding author: Collected specimens in the field, carried out morphological examinations and measurements, produced taxonomic descriptions, images, key and distributional maps and wrote manuscript.

Signed

Date 18th Feb 2015

Alan N. Andersen

Provided specimens and advised on morphological species concepts for the group, produce Table 1 and critically reviewed the manuscript. I give consent for Kathryn Sparks (candidate) to include this paper for examination towards the degree of Doctor of Philosophy.

Signed

Date 17 Feb 2014

Andrew D. Austin

Provided supervision over the direction of the study, assisted with obtaining funding, provided advice on taxonomic conventions and critically reviewed the manuscripts. I give consent for Kathryn Sparks (candidate) to include this paper for examination towards the degree of Doctor of Philosophy.

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CHAPTER V: General Discussion

5.1 Synthesis

This study set out to explore the species diversity of *Monomorium* in Australia and the relationships among those species. It was the first to use a molecular phylogenetic approach to investigate problematic issue of species boundaries and the results of this study will serve as a framework for future systematic work on species of *Monomorium* and *Chelaner* in the region.

Prior to this study, the Australasian species of *Monomorium* were a poorly defined paraphyletic assemblage of lineages in need of systematic reassessment (Ward et al. 2014). The Australian species were distributed across loosely defined species-groups (Heterick 2001) and there was taxonomic disagreement around what constituted a species for the genus (Andersen 2007). This study has developed a phylogenetic framework that segregates the known endemic species into two separate lineages. *Monomorium* now encompasses those species with 11-segmented antennae as well as *M. sordidum* and the *M. rothsteini* complex, both with 12-segmented antennae. *Chelaner* was brought out of synonymy to encompass a monophyletic group of species that occur across Australia, New Caledonia, New Guinea and New Zealand and have 12-segmented antennae. Only one of the Australian *Monomorium* species-groups (*monomorium*-group) was supported by the analyses herein while four were found to be para- or polyphyletic while two could not be tested due to lack of samples.

Using multiple specimens from different colonies and morphotypes of what were purportedly the same species, uncovered evidence of unrecognised species diversity across the two genera. Specimens of *M. fieldi*, *M. laeve*, *M. sydneyense*, *M. stictonotum* and *C. leae* did not form monophyletic lineages but specimens from specific morphotypes within four of these species did form monophyletic clades. This study has highlighted the difficulty in defining species boundaries in groups where there is a confusing overlap of morphology, and the benefit of using independent evidence such as molecular data to support taxonomic decisions.

A major focus for this study was to test the single species hypothesis for *M. rothsteini*. The mitochondrial DNA marker *COI* was sequenced for a large number of
samples across both the geographic and morphological range of the taxon. The phylogenetic results, combined with morphological and geographic data, provided persuasive evidence for many separately evolving lineages within this broadly defined species. Many of the lineages were morphologically distinct from their closest relative or in their own right. In addition, some lineages that occurred in close sympatry were found to be distantly related genetically and morphologically distinct from one another, while samples that were morphologically indistinguishable across vast geographic distances were also closely related in terms of their mitochondrial DNA. Based on these results it was concluded that *M. 'rothsteini'* was a remarkable radiation of many separately evolving species, many with a unique combination of morphological characters as well as a number of cryptic species for which no morphological characters could be found to separate them.

The results of the species delimitation study of the *M. 'rothsteini'* complex were formalised in a taxonomic revision in which 18 new species were described and four names were brought out of synonymy to bring the total number of described species in the complex to 23.

### 5.2 Future directions

**Systematic resolution of Monomorium s.l. and its relatives**

This study has added significantly to the knowledge of relationships among Australasian *Monomorium* and resolved one of the problematic areas of the Solenopsidini by bringing *Chelaner* out of synonymy. However, *Monomorium* remains a polyphetic assemblage comprising three clades (Ward et al. 2014): *Monomorium* sensu stricto, a clade found in the Paleotropics, and one restricted to the Neotropics. Ward et al. (2014) resurrected *Syllophosis* to unite the species formally belonging to the *M. fossulatum*– and *M. hildebrandti*-groups. These two cryptobiotic groups were only moderately supported as a monophyletic lineage in their analysis and not at all in this study. A greater sample of closely related taxa than was possible in the Ward et al. (2014) study and greater diversity of markers than were used in this study may be required to fully resolve the position of the species currently housed under *Syllophosis*. A third lineage of cryptobiotic, ‘*Monomorium*-like’ ants from Australia was revealed in Chapter 2 that most likely represents a new genus. Only three colonies from potentially two species were available for this study which was deemed
insufficient to formally describe the new genus. Future collecting efforts will be required that target the specific subterranean habitat of this enigmatic lineage. The subterranean ant fauna is largely unstudied in Australia and including this particular habitat in future collecting efforts, through the use of subterranean traps, is likely to reveal numerous new species (Wilkie et al. 2007, Andersen & Brault 2010).

The resolution of this problematic genus will require much broader sampling across the major continents and employ a larger selection of genetic markers than was possible in this study. In addition, a morphological assessment of the clades will be required to adequately define them and stabilize their taxonomy. The effort involved in these two tasks will be significant and should not be understated, but utilizing molecular phylogenetic analyses as a guide to morphology and to inform taxonomic decisions is a method that has been employed successfully in this study and others (eg. (Fisher & Smith (2008), Blaimer (2012d), Blaimer & Fisher (2013)) and can be replicated across other problematic ant groups.

**Taxonomy of the Australian species**

The scope of this study was limited to the Australian *Monomorium* and their close geographic allies. However, rarity of many species meant that even within a restricted geographic region, only slightly more than one-third of the species (22 out of a potential 61) could be included in the molecular analysis. The relationships among many of the species in the two genera therefore remain unknown. The monophyly of the *falcatum*-group could not be tested as only a single representative, *C. decuria* was included in this study. *Chelaner decuria* is the only species in the genus with 10-segmented antennae and its position was not resolved with consistent good support. The addition of more markers that are informative at a deeper level of divergence will be needed to fully resolve the placement of this unique species.

Species delimitation in Australian *Monomorium* and *Chelaner* is hampered by simplified and overlapping morphology in what are typically very small ants. This study has provided evidence that the number of species for both genera is likely to increase when molecular data, in combination with morphology, is applied to the task. The evidence for paraphyletic species in *Monomorium* is clear (Chapter 2) with four species (and potentially many more based on morphological assessment, (Andersen 2007)) requiring revision. The evidence for species paraphyly in *Chelaner*
is less compelling from this study as, in most cases, only one specimen per species was available for the analysis. *Chelaner leae* is one species for which multiple morphotypes were available and there is evidence that these are also representative of distinct species on a molecular level.

Thus, although results presented here provide some insights into the relationships among species and the taxon names that may conceal multiple species, there are still many species about which very little is known. A thorough systematic revision of Australian *Monomorium* and *Chelaner* would need to include not only all the recognized species but also the different morphotypes within those species across their geographic ranges. In this respect, the molecular systematic framework provided in Chapter 2, could act as a guide to future taxon sampling facilitating smaller projects that focus on discrete clades within the two genera.

**The *Monomorium rothsteini* complex**

The species delimitation study in Chapter 3 uncovered 38 separately evolving lineages, but only 22 were included in the taxonomic revision of the complex. The remaining lineages lacked sufficient sampling to adequately describe their morphological and genetic variability, however it is highly likely that the number of species in the complex will exceed 30. Formally describing additional lineages will require further sampling across many parts of Australia with subsequent sequence generation and analysis. The more common and distinctive species were described (Chapter 4) and the sequences that were generated along with the identification key and images are important resources that can be used for the identification of many of the frequently encountered taxa in this difficult group.

**Ant diversity and species discovery in Australia**

Australia has an exceptionally diverse ant fauna but much of it is undescribed. Species richness at local scales can be very high (Andersen et al. 2013a) but documenting the fauna on a regional level has been an unattainable goal thus far and estimates for the fauna vary markedly. There are around 1300 described species but the true figure is expected to be double (CSIRO 2015), or perhaps even five times this number (Andersen 2007). Molecular systematics studies of Australian ants at the species level have been very few (Lucky 2011, Andersen et al. 2013b) and the
current understanding of ant species diversity in Australia is based almost entirely on morphological species concepts. It is unclear whether the species paraphyly and cryptic speciation discovered as part of this study is specific to *Monomorium* or indicative of other ant genera in Australia but it is highly probable for the morphologically challenging and megadiverse *Iridomyrmex, Melophorus, Pheidole* and *Camponotus*. An understanding of the drivers of speciation in such diverse groups as these in the Australian context is yet to be reached and remains a fundamental gap in our knowledge of Australian ant biology. The fluctuating paleoclimate in what is the present day Australian arid zone, characterised by an oscillating pattern of mesic and desertification is one potential driver of speciation in a diversity of phyla (Byrne et al. 2008). Andersen (2003) has suggested that high levels of carbohydrate and moisture availability in the form of plant exudates (from the ecologically dominant *Acacia* in particular) and homopterans in the Australian arid zone have shaped a very productive environment conducive to high levels of specialization and speciation. However, central to testing this hypothesis is a deeper understanding of the diversity of ant species and their ecological requirements.

The significant leaps that have been made in higher level ant systematics through the use of molecular phylogenetics have not been accompanied by an equally significant advance in species discovery and description, despite increasing numbers of species being discovered using DNA sequences across other taxonomic groups (eg. Smith et al. 2013, Bertrand et al. 2014). Next generation sequencing that enables faster and cheaper generation of well-established markers like *COI* for barcoding (Shokralla et al. 2014) has the potential to increase the rate of species discovery in taxonomically difficult groups. However, emerging molecular techniques using high through-put sequencing to identify novel markers amenable for resolving species-level issues are clearly needed to ameliorate the shortcomings of using *COI* alone (Hedin et al. 2012, Lemmon et al. 2012, Kawahara & Breinholt 2014).

Species discovery at a molecular level is becoming faster and less expensive, and is an important first step in understanding species richness at local and regional scales, as well as for untangling tricky morphologically cryptic complexes. However, it is the crucial second step, the meticulous and time consuming morphological description necessary for publishing species names that is lagging behind, and it is this step that
ensures biodiversity knowledge is accessible to more applied areas of research and the wider community. The decline of taxonomic expertise is recognised as an obstacle to this (House of Lords 2008, Sluys 2013) although there exists some debate around how significant is the decline and what effect it has on species discovery and description (Costello et al. 2013, Mora et al. 2013). The narrative evidence from the UK and Europe indicates that not only are there fewer taxonomists working in universities, museums and herbaria but those that are in paid employment are approaching retirement. The decline in overall number of taxonomists is, however, contested by data presented in Joppa et al. (2011) indicating the number of taxonomists is actually increasing exponentially and that the number of species described per taxonomist has been in decline since 1900. It is difficult to ascertain from their data whether they considered all authors of a taxonomic paper to be “taxonomists” which is often not the case, but multi-authored papers aside, it is not unexpected that fewer species are being described relative to the number of taxonomists. Species descriptions are generally very comprehensive, often with the inclusion of detailed images, distributional information and molecular data, taking many weeks to complete. Compare this to the single paragraph descriptions of 100 years ago. Contrary to the claims of Joppa et al. (2011) it is rarely easier to circumscribe taxa when increasing numbers of species are known; rather there are more comparisons that must be made to be certain that new species are in fact ‘new’. The debate over declining efficiency aside, the situation for taxonomy in Australia seems particularly acute with an unfathomably large invertebrate fauna to be documented and an aging taxonomic workforce and declining opportunities for funding and early career researchers with which to do it. Using ants as an example there are just three ant researchers with full employment in Australia and none are employed specifically as taxonomic researchers. Museums, herbaria and the peak national research body, CSIRO, are under increasing financial pressures that inevitably result in fewer research positions. However, documenting species’ morphology, distribution and ecological requirements will be necessary before any detailed understanding can be achieved of the role different species play in the environment or the evolutionary drivers of speciation in a hyperdiverse group like ants in the Australian context.
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