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Barcoding of mygalomorph spiders (Araneae : Mygalomorphae) in the Pilbara bioregion of Western Australia reveals a highly diverse biota

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Abstract. The Pilbara bioregion of Western Australia is an area that contains vast mineral deposits and unique ecosystems. To ensure that mineral deposits are mined with minimal impact on the natural environment, impact assessment surveys are required to determine what fauna and flora species are located within proposed development areas, in particular, by determining the distributions of short-range endemic species (SREs). One infraorder of Arachnida, the Mygalomorphae (trapdoor spiders and their kin), are frequently identified as SREs. These identifications are traditionally performed using morphological techniques; however, only males can be reliably identified to species. Furthermore, the majority of species have not been formally described and males comprise only ~5% of specimens collected. To assess mygalomorph diversity and the distribution of species in the Pilbara, we employed a molecular barcoding approach. Sequence data from the mitochondrial DNA cytochrome *c* oxidase subunit I (COI) gene were obtained from 1134 specimens, and analysed using Bayesian methods. Only a fraction of the total mygalomorph fauna of the Pilbara has been documented, and using a species boundary cut-off of 9.5% sequence divergence, we report an increase in species richness of 191%. Barcoding provides a rapid, objective method to help quantify mygalomorph species identifications and their distributions, and these data, in turn, provide crucial information that regulatory authorities can use to assess the environmental impacts of large-scale developments.

Additional keywords: Actinopodidae, Barychelidae, Ctenizidae, Dipluridae, Idiopidae, mitochondrial DNA, Nemesiidae, speciation, Theraphosidae.

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Introduction

The Pilbara bioregion is located in north-western Australia (Fig. 1) and contains a wide array of biota that includes many diverse clades with extremely high levels of endemism. Molecular studies of both Pilbara invertebrates (Finston and Johnson 2004; Harvey *et al*. 2008; Humphreys 2001; Guthrie *et al*. 2010; Volschenk *et al*. 2010) and vertebrates (Doughty *et al*. 2011; Pepper *et al*. 2011, 2013) provide evidence of high levels of speciation, endemism, cryptic diversity and/or complex genetic patterns in different environments, including both surface and subterranean systems. This may, in part, be a reflection of the great age of the Pilbara bioregion (Pepper *et al*. 2013), with many current geological structures, such as basins and terranes, having formed over two billion years ago (Hickman 2012).

Mygalomorph spiders, due to their longevity, habitat specialisation and usually limited powers of dispersal, are of long-standing and continuing conservation significance in many

regions of the world (Pedersen and Loeschcke 2001; Arnedo and Ferrández 2007; Bond and Stockman 2008). Their conservation status has recently been called into focus in Western Australia (see Harvey *et al*. 2011), where the distributions of numerous short-range endemic (SRE) trapdoor spiders continually intersect with current or planned mining operations. To ensure the protection of threatened species and core biodiversity values across Australia, State and Commonwealth legislation is increasingly recognising the conservation significance of invertebrate taxa and the need to conserve SRE species and/or populations (Harvey *et al*. 2011). This was recently exemplified by the listing of the Western Australian shield-backed trapdoor spider (*Idiosoma nigrum* Main, 1952) as vulnerable under the *Commonwealth Environmental Biodiversity and Conservation (EPBC) Act* (http://www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id = 66798).

Providing legislative protection to species that are unnamed is clearly problematic, and for mygalomorph spiders in the Pilbara only eight have been formally named in the scientific literature, despite the Western Australian Museum housing over 2300 specimens from across the Pilbara. The first was *Aganippe occidentalis* Hogg 1903, based on an adult male from Roebourne (Hogg 1903). Ninety-two years later, Faulder (1995) included a Pilbara specimen of *Missulena rutraspina* Faulder, 1995, along with others from Victoria and South Australia. Harvey *et al*. (2012) described four new species of the genus *Aname* (Nemesiidae) using barcoding data to support morphological differences found in adult males. One of these species, *A. mellosa* Harvey *et al*., 2012, was also found in other parts of Western Australia. Most recently, Harms and Framenau (2013) described two new species of *Missulena* from the Pilbara, also utilising morphological and COI data.

Over the past decade, numerous unpublished environmental surveys have been conducted in the Pilbara bioregion to aid in the planning of large-scale developments such as mines, roads, railway lines and ports. As part of these surveys, proponents have been obliged to conduct surveys for flora and fauna, including SRE fauna (Environmental Protection Authority 2009). Mygalomorph spiders are a relatively common component of the biota of the region and have been targeted for survey to assist with environmental assessments and planning. As part of these environmental assessments, a requirement of the Western Australian Environmental Protection Act (EPA), specimens that have been collected are required to be identified to species or an equivalent level (when no formal classification is available), which for mygalomorph spiders is based on male reproductive anatomy. However, only 5% or so of mygalomorph spiders collected during field trips are adult males, the remaining females and juveniles mostly lack the diagnostic features that are used to identify species. Certainly, the low number of male specimens has hampered the assessment phase of project development in the region, usually due to the absence of enough data to make an informed decision. For example, there are frequently cases where an adult male of an unnamed species was collected from a potential impact site, but could not be verified from elsewhere due to the lack of other adult males. It became apparent to us that a different approach was needed.

In this study we adopted DNA barcoding methodologies to undertake an initial assessment of the mygalomorph fauna of the Pilbara, and to test the utility of COI barcodes for delimiting male morpho-types and thus uniting conspecific females and juveniles. The technique has been widely applied to assist with the identification of many groups of organisms (e.g. Borges *et al*. 2012; Martínez *et al*. 2012; Meiklejohn *et al*. 2012; Zhou *et al*. 2012) and can be particularly useful in matching adult and juvenile life stages (Armstrong and Ball 2005). We sequenced the barcoding fragment of a range of mygalomorph spiders from both the Pilbara and from outlying regions of Western Australia, and further used these data to explore their diversity and to a lesser extent phylogenetic relationships.

Materials and methods

Ingroup taxa

Mygalomorph spiders were extensively sampled within the Pilbara and surrounding Interim Biogeographic Regionalisation for Australia (IBRA) bioregions (Thackway and Cresswell 1995) (Figs 1, 2). These specimens were collected either using dry pit-fall traps or excavated directly from burrows. Dry pits were most successful at capturing wandering males after rainfall events but would also capture dispersing juveniles and sub-adults at other times. Burrows were located by targeted searches within micro-habitats known to support specific taxa. Unbounded area searches, occasionally assisted by the use of a 'blower-vac' to remove surface debris, were conducted to supplement the targeted searches. Wandering males were also occasionally collected by nocturnal searches following or during rain events.

All ingroup taxa are vouchered at the Western Australian Museum (Table S1, available as supplementary material on the journal website). Putative morpho-species were assigned individual and consecutively numbered codes ('MYG001', 'MYG002' etc.) to facilitate comparisons, which invariably relied on the adult male morphology. When a male was not present, but a unique genetic lineage was found to exist, the individuals within that lineage were provided with the same code but with a '-DNA' suffix ('MYG003-DNA', 'MYG004-DNA' etc.).

Outgroup taxa

The tree was rooted on the Atlantic horseshoe crab, *Limulus polyphemus* (Linnaeus, 1758), and also included three other outgroups, the amblypygid *Damon diadema* (Simon, 1876) and the spiders *Heptathela hangzhouensis* Chen, Zhang & Zhu, 1981 (Liphistiidae) and *Hypochilus thorelli* Marx, 1888 (Hypochilidae). The four outgroup taxa were from outside of Western Australia and obtained from GenBank (www.ncbi.nlm.nih.gov; Table S1).

Molecular methods

Prior to July 2012, total genomic DNA was isolated from alcohol-preserved specimens using the Qiagen DNeasy Blood and Tissue extraction kit (www.qiagen.com). One or two legs were removed from each specimen for DNA extraction, and the remaining animal was returned to ethanol. Extractions were carried out using the spin-column protocol for animal tissue, following the manufacturer's instructions with the following exception: the DNA was eluted to a final volume of 30 µL, to

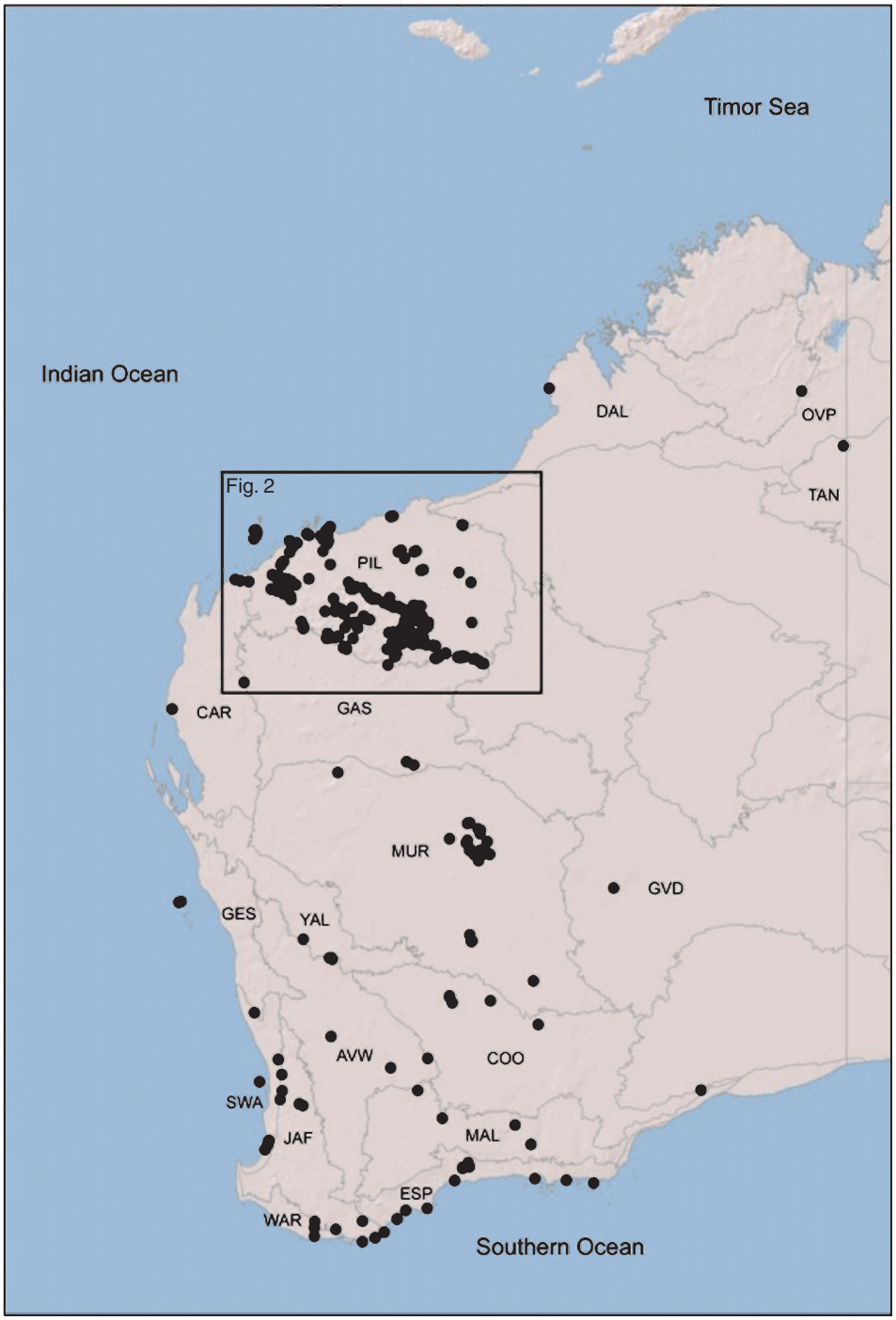

Fig. 1. Map of Western Australia showing the distribution of mygalomorph spider specimens that were sequenced for this study. Interim Biogeographic Regionalisation for Australia (IBRA) 7.0 bioregions that contain collected specimens have been abbreviated as follows: AVW, Avon Wheatbelt; CAR, Carnarvon; COO, Coolgardie; DAL, Dampierland; ESP, Esperance Plains; GAS, Gascoyne; GES, Geraldton Sandplains; GVD, Great Victoria Desert; JAF, Jarrah Forest; MAL, Mallee; MUR, Murchison; OVP, Ord Victoria Plain; PIL, Pilbara; SWA, Swan Coastal Plain; TAN, Tanami; WAR, Warren; YAL, Yalgoo.

increase DNA concentration. A second 100 µL elution was also collected for each sample to increase yield. Post July 2012, a non-destructive DNA extraction method, ANDE (Castalanelli *et al*. 2010) (www.ande.com.au) was used to extract the DNA.

The mitochondrial cytochrome *c* oxidase subunit I (COI) barcoding fragment (658 bp) was amplified using LCO1490 and HCO2198 (Folmer *et al*. 1994). Polymerase chain reaction amplifications with the Folmer primers were performed in a 25 µL

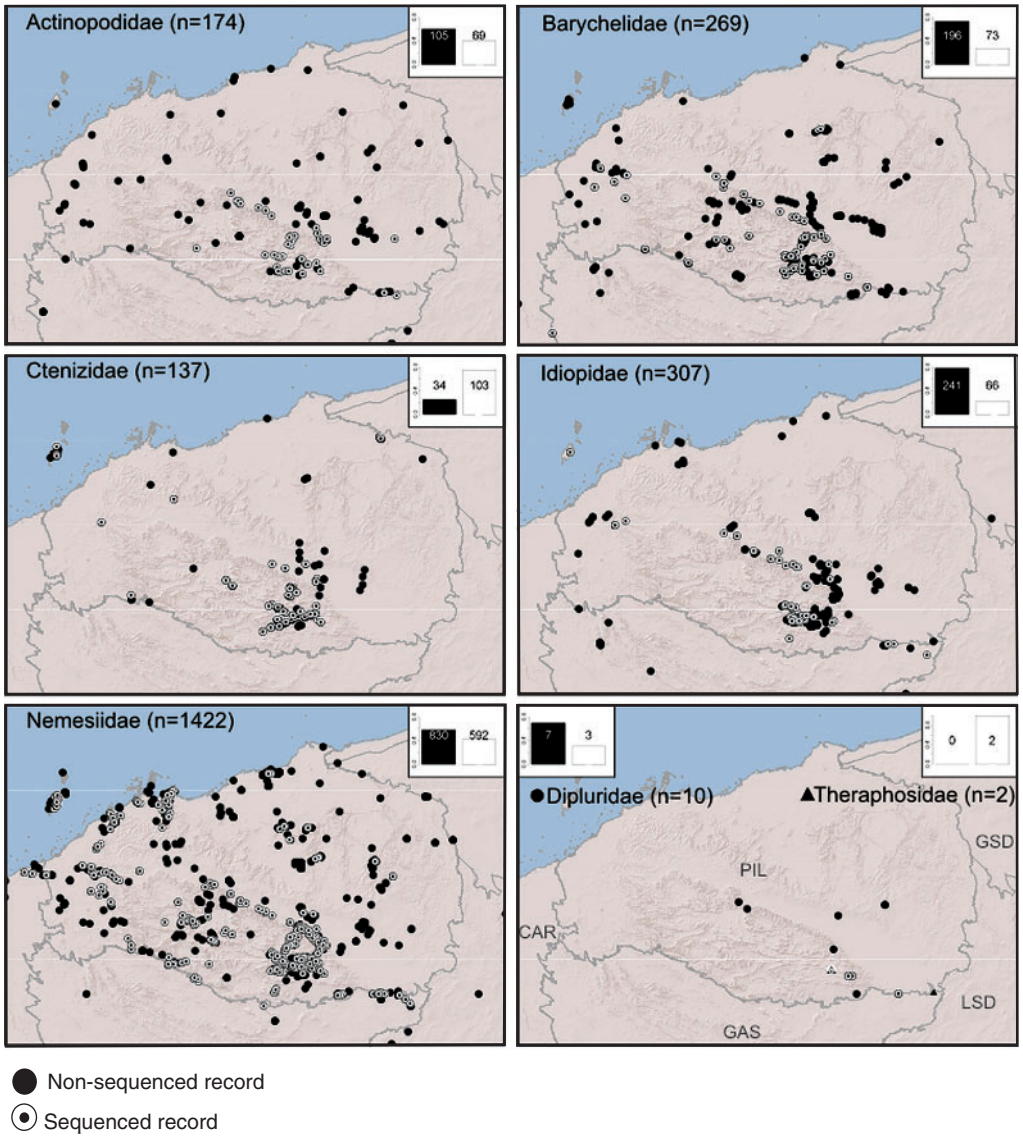

Fig. 2. Maps of the Pilbara bioregion of Western Australia (see Fig. 1) showing the distributions of mygalomorph spider specimens held by the Western Australian Museum. Maps are arranged according to family, with sequenced and non-sequenced records highlighted. Interim Biogeographic Regionalisation for Australia 7.0 bioregions are as follows: CAR, Carnarvon; GAS, Gascoyne; GSD, Great Sandy Desert; LSD, Little Sandy Desert; PIL, Pilbara.

volume containing: 0.4 μM primer, 0.02 μM dNTPs, 2.0 μM $MgCl_2$, 1 × PCR buffer, 1.38U Tth Plus DNA Polymerase (Fisher Biotec, Wembley, WA, Australia). The following cycling conditions were used to amplify the reaction: one initial denaturing cycle of 3 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 48°C and 30 s at 72°C; followed by a final elongation cycle of 2 min at 72°C.

Unpurified PCR products were submitted to the Australian Genome Research Facility (AGRF) in Perth, Western Australia, where sequencing reactions were carried out using the BDT v3.1 reaction mix (ABI Prism, USA). The products were sequenced in both directions on an AB 3730xl automated sequencer (Applied Biosystems, USA) at the AGRF facility.

Data analysis

The COI sequences (accession numbers KJ744382 – KJ745514) were edited using Geneious v. 5.6.5 (Geneious) and each locus aligned using a built-in version of MUSCLE (Edgar 2004). Geneious was used to: (i) detect the presence of nuclear mitochondrial DNA (NUMTs) by translating each COI sequence with the standard invertebrate mitochondrial code; and (ii) calculate net divergences between mitochondrial sequences using uncorrected p-distance.

To determine the efficacy of detecting mygalomorph species using DNA barcoding, and to provide a molecular phylogenetic framework for conservation decision-making under the Western

Australian EPA Guidance Statement, a cut-off value of 9.5% was selected and broadly applied across all families. Although studies on mygalomorph spiders have evidenced variable cut-off values depending on the taxon studied (e.g. Bond and Stockman 2008; Hamilton *et al.* 2014), this value was considered appropriate as a baseline for Pilbara species, given a preliminary analysis of COI pairwise divergence values across available male morpho-types. We acknowledge that the 9.5% figure may be considered arbitrary, but it is important to implement protocols for conservation planning, before the application of larger multi-locus studies.

Unlike standard barcoding papers (Hebert *et al.* 2003; Meiklejohn *et al.* 2012), we employed a Bayesian approach to phylogenetic analysis (e.g. Delsinne *et al.* 2012). Bayesian trees were generated using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). The data were partitioned in three according to codon position, with each partition allowed to evolve independently under a general time reversal (GTR) model (i.e. 'Lset nst = 6 rates = invgamma) as determined by jModelltest2 (Darriba *et al.* 2012). The program was run for 10 million generations, sampling every 1000 generations, with the first 2500 trees discarded as burn-in.

Results

This study sequenced COI from a total of 1134 mygalomorph spider specimens and included four additional outgroup sequences. Of the 1134 sequences, 908 were from the Pilbara (Fig. 1; Table 1). Currently the Western Australian Museum holds 2321 mygalomorph specimens from this region, of which 39.1% have now been sequenced (Fig. 2). Although most specimens had 658 bp in the barcoding fragment, three specimens, identified as *Aname* MYG271-DNA, contained an additional amino acid at bp position 77. The 661 bp alignment contained 451 polymorphic sites, 40 parsimony informative sites, and 838 unique sequences. The polymorphisms observed within the barcoding fragment resulted in no stop codons being translated.

While the back-bone of the resulting tree is poorly resolved (Fig. 3), five of the seven mygalomorph families were recovered as monophyletic: Dipluridae, Theraphosidae, Barychelidae, Actinopodidae and Ctenizidae. The only family level sister-group relationship was between Barychelidae and Theraphosidae, a clade that is well supported morphologically (Raven 1985) and molecularly (Bond *et al.* 2012). The genus *Cethegus* (Dipluridae) was found to be sister to the remaining mygalomorphs, a result that was also found in the morphological analysis of Goloboff (1993).

The two largest families sampled in this study, Nemesiidae and Idiopidae were not recovered as monophyletic groups (Fig. 3). The Nemesiidae comprised 15 separate clades, whereas the Idiopidae comprised two separate clades. Despite the expected lack of resolution for family level relationships, four of the eight nemesiid genera were recovered as monophyletic (*Chenistonia*, *Pseudoteyl*, *Stanwellia* and *Yilgarnia*). The other

Table 1. Summary statistics showing the number of COI sequences obtained from different mygalomorph families and genera, and the number of inferred genetic clades using a 9.5% species level cut-off

Morphological and molecular congruence is defined as the proportion of male morpho-species defined by concordant genetic clades

Family	Genus	Genetic clades (9.5% species cut-off)	Male morphological species (morphological and molecular congruence as percentage)	Sequences	Sequences from Pilbara
Actinopididae	*Missulena*	13	11 (72%)	86	69
Barychelidae		19	10 (90%)	80	73
	Aurecocrypta	7	1 (100%)	16	
	Idiommata	4	4 (75%)	8	
	Synothele	8	5 (100%)	55	
Ctenizidae	*Conothele*	13	1 (0%)	105	103
Dipluridae	*Cethegus*	3	1 (100%)	4	3
Idiopidae		23	13 (100%)	82	66
	Aganippe	16	10 (100%)	31	
	Anidiops	3	1 (100%)	46	
	Euoplos	3	1 (100%)	4	
	Idiosoma	1	1 (100%)	1	
Nemesiidae		90	45 (95%)	785	592
	Aname	49	29 (95%)	718	
	Chenistonia	6	4 (100%)	5	
	Kwonkan	15	4 (100%)	31	
	Merredinia	3	1 (100%)	3	
	Pseudoteyl	2	0 (0%)	4	
	Stanwellia	2	1 (100%)	7	
	Teyl	4	4 (100%)	7	
	Yilgarnia	7	2 (100%)	10	
Theraphosidae		1	0 (0%)	2	2
Total		155	81 (92%)		

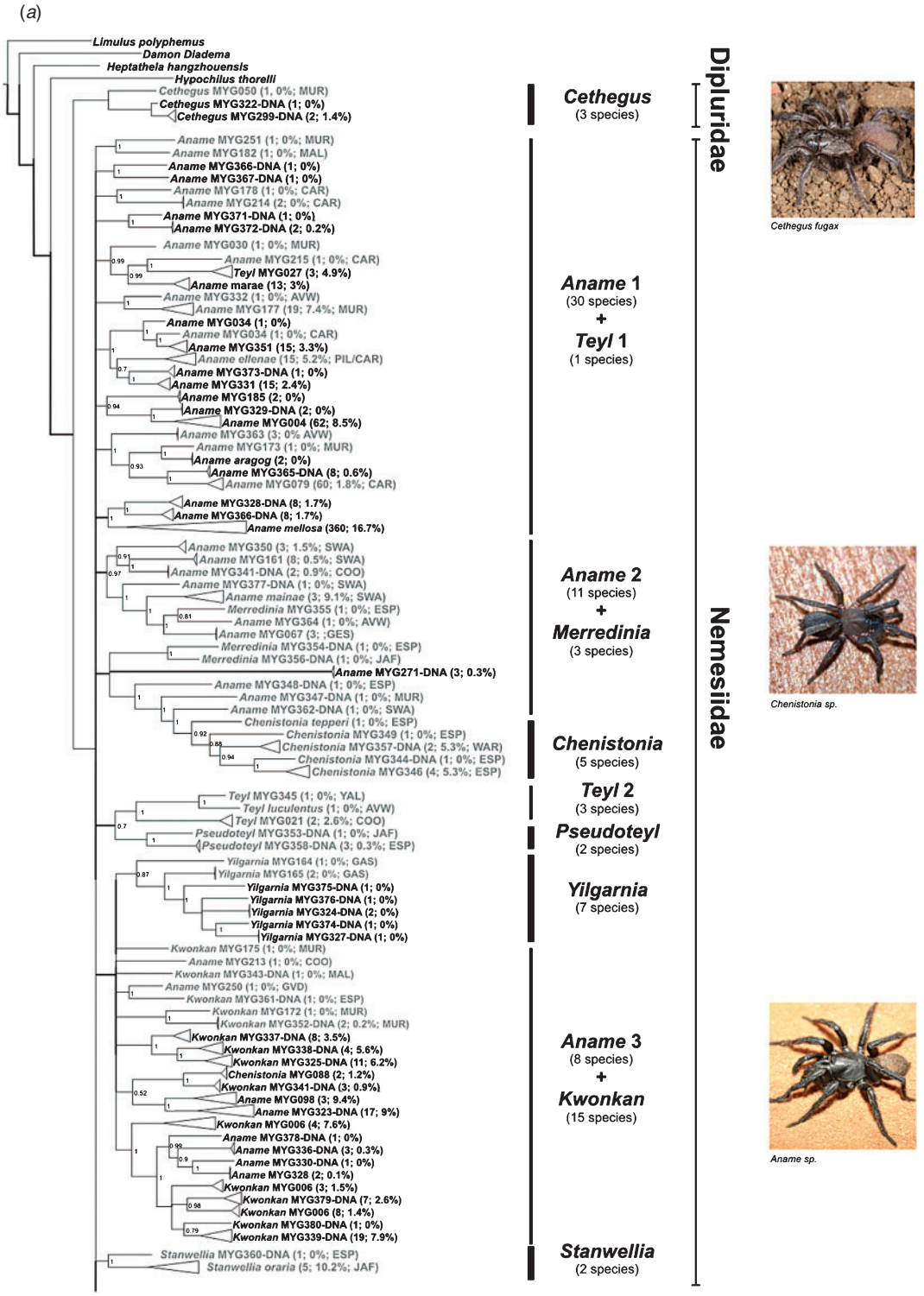

Fig. 3. Bayesian majority-rule consensus tree resulting from a partitioned phylogenetic analysis of 1138 cytochrome *c* oxidase subunit I (COI) gene sequences from Mygalomorphae. Tree terminals represent genetic clades at the 9.5% species level cut-off, with brackets denoting: the number of specimens in each clade; intra-clade pairwise divergences; and the Interim Biogeographic Regionalisation for Australia 7.0 bioregion/s from which specimens were collected. Grey branches denote genetic clades not occurring in the Pilbara. Families are shown on the right, with generic groups and the number of species in each shown in the middle, between the two bars. Thick left bars denote monophyletic genera. Posterior probability values are shown next to the nodes where the value is >50%.

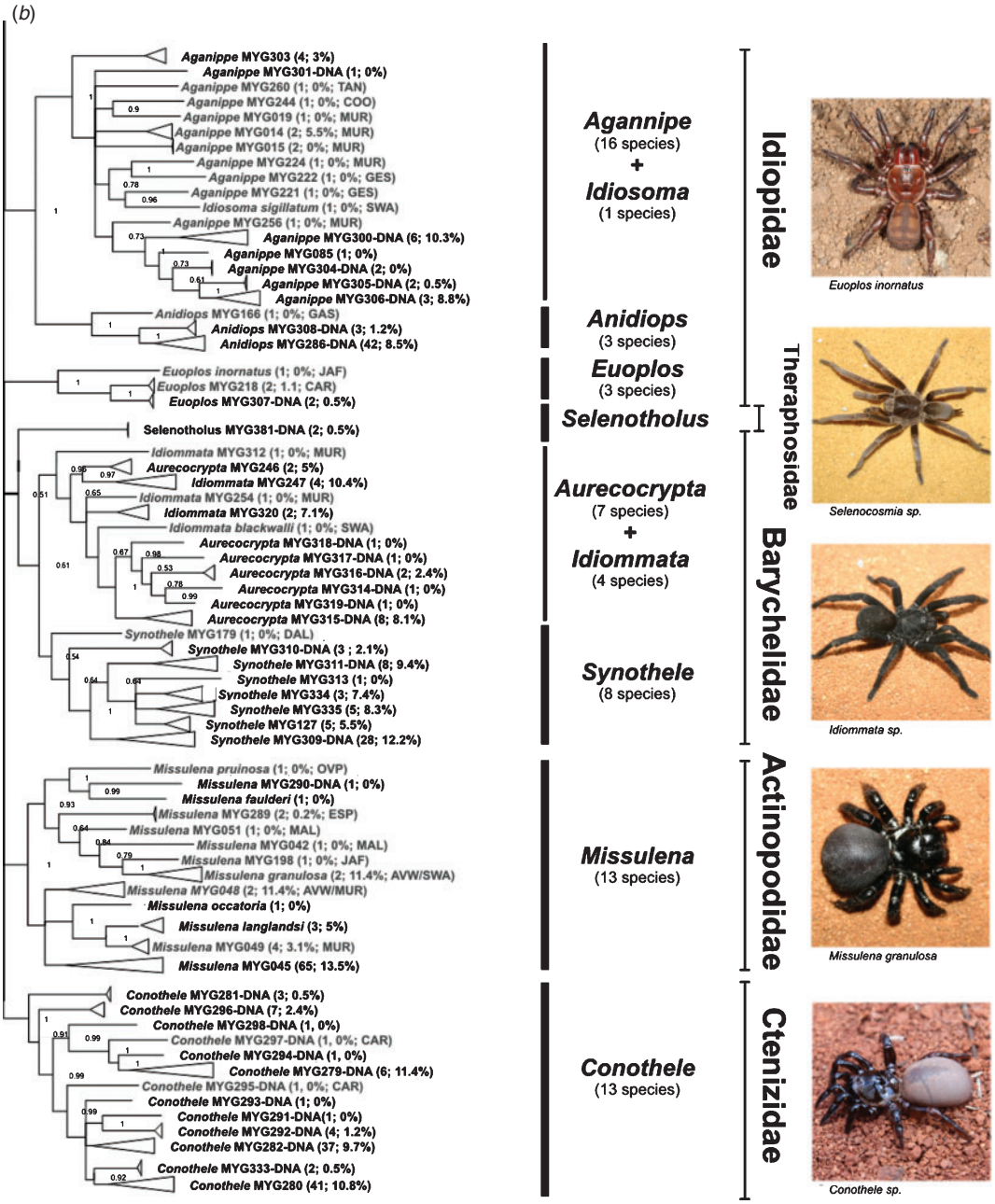

Fig. 3. (*continued*)

nemesiid genera (*Kwonkan*, *Merredinia* and *Teyl*) were not monophyletic and were embedded within clades of the highly polyphyletic *Aname*. The two idiopid clades correspond to the two Australian tribes of Arbanitinae recognised by Main (1985*b*) and Raven (1985), with *Aganippe*, *Anidiops* and *Idiosoma* (Arbanitinae) forming one clade and *Euoplos* (Arbanitini) forming the other. All idiopid genera were monophyletic apart from *Aganippe* which was rendered paraphyletic by *Idiosoma*. Likewise, for the Barychelidae, *Idiommata* and *Aurecocrypta* were intermixed, but *Synothele* was monophyletic. Only single genera were analysed from the families Actinopodidae, Ctenizidae, Dipluridae and Theraphosidae.

Species

Using the proposed 9.5% cut-off value we detected 161 distinct lineages (Table 1). The majority of lineages were represented by >1 individual and 81 lineages contained a morphological species based on the presence of a male specimen. More importantly the barcoding technique revealed an additional 80 species, an increase of 198%. Using a 9.5% cut-off, 92% of the morphological species were congruent with the molecular species boundary. There were, however, exceptions and eight previously identified species had an intra-specific diversity >9.5%. These included two named species (*Aname mellosa*

and *Missulena occatoria* Walckenaer, 1805), four taxa based on morpho-typed males (*Conothele* MYG280, *Idiommata* MYG247, *Missulena* MYG045 and *Missulena* MYG048) and two DNA lineages (*Aganippe* MYG300-DNA and *Synothele* MYG309-DNA).

Most species were represented by relatively few specimens, and little can be inferred of their total distributions. However, of particular interest was the largest lineage observed during this study, that of *Aname mellosa* (360 specimens), which had an intraspecific diversity of 16.7%, and high levels of population structure with 10 distinct clades. The 10 clades showed distinct geographical clustering and in most cases each clade was in peripatry (clades 1 to 7) and three were in sympatry (clades 8 to 10; Fig. 4). Intra-clade diversity was 2–5% and inter-clade diversity 5–9%.

Discussion

This study analysed the barcoding gene COI from 1134 newly sequenced specimens of seven different mygalomorph families, to explore the diversity of species in the Pilbara bioregion of Western Australia, to test the genetic relationships between and within species, and assess molecular results against morpho-type designations. The results affirmed monophyly for many genera and families, but failed to adequately resolve relationships in numerous taxa due to the low resolving power of a single mitochondrial gene. This study is therefore preliminary as a regional phylogenetic assessment, but there are numerous outcomes that suggest that the data obtained provide insights into the composition and relationships of the trapdoor spider fauna of the Pilbara, and that the data are highly relevant to industry and regulatory agencies when assessing development projects in the region. At present, the majority (92%) of the morpho-types that have been previously recognised based on adult male morphology were recovered using sequence data, showing the utility of barcoding in mygalomorph spiders. Our decision to use the 9.5% threshold was generally upheld as at this level the adult males showed consistent morphological differences in features (e.g. pedipalp, first leg, colouration), and these characters are often used to hypothesise species status for a trapdoor spider taxon (e.g. Main 1985*b*; Raven 1994; Hendrixson and Bond 2005; Wishart and Rowell 2008; Harvey *et al*. 2012). While a different cut-off value may also delimit species lineages in the Pilbara, we usually lacked multiple male specimens of any one morpho-type to adequately test and optimise a cut-off value for mygalomorph spiders. For example, in 31 instances where multiple males existed for a particular morpho-type, eight 'species' had intraspecific divergences >9.5% (range 10.2 to 18.1%), and 23 had intraspecific divergences <9.5% (range 0 to 8.5%). Clearly, future studies should concentrate on sequencing additional exemplar males for each morpho-type, to test the preliminary 9.5% hypothesis promoted here.

Some species, however, showed different results. The most striking example is the *A. mellosa* complex, which comprised 10 distinct genetic lineages in the Pilbara and northern Gascoyne (Fig. 4), but which could not be distinguished using morphology alone. This confirms the result of Harvey *et al*. (2012) who also found high COI divergence levels in the same species, albeit from a much smaller number of samples. We sequenced 360 specimens with an overall divergence level of 16.7% (Fig. 3). This rate is likely to be higher as we have not included specimens from localities other than the Pilbara and northern Gascoyne. Interestingly, the 10 lineages showed distinct geographical

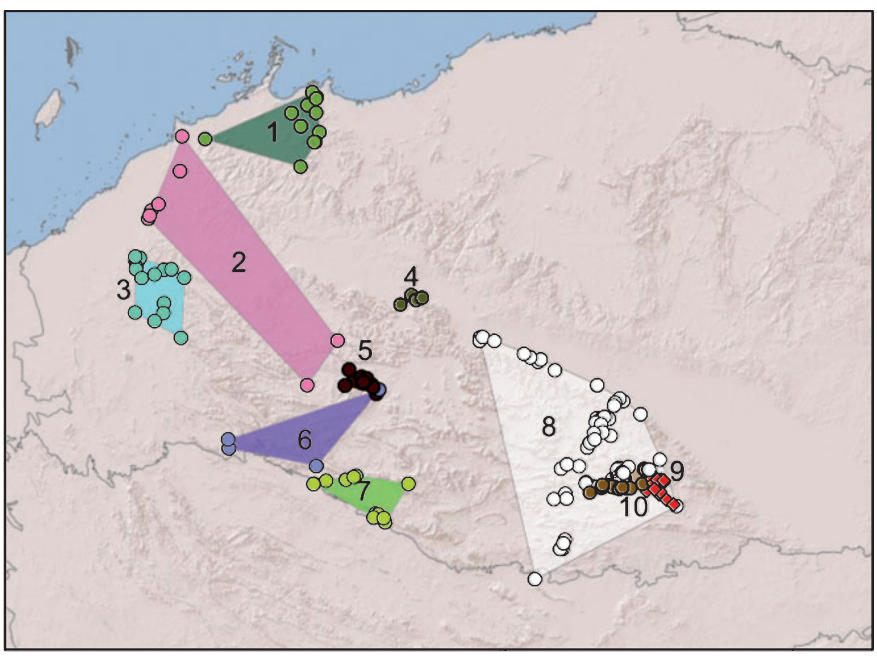

Fig. 4. Map of the western Pilbara and northern Gascoyne showing the distribution of specimens morphologically identified as *Aname mellosa*. Each colour represents the 10 distinct genetic clades, denoted 1 to 10. See text for details.

clustering (Fig. 4) and in most cases each clade was in peripatry. Future studies, such as using microsatellites (e.g. Cheng *et al*. 2013) or multi-locus data, will be needed to tease apart the relationship between the 10 *A. mellosa* clades or even the other groups that have divergences >9.5%. This may help determine whether they are indeed one or several cryptic species and further test the utility of the 9.5% threshold as a species boundary.

Taxonomic considerations

This study raises several interesting taxonomic issues which cannot be resolved within the framework of a barcoding study. Although additional loci are needed to more fully explore the phylogenetic relationships of all of these taxa, the results obtained here suggest that taxonomic changes might be needed in the future.

The Australian Barychelidae were revised by Raven (1994) who recognised 11 genera and 105 species from Australia. While we recovered a monophyletic *Synothele*, the barcoding data could not reliably segregate *Idiommata* and *Aurecocrypta*. Although Raven (1994) recognised the two genera as morphologically distinct, the differences are slight and are based on scopula density and size of claw tufts. In addition, these two genera are the only barychelids that have been observed to have a cheliceral lyra (Raven 1994). While increasing the number of genes may resolve each genus as reciprocally monophyletic, both the genetic and morphological results suggest that they are indeed closely related.

The Ctenizidae are represented by only a single named species in Australia, *Conothele doleschallii* Thorell, 1881 (Australian Faunal Directory, accessed 18 October 2013), but many additional species have been identified in museum collections (R. Raven, B. Y. Main, pers. comm.). We have found 12 taxa in our study, including 11 from the Pilbara, but only one has been matched to a species code name based on an adult male. While this suggests that the family is highly diverse in arid Australia and that a substantial systematic project is required to document the fauna, it is interesting to note that very few males are ever collected and a different collecting strategy may be required.

The Idiopidae was represented by four genera, of which *Anidiops* and *Euoplos* were monophyletic, but *Aganippe* was paraphyletic with *Idiosoma sigillatum* (O. P.-Cambridge, 1870) nested deeply within the genus. The morphological differences between *Aganippe* and *Idiosoma* are very slight, with *Idiosoma* having abdominal coriaceous corrugations which are lacking in *Aganippe* (Main 1985*b*; Raven 1985). The similarity between these genera was first highlighted by Pocock (1897: 112), who noted that 'In spite of the differences observable between them in the arrangement of the eyes and the structure of the abdomen, the genera *Aganippe* and *Idiosoma* cannot, I think, be far separated in a natural classification of spiders'. It appears that *Idiosoma* may simply be a highly derived sub-clade of *Aganippe*.

Although five genera of Nemesiidae (*Chenistonia*, *Merredinia*, *Pseudoteyl*, *Stanwellia* and *Yilgarnia*) were found to be monophyletic, the remaining genera (*Aname*, *Kwonkan* and *Teyl*) consisted of a mixture of paraphyletic and polyphyletic clades. The polyphyletic nature of *Teyl* was unexpected as the tribe to which it belongs, Teylini (Main 1985*a*), is easily recognised due to almost all males having a spherical bulb and an embolus arising equatorially (Main 2004). However, the two teyline genera included in this analysis, *Teyl* and *Pseudoteyl*, were actually recovered as monophyletic with the exception of the only *Teyl* specimen from the Pilbara, *Teyl* MYG027. Clearly, more work is needed to determine whether the equatorially arising embolus is a feature that has arisen more than once. *Aname* species were interspersed throughout the *Kwonkan* lineages. These two genera are often mistaken due to their being separated by only a single character. *Aname* (like most nemesiids) lacks spines on any tarsi, but tarsal spines are present in *Kwonkan* (Main 1983); this represents the most reliable character to segregate the two genera. These three paraphyletic and polyphyletic genera highlight the uncertain systematic relationships of nemesiid spiders within Australia.

Management considerations

This study has shown that the barcoding fragment of the mitochondrial gene COI is of great utility to associate otherwise unidentifiable juveniles and females with adult males, which tend to have species-specific distinctive, diagnosable morphologies. Alternatively, this technique has uncovered many novel genetic clades that could not yet be matched to adult males even though unsequenced males might be present in the collection of the Western Australian Museum. With adult males incorporating only 5% of the specimens collected during surveys in the Pilbara, DNA barcoding has allowed many more specimens to be incorporated into environmental assessments of developments such as mines, mine infrastructure and roads than could be assessed using traditional morphological techniques. This, in turn, provides information that regulatory authorities can use to assess the environmental impacts of these large-scale developments (Harvey *et al*. 2008; Environmental Protection Authority 2009).

We advocate the adoption of a baseline barcoding technique and the framework outlined here for the Pilbara when sampling for trapdoor spiders, as it provides a rapid, objective method to quantify species identifications and their distributions, especially as so few male specimens are available and so few species have been formally named in the scientific literature.

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