Molecular Phylogenetics and Evolution 62 (2012) 375–396

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogeny and historical biogeography of ancient assassin spiders (Araneae: Archaeidae) in the Australian mesic zone: Evidence for Miocene speciation within Tertiary refugia

Michael G. Rix [a,*], Mark S. Harvey [a,b,c,d]

[a] Department of Terrestrial Zoology, Western Australian Museum, Locked Bag 49, Welshpool DC, Perth, Western Australia 6986, Australia
[b] School of Animal Biology, University of Western Australia, 35 Stirling Highway, Crawley, Perth, Western Australia 6009, Australia
[c] Division of Invertebrate Zoology, American Museum of Natural History, New York, NY 10024, USA
[d] California Academy of Sciences, 55 Music Concourse Drive, San Francisco, CA 94118, USA

ARTICLE INFO

Article history:
Received 28 July 2011
Revised 11 October 2011
Accepted 13 October 2011
Available online 21 October 2011

Keywords:
Arachnida
Palpimanoidea
Araneomorphae
Systematics
Rainforest
Short-range endemism
Great Dividing Range

ABSTRACT

The rainforests, wet sclerophyll forests and temperate heathlands of the Australian mesic zone are home to a diverse and highly endemic biota, including numerous old endemic lineages restricted to refugial, mesic biomes. A growing number of phylogeographic studies have attempted to explain the origins and diversification of the Australian mesic zone biota, in order to test and better understand the mode and tempo of historical speciation within Australia. Assassin spiders (family Archaeidae) are a lineage of iconic araneomorph spiders, characterised by their antiquity, remarkable morphology and relictual biogeography on the southern continents. The Australian assassin spider fauna is characterised by a high diversity of allopatric species, many of which are restricted to individual mountains or montane systems, and all of which are closely tied to mesic and/or refugial habitats in the east and extreme south-west of mainland Australia. We tested the phylogeny and vicariant biogeography of the Australian Archaeidae (genus *Austrarchaea* Forster & Platnick), using a multi-locus molecular approach. Fragments from six mitochondrial genes (COI, COII, tRNA-K, tRNA-D, ATP8, ATP6) and one nuclear protein-coding gene (Histone H3) were used to infer phylogenetic relationships and to explore the phylogeographic origins of the diverse Australian fauna. Bayesian analyses of the complete molecular dataset, along with differentially-partitioned Bayesian and parsimony analyses of a smaller concatenated dataset, revealed the presence of three major Australian lineages, each with non-overlapping distributions in north-eastern Queensland, mid-eastern Australia and southern Australia, respectively. Divergence date estimation using mitochondrial data and a rate-calibrated relaxed molecular clock revealed that major lineages diverged in the early Tertiary period, prior to the final rifting of Australia from East Antarctica. Subsequent speciation occurred during the Miocene (23–5.3 million years ago), with tropical and subtropical taxa diverging in the early-mid Miocene, prior to southern and temperate taxa in the mid-late Miocene. Area cladograms reconciled with Bayesian chronograms for all known Archaeidae in southern and south-eastern Australia revealed seven potentially vicariant biogeographic barriers in eastern Queensland, New South Wales and southern Australia, each proposed and discussed in relation to other mesic zone taxa. Five of these barriers were inferred as being of early Miocene age, and implicated in the initial vicariant separation of endemic regional clades. Phylogeographic results for Australian Archaeidae are congruent with a model of sequential allopatric speciation in Tertiary refugia, as driven by the contraction and fragmentation of Australia's mesic biomes during the Miocene. Assassin spiders clearly offer great potential for further testing historical biogeographic processes in temperate and eastern Australia, and are a useful group for better understanding the biology and biogeography of the Australian mesic zone.

1. Introduction

The island continent of Australia is an ancient, dynamic landscape, with a temperate Gondwanan heritage (Hopper et al., 1996; Crisp et al., 2004) and a more recent climatic history dominated by widespread and ongoing aridification (Crisp et al., 2004; Byrne et al., 2008). The Australian biota is thus a mixture of relictual mesic elements, later colonisers and more recently evolved arid-adapted taxa (Crisp et al., 2004), the latter having diversified spectacularly since the mid-Miocene (Byrne et al., 2008). Australia's remaining 'mesic biomes' are broadly restricted to the

* Corresponding author. Fax: +61 8 9212 3882.
E-mail address: michael.rix@museum.wa.gov.au (M.G. Rix).

1055-7903/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2011.10.009

continent's southern, eastern and tropical northern coastlines (Crisp et al., 2004; Ponniah and Hughes, 2004; Byrne et al., 2008) and are largely concordant with the distribution of rainforests and closed and tall open forests in Australia's east and extreme south-west (see Specht, 1981; Webb and Tracey, 1981; Adam, 1992). These refugial, mesic habitats, despite having undergone enormous contractions since the Miocene, are home to a significant proportion of Australia's biodiversity (Webb and Tracey, 1981; Yeates et al., 2002) and include a highly endemic biota (e.g. Schneider et al., 1998; Webb and Tracey, 1981; Moritz et al., 2000; Rix et al., 2009; Bell et al., 2010; Cooper et al., 2011; Hugall and Stanisic, 2011). Rigorous molecular phylogenetic studies are critical to testing biogeographic hypotheses and understanding alternative patterns of speciation and divergence in a complex Australian context (Crisp et al., 2004) and a growing number of phylogeographic studies have attempted to explain the origins and diversification of the Australian mesic zone biota, for both vertebrates (e.g. Cracraft, 1991; Joseph and Moritz, 1994; Joseph et al., 1993, 1995; Schneider et al., 1998; Schneider and Moritz, 1999; Schäuble and Moritz, 2001; Hoskin et al., 2003; Jennings et al., 2003; O'Connor and Moritz, 2003; Knowles et al., 2004; Chapple et al., 2005; Moussalli et al., 2005; Couper et al., 2008; Hugall et al., 2008; Colgan et al., 2009; Bell et al., 2010) and invertebrates (e.g. Ward, 1980; Hugall et al., 2003; Sota et al., 2005; Ponniah and Hughes, 2004, 2006; Baker et al., 2008; Schultz et al., 2009; Hugall and Stanisic, 2011; Lucky, 2011; Cooper et al., 2011). Many of these invertebrate studies have focused on relatively few insect, mollusc and crustacean taxa (e.g. ants, snails and freshwater crayfish), and additional studies are required to complement and test prevailing biogeographic hypotheses born out of recent research on birds, reptiles and amphibians.

The assassin spiders of the family Archaeidae (Fig. 1) are an ancient and iconic lineage of araneomorph spiders, remarkable for their extraordinary appearance, highly specialised ecology, evolutionary antiquity and endemism on the southern continents (Forster and Platnick, 1984; Harvey, 2002a; Wood et al., 2007; Wood, 2008; Rix and Harvey, 2011). Assassin spiders are obligate predators of other spiders, and all possess a modified, grossly-elevated cephalothorax and 'head' region bearing long, spear-like chelicerae (Fig. 1), used to hunt and capture their spider prey (Legendre, 1961; Forster and Platnick, 1984; Wood et al., 2007; Wood, 2008; Rix and Harvey, 2011). This highly specialised araneophagic morphology is unique among arachnids, making the Archaeidae among the most instantly-recognisable of all spider families. They are the icon of Madagascar's rich spider fauna (Griswold, 2003; Wood, 2008), and while long considered among the rarest and most enigmatic of spiders (Forster and Platnick, 1984), recent dedicated research in South Africa, Madagascar and Australia has revealed diverse and highly endemic faunas in all three countries, each of considerable evolutionary and biogeographic significance (Platnick, 1991a, 1991b; Lotz, 1996, 2003, 2006; Harvey, 2002a; Wood et al., 2007; Wood, 2008; Rix and Harvey, 2011).

The history of the discovery and documentation of living and fossil assassin spiders highlights the great antiquity of the family, and points to the utility of the Archaeidae for testing

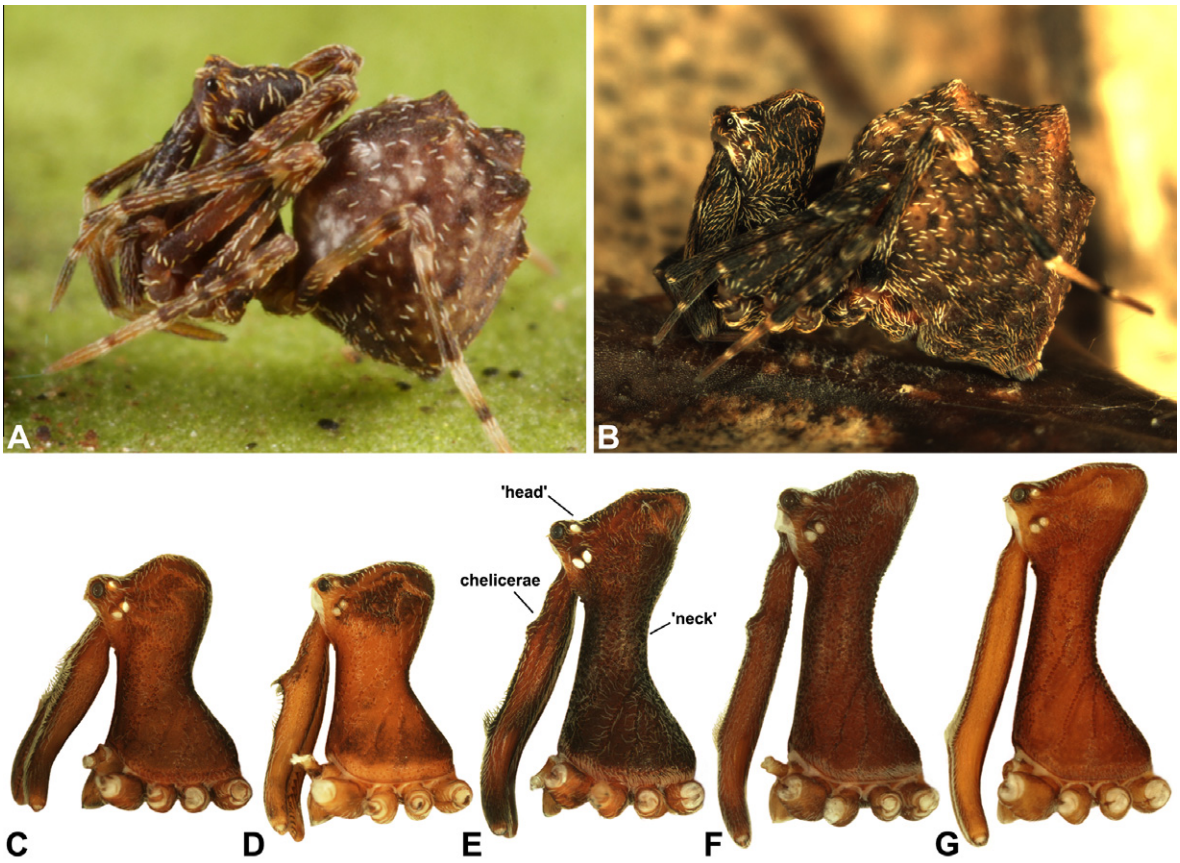

Fig. 1. Australian assassin spiders (family Archaeidae). (A-B) Habitus images of live specimens: (A) juvenile *Austrarchaea raveni* Rix and Harvey, 2011 from Mount Glorious, Queensland; (B) female *Austrarchaea* sp. nov. (WA-4) from Karri Valley, Western Australia. (C–G) Lateral carapace profiles of male *Austrarchaea* spp., showing variation in the height of the carapace and length of the 'neck': (C) *Austrarchaea* sp. nov. (VIC-2) from Acheron Gap, Victoria; (D) *Austrarchaea* sp. nov. (WA-1) from Mount Hassell, Western Australia; (E) *A. christopheri* Rix and Harvey, 2011 from Dorrigo National Park, New South Wales; (F) *A. nodosa* (Forster, 1956) from Lamington National Park, south-eastern Queensland; (G) *Austrarchaea* sp. nov. from Mount Elliot, north-eastern Queensland. Image (A) by Greg Anderson, used with permission; image (B) by M. Rix.

historical biogeographic hypotheses. The first assassin spider, aptly-named *Archaea paradoxa* by Koch and Berendt (1854), was described from Tertiary (Paleogene) Baltic amber (Dunlop et al., 2011), and remained the only described archaeid species for over a quarter of a century (Forster and Platnick, 1984; Harvey, 2002a; Wood et al., 2007; Rix and Harvey, 2011). Living representatives of the family were first discovered in the forests of Madagascar in the late 19th century (see Pickard-Cambridge, 1881), with the first mainland African species described in 1919 (Hewitt, 1919) and the first Australian species in 1929 (Butler, 1929). Since 1881, additional living Archaeidae have been found only in Madagascar, southern Africa and mainland Australia (Platnick, 2011), although a rich fossil record dates back to at least the mid-Mesozoic (Penney, 2003; Selden et al., 2008; Selden and Penney, 2010) with records from Baltic, Bitterfeld and Myanmar ambers, Madagascar copal and from sedimentary deposits of the Jurassic of Karatau (Kazakhstan) and Daohugou (China) (Dunlop et al., 2011). Indeed, the currently restricted, apparently Gondwanan distribution of Archaeidae is in stark contrast to the formerly pancontinental (and probably Pangaean) distribution of the family, lending support to an 'ousted relicts' hypothesis and a largely relictual distribution across the Southern Hemisphere (see Eskov and Golovatch, 1986; Penney, 2003). Recent taxonomic and phylogenetic research has further uncovered an unexpected diversity of extant species in all three countries, with phylogenetic evidence for east–west geographic separation and pronounced morphological convergence in Malagasy taxa (Wood et al., 2007), and preliminary taxonomic and phylogenetic evidence for strongly allopatric, relictual short-range endemic distributions of Australian species (Rix and Harvey, 2011). Clearly, the currently relictual distributions of speciose extant taxa, combined with the deep persistence of assassin spiders throughout the Cenozoic, renders the Archaeidae of considerable interest to biogeographers.

The Australian assassin spider fauna consists of 22 described and numerous undescribed species of *Austrarchaea* Forster and Platnick, 1984 (Fig. 1), found throughout the mesic rainforests, wet sclerophyll forests and temperate heathlands of south-western, south-eastern and north-eastern Australia (Rix and Harvey, 2011) (see inset Figs. 2 and 8–10). Prior to the revisionary taxonomic work of Rix and Harvey (2011), only five species of Archaeidae had been described from opposite corners of mainland Australia, despite the presence of dozens of undescribed species sitting unworked in museum collections (Rix and Harvey, 2011). Rix and Harvey (2011) described 17 new species from the rainforests of south-eastern Queensland and eastern New South Wales, providing the first compelling taxonomic and molecular phylogenetic evidence for the significant diversity of Archaeidae present within Australia. Dedicated field surveys throughout south-western and eastern Australia, combined with recent advances in our understanding of archaeid biology and ecology, have also revealed an Australian assassin spider fauna that is far more widespread than expected even 10 years ago (Rix and Harvey, 2011). This fauna is characterised by mostly short-range endemic (Harvey, 2002b; Harvey et al., 2011) allopatric taxa, many of which are restricted to individual mountains or montane systems, and all of which are closely tied to mesic and/or refugial habitats (see Rix and Harvey, 2011).

The current study aims to test the phylogeny and vicariant biogeography of the Australian assassin spiders, using a multi-locus molecular approach. It is the first comprehensive phylogenetic analysis of Australian Archaeidae, and one of a growing number of studies to explore the phylogeography of Australia's mesic zone using an invertebrate taxon. Our specific aims were to: (i) infer the phylogenetic interrelationships among species of *Austrarchaea*; and (ii) explore the phylogeographic origins of the diverse Australian fauna, reconciling topology and geography with divergence date estimation. Results are compared and discussed in relation to other sympatric taxa, with the overall aim of providing further insights into the historical biogeography of the continent's remaining rainforests, wet sclerophyll forests and southern-temperate heathlands.

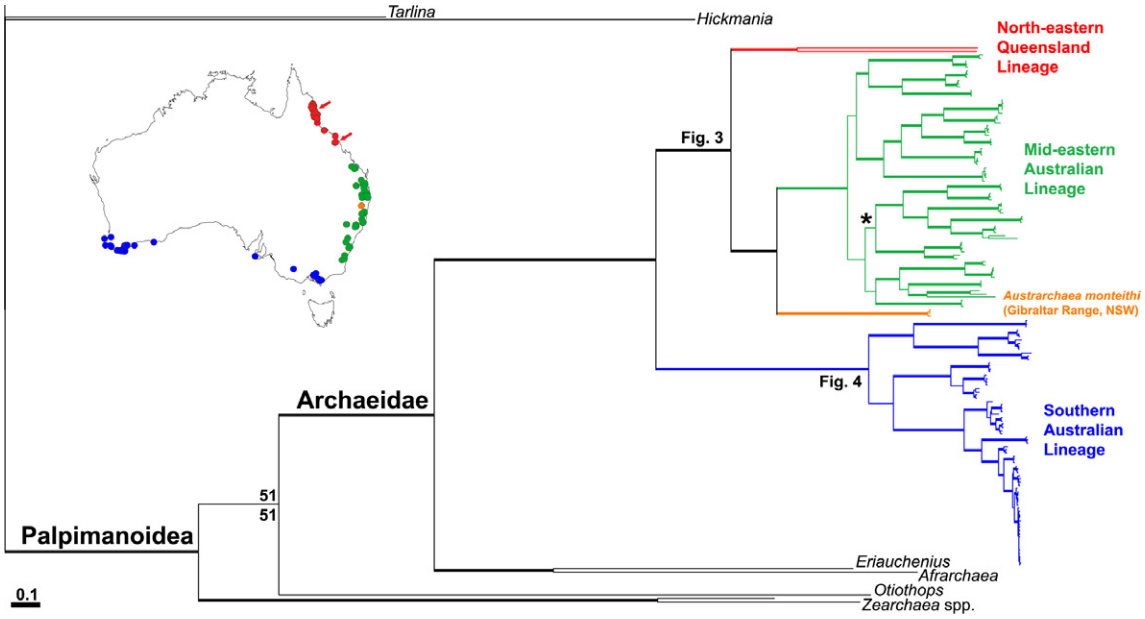

Fig. 2. Bayesian majority-rule consensus tree with re-estimated branch lengths, resulting from a combined, gene-partitioned phylogenetic analysis of the 'complete' dataset (168 taxa; 2591 bp; 40 million generations). This tree was largely identical (except where highlighted*) to that resulting from a combined, gene-partitioned analysis of the 'core' dataset with <10% missing data (148 taxa; 2585 bp; 40 million generations). Major Australian clades are coloured according to their distribution (see inset), with further resolution for Australian taxa provided in Figs. 3 and 4. Clades with >95% posterior probability support are denoted by thickened branches, with lower individual clade support values shown above nodes (for the 'complete' dataset) or below nodes (for the 'core' dataset). Red arrows on inset map denote the sampling localities for two species in the north-eastern Queensland lineage.

2. Material and methods

2.1. Taxa

Specimens of Archaeidae were collected throughout eastern and south-western Australia as part of ongoing monographic research (see Rix and Harvey, 2011) and preserved in 95% ethanol for long-term storage. Molecular exemplar specimens were available from most known populations for 29 of 31 recorded species from Western Australia, South Australia, Victoria, New South Wales and south-eastern Queensland (Figs. 8–10), with only two recognised species not available for sequencing: *A. clyneae* Rix and Harvey, 2011 from Mount Clunie (New South Wales) (Fig. 9) and an undescribed species from the Grampians National Park (Victoria) (Fig. 8). Two additional exemplar specimens were sequenced from a diverse, as yet unstudied clade of Archaeidae from tropical north-eastern Queensland (Fig. 2).

Five outgroup taxa were sequenced from the superfamily Palpimanoidea, including two species of *Zearchaea* (family Mecysmaucheniidae), one species of *Otiothops* (family Palpimanidae) and two species of African/Malagasy Archaeidae, representing each of the two currently recognised Old World genera: *Eriauchenius workmani* O. Pickard-Cambridge, 1881 from Ranomafana, Madagascar; and *Afrarchaea woodae* Lotz, 2006 from Kei Mouth, South Africa. Trees were rooted with the taxa *Hickmania troglodytes* (Higgins and Petterd, 1883) and *Tarlina smithersi* Gray, 1987 in Forster et al. (1987) (both in the superfamily Austrochiloidea), shown to be sister or closely-related to the Palpimanoidea in recent higher-level phylogenetic studies (see Griswold et al., 2005; Rix et al., 2008; Rix and Harvey, 2010, 2011).

In total, nucleotide sequences were obtained from 168 specimens, including 161 assassin spiders from Australia (see Table 1; Supplementary material 1). All specimens are vouchered and deposited in museum institutions, and all specimens are identifiable by unique isolate codes and/or museum voucher registration numbers, with sequences further deposited in GenBank (Table 1).

2.2. Genes

A multi-locus molecular approach to phylogenetic inference was employed in this study, with nucleotide sequences obtained from six mitochondrial genes and one nuclear protein-coding gene. The contiguous mitochondrial loci cytochrome *c* oxidase subunit I (COI), cytochrome *c* oxidase subunit II (COII), transfer RNA (tRNA) lysine (tRNA-K), tRNA aspartic acid (tRNA-D), ATP synthase subunit 8 (ATP8) and ATP synthase subunit 6 (ATP6) were sequenced to provide a rigorous selection of relatively rapidly-evolving mitochondrial markers, each phylogenetically informative at the population, species and/or genus level. The nuclear marker Histone H3 (H3) was also included for its non-maternal inheritance, resolution at marginally deeper phylogenetic nodes relative to mitochondrial loci and utility using a multi-locus molecular approach (Colgan et al., 1998; Boyer and Giribet, 2007; Sharma and Giribet, 2009; Crews et al., 2010; Pola and Gosliner, 2010; Yoshizawa and Johnson, 2010). Nuclear ribosomal RNA (rRNA) genes and internal transcribed spacers (e.g. 18S rRNA, 28S rRNA, ITS2) were not sequenced for this study, due to the presence of multiple length-variable rDNA amplicons and strong evidence for the non-concerted evolution of nuclear rDNA in Australian Archaeidae (see also Rix et al., 2008).

2.3. Molecular and laboratory methods

Between two and seven legs of each individual were removed for DNA extractions, and whole genomic DNA was extracted from leg tissue samples using the Qiagen DNeasy Blood and Tissue Kit protocol for animal tissues. Polymerase chain reaction (PCR) amplification of target gene regions was achieved using Invitrogen Platinum *Taq* Polymerase chemistry, in an Eppendorf Mastercycler ep gradient S thermal cycler. For each PCR reaction, 2 μl of extracted DNA, 0.25 μl of Platinum *Taq* (at 5 u/μl), 2–3 μl of $MgCl_2$ (at 50 mM), 2.5 μl of 10× PCR buffer, 5 μl of dNTPs (at 1 mM), 5 μl of each primer (at 2 μM) and purified H_2O were used in every 25 μl reaction.

A 1071 bp fragment of the mitochondrial COI gene, along with a 535–541 bp fragment of the adjacent COII gene (~1609 bp in total) were amplified using the primers ArCO1 (newly-designed for this study) plus C2-N-3661b (modified from Simon et al., 1994), or variants thereof (Table 2). The alternative forward primers LCO (Folmer et al., 1994) and C1-J-1718spF (Simon et al., 1994; Rix et al., 2010) were also used in several taxa (Table 2). Sequencing of COI–COII amplicons was achieved using PCR primers plus overlapping, internal sequencing primers, the latter also used as PCR primers in certain taxa (see Table 2; Supplementary material 2). A 71–77 bp fragment of the COII gene, an adjacent 90–113 bp region including the partially overlapping tRNA genes tRNA-K and tRNA-D, an adjacent 137–146 bp region including the entire ATP8 gene, along with an adjacent 335 bp fragment of the ATP6 gene (~640 bp in total) were amplified using the primers C2-N-3661R (modified from Simon et al., 1994) plus ATP6a (newly-designed for this study), or variants thereof (Table 2). A final 304 bp fragment of the nuclear H3 gene was amplified with the primers H3F1 (newly-designed for this study) or H3aF (Colgan et al., 1998) plus H3aR (Colgan et al., 1998) (Table 2). The PCR protocol used was: 94 °C for 1 min; 35–40× (94 °C for 30 s, 48–52.1 °C for 30 s, 72 °C for 1 min); 72 °C for 5 min. The presence of PCR products in PCR reactions was confirmed using standard agarose gel electrophoresis; if PCR products were detected, PCR reactions were then purified using the MoBio UltraClean PCR Clean-up Kit. Bidirectional sequencing of purified PCR products was performed by Macrogen Corporation (South Korea), using supplied PCR primers and additional internal sequencing primers for COI–COII (Table 2; Supplementary material 2).

2.4. Sequence annotation and alignment

Sequence (.ab1) files for the coding and non-coding strands were assembled automatically as anti-parallel contigs, and visualised using Sequencher 4.8 (Demonstration Version). All chromatograms were inspected and annotated by eye, with ambiguous bases denoted by IUPAC codes. Annotated sequences were saved as text files and imported into ClustalX Version 1.83 (Thompson et al., 1997) for alignment. Data were aligned in combination, using a default gap opening cost of 15 and a gap extension cost of 50, with minor manual adjustments made to the position of small (⩽9 bp) amino acid insertions/deletions (indels) according to known open reading frames. All protein-coding genes (i.e. COI, COII, ATP8, ATP6, H3) were either length invariable or possessed only minor (⩽3 amino acid) indels in some taxa, with significant length variation present only within mitochondrial tRNAs. In total, nearly 2.6 kilobases of aligned nucleotide data were obtained from 168 taxa (Table 1), with final alignments saved as nexus files in ClustalX.

2.5. Phylogenetic inference

To infer phylogenetic interrelationships among sequenced specimens of Archaeidae, two combined, gene-partitioned Bayesian analyses were executed in MrBayes Version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). A first analysis – of the 'complete' dataset – incorporated all 168 sequenced

Table 1
Specimens sequenced as part of this study.

Species name	Specimen code	Museum reg. no.	GenBank accession numbers		
			COI-COII	COII-ATP6	H3
OUTGROUPS					
Hickmania troglodytes	N.A.[b]	WAM T79989	JF909360	JN715992	JN716156
Tarlina smithersi	N.A.[b]	WAM T112581	JF909361	JN715993	JN716157
OTHER PALPIMANOIDEA					
Otiothops birabeni	N.A.[b]	MACN Ar11491	JF909362	JN715994	JN716158
Zearchaea sp. 1	N.A.[a]	WAM T79990	JF909363	JN715995	JN716159
Zearchaea sp. 2	N.A.[b]	WAM T112582	JF909364	JN715996	JN716160
OLD WORLD ARCHAEIDAE					
Eriauchenius workmani	N.A.[b]	CASENT 9018984	JF909365	JN715997	JN716161
Afrarchaea woodae	N.A.[a]	CASENT 9018957	JN715918	JN715998	JN716162
QUEENSLAND ARCHAEIDAE					
Austrarchaea alani	KT-63-F[b]	WAM T112550	JF909379	JN715999	JN716163
	KT-64-J	WAM T112550	JF909380	JN716000	JN716164
	KT-65-J	WAM T112550	JF909381	JN716001	JN716165
	KT-66-M	WAM T112551	JF909382	JN716002	JN716166
	KT-67-J	WAM T112551	JF909383	JN716003	JN716167
Austrarchaea aleenae	BUL-68-M[b]	WAM T112552	JF909384	JN716004	JN716168
	BUL-69-J	WAM T112552	JF909385	JN716005	JN716169
	BUL-70-J	WAM T112552	JF909386	JN716006	JN716170
Austrarchaea cunninghami	Ar55-89-F[b]	WAM T112555	JF909393	JN716007	JN716171
	Ar55-90-J	WAM T112555	JF909394	JN716008	JN716172
	Ar55-91-J	WAM T112555	JF909395	JN716009	JN716173
Austrarchaea dianneae	Ar56-54-M	WAM T112556	JF909399	JN716010	JN716174
	Ar56-55-J	WAM T112556	JF909400	JN716011	JN716175
	Ar56-56-J	WAM T112556	JF909401	JN716012	JN716176
	Ar59-60-M[b]	WAM T112557	JF909396	JN716013	JN716177
	Ar59-61-J	WAM T112557	JF909397	JN716014	JN716178
	Ar59-62-J	WAM T112557	JF909398	JN716015	JN716179
Austrarchaea harmsi	Ar71-71-J	WAM T112560	JF909409	JN716016	JN716180
	Ar71-72-J	WAM T112560	JF909410	JN716017	JN716181
	Ar70-73-M[b]	WAM T112559	JF909406	JN716018	JN716182
	Ar70-74-J	WAM T112559	JF909407	JN716019	JN716183
	Ar70-75-J	WAM T112559	JF909408	JN716020	JN716184
Austrarchaea judyae	Ar67-76-F[b]	WAM T112563	JF909414	JN716021	JN716185
	Ar67-77-J[a]	WAM T112563	JN715919	JN716022	JN716186
	Ar67-78-J	WAM T112563	JF909415	JN716023	JN716187
	Ar66-79-J	WAM T112562	JF909416	JN716024	JN716188
	Ar68-80-M[a]	WAM T112564	JF909417	N.A.	JN716189
	Ar68-81-J[a]	WAM T112564	JF909418	N.A.	JN716190
	Ar68-82-J[a]	WAM T112564	JF909419	N.A.	JN716191
Austrarchaea nodosa	QLD-42-J[a]	WAM T89592	JN715920	JN716025	JN716192
	Ar57-46-J	WAM T112572	JF909377	JN716026	JN716193
	LAM-51-J[b]	WAM T89592	JF909375	JN716027	JN716194
	Ar58-53-J	WAM T112573	JF909378	JN716028	JN716195
	Ar56-58-J	WAM T112571	JF909376	JN716029	JN716196
Austrarchaea raveni	Ar73-83-F[b]	QMB S90192	JF909435	JN716030	JN716197
	Ar73-84-J	WAM T112574	JF909436	JN716031	JN716198
	Ar73-85-J	WAM T112574	JF909437	JN716032	JN716199
	Ar69-86-M	WAM T112575	JF909438	JN716033	JN716200
	Ar69-87-J	WAM T112575	JF909439	JN716034	JN716201
	Ar69-88-J	WAM T112575	JF909440	JN716035	JN716202
Austrarchaea sp. nov. (NEQ-1)	MG-45-J[b]	WAM T97462	JF909374	JN716036	JN716203
Austrarchaea sp. nov. (NEQ-2)	EU-172-J[a]	AMS KS106561	JN715921	JN716037	JN716204

(*continued on next page*)

Table 1 (*continued*)

Species name	Specimen code	Museum reg. no.	GenBank accession numbers		
			COI-COII	COII-ATP6	H3
NEW SOUTH WALES ARCHAEIDAE					
Austrarchaea binfordae	Ar46-106-M[b]	AMS KS114969	JF909402	JN716038	JN716205
Austrarchaea christopheri	Ar49-95-M[b]	AMS KS114968	JF909387	JN716039	JN716206
	Ar49-96-J	WAM T112554	JF909388	JN716040	JN716207
	Ar49-97-J	WAM T112554	JF909389	JN716041	JN716208
	Ar50-98-J	WAM T112553	JF909390	JN716042	JN716209
	Ar50-99-J[a]	WAM T112553	JF909391	JN716043	N.A.
	Ar50-100-J	WAM T112553	JF909392	JN716044	JN716210
Austrarchaea helenae	Ar30-124-J[a]	WAM T112561	JF909411	N.A.	JN716211
	Ar30-125-J[b]	WAM T112561	JF909412	JN716045	JN716212
	Ar30-126-J	WAM T112561	JF909413	JN716046	JN716213
Austrarchaea mascordi	Ar41-48-F[b]	AMS KS114973	JF909420	JN716047	JN716214
	Ar41-113-J	WAM T112566	JF909421	JN716048	JN716215
	Ar41-114-J	WAM T112566	JF909422	JN716049	JN716216
	Ar40-115-M	WAM T112565	JF909423	JN716050	JN716217
Austrarchaea mcguiganae	Ar28-47-J[b]	WAM T112567	JF909424	JN716051	JN716218
	Ar28-127-F[a]	AMS KS114975	JN715922	JN716052	JN716219
	Ar28-128-J	WAM T112567	JF909425	JN716053	JN716220
Austrarchaea milledgei	Ar43-107-F	WAM T112568	JF909426	JN716054	JN716221
	Ar43-108-J	WAM T112568	JF909427	JN716055	JN716222
	Ar43-109-J	WAM T112568	JF909428	JN716056	JN716223
	Ar42-110-J	WAM T112569	JF909429	JN716057	JN716224
	Ar42-111-J	WAM T112569	JF909430	JN716058	JN716225
	Ar42-112-J[b]	WAM T112569	JF909431	JN716059	JN716226
Austrarchaea monteithi	Ar52-92-F[b]	AMS KS114976	JF909432	JN716060	JN716227
	Ar52-93-J	WAM T112570	JF909433	JN716061	JN716228
	Ar52-94-J	WAM T112570	JF909434	JN716062	JN716229
Austrarchaea platnickorum	Ar51-101-M	WAM T112558	JF909403	JN716063	JN716230
	Ar51-102-F	WAM T112558	JF909404	JN716064	JN716231
	Ar51-103-J[b]	WAM T112558	JF909405	JN716065	JN716232
Austrarchaea smithae	Ar32-116-F[b]	WAM T112576	JF909441	JN716066	JN716233
	Ar32-117-J	WAM T112576	JF909442	JN716067	JN716234
	Ar32-118-J	WAM T112576	JF909443	JN716068	JN716235
Austrarchaea sp. indet.	Ar47-104-J	WAM T112580	JF909444	JN716069	JN716236
	Ar47-105-J[b]	WAM T112580	JF909445	JN716070	JN716237
Austrarchaea sp. indet.	Ar33-119-J	WAM T112578	JF909446	JN716071	JN716238
	Ar33-120-J	WAM T112578	JF909447	JN716072	JN716239
	Ar33-121-J[b]	WAM T112578	JF909448	JN716073	JN716240
	Ar34-122-J	WAM T112579	JF909449	JN716074	JN716241
	Ar34-123-J	WAM T112579	JF909450	JN716075	JN716242
Austrarchaea sp. indet.	Ar27-129-J	WAM T112577	JF909451	JN716076	JN716243
	Ar27-130-J	WAM T112577	JF909452	JN716077	JN716244
	Ar27-131-J[b]	WAM T112577	JF909453	JN716078	JN716245
VICTORIAN ARCHAEIDAE					
Austrarchaea sp. nov. (VIC-1)	Ar13-135-F	WAM T114024	JN715923	JN716079	JN716246
	Ar13-136-J	WAM T114024	JN715924	JN716080	JN716247
	Ar13-137-J	WAM T114024	JN715925	JN716081	JN716248
	Ar18-138-M	MV	JN715926	JN716082	JN716249
	Ar18-139-J[b]	WAM T114025	JN715927	JN716083	JN716250
	Ar18-140-J	WAM T114025	JN715928	JN716084	JN716251
	Ar16-141-J	WAM T114026	JN715929	JN716085	JN716252
Austrarchaea sp. nov. (VIC-2)	Ar14-49-F[b]	WAM T112583	JF909366	JN716086	JN716253
	Ar14-133-J	WAM T112583	JN715930	JN716087	JN716254
	Ar14-134-J	WAM T112583	JF909367	JN716088	JN716255
SOUTH AUSTRALIAN ARCHAEIDAE					
Austrarchaea sp. nov. (SA-1)	Ar77-50-F[b]	SAM	JN715931	JN716089	JN716256

Table 1 (*continued*)

Species name	Specimen code	Museum reg. no.	GenBank accession numbers		
			COI-COII	COII-ATP6	H3
	Ar77-142-J	WAM T114027	JN715932	JN716090	JN716257
	Ar77-143-J	WAM T114027	JN715933	JN716091	JN716258
WESTERN AUSTRALIAN ARCHAEIDAE					
Austrarchaea mainae	TO-1-J	WAM T89566	JN715934	JN716092	JN716259
	TO-2-J	WAM T89566	JN715935	JN716093	JN716260
	TO-3-J	WAM T89568	JN715936	JN716094	JN716261
	TO-4-J	WAM T89568	JN715937	JN716095	JN716262
	WF-5-J[a]	WAM T89569	JN715938	JN716096	JN716263
	WF-6-J	WAM T89569	JN715939	JN716097	JN716264
	WF-8-F	WAM T89571	JN715940	JN716098	JN716265
	WF-9-F[b]	WAM T89572	JF909368	JN716099	JN716266
	WF-10-J[a]	WAM T89573	JN715941	JN716100	JN716267
	WF-11-F	WAM T89574	JN715942	JN716101	JN716268
	WF-12-J	WAM T89575	JN715943	JN716102	JN716269
	WF-13-J	WAM T89576	JN715944	JN716103	JN716270
	GI-14-F	WAM T89577	JN715945	JN716104	JN716271
	GI-15-J	WAM T89577	JN715946	JN716105	JN716272
	GR-16-F	WAM T89578	JN715947	JN716106	JN716273
	GR-17-J	WAM T89578	JF909369	JN716107	JN716274
	MB-20-F	WAM T89581	JN715948	JN716108	JN716275
	MB-21-J	WAM T89582	JN715949	JN716109	JN716276
	MB-23-F	WAM T89584	JN715950	JN716110	JN716277
	WF-24-F	WAM T89585	JN715951	JN716111	JN716278
	WF-25-J	WAM T89586	JN715952	JN716112	JN716279
	WF-26-J	WAM T89587	JN715953	JN716113	JN716280
	WF-28-J	WAM T89589	JN715954	JN716114	JN716281
	WF-29-J	WAM T89589	JN715955	JN716115	JN716282
	MH-30-J	WAM T89590	JN715956	JN716116	JN716283
	WB-31-J	WAM T89591	JN715957	JN716117	JN716284
	BB-34-F	WAM T89563	JN715958	JN716118	JN716285
	BB-35-J[a]	WAM T89563	JN715959	JN716119	JN716286
	WA-36-J	WAM T89564	JN715960	JN716120	JN716287
	WA-37-J	WAM T89564	JN715961	JN716121	JN716288
	BB-147-J	WAM T97463	JN715962	JN716122	JN716289
	WA-162-F	WAM T114037	JN715963	JN716123	JN716290
	PO-166-F	WAM T97465	JN715964	JN716124	JN716291
	PO-167-J	WAM T97465	JN715965	JN716125	JN716292
	PO-168-F[a]	WAM T114028	JN715966	JN716126	N.A.
	WB-169-F	WAM T114029	JN715967	JN716127	JN716293
	WB-170-J	WAM T114030	JN715968	JN716128	JN716294
	TO-171-J	WAM T89567	JN715969	JN716129	JN716295
Austrarchaea robinsi	EP-40-J[a]	WAM T89558	JF909370	JN716130	N.A.
	EP-41-J[a]	WAM T89558	JF909371	JN716131	N.A.
	BK-148-J[a]	WAM T114031	JN715970	JN716132	N.A.
	BK-149-J[a]	WAM T114032	JN715971	JN716133	N.A.
Austrarchaea sp. nov. (WA-1)	HA-150-J	WAM T97467	JN715972	JN716134	JN716296
	HA-151-J	WAM T97467	JN715973	JN716135	JN716297
	TP-152-F[b]	WAM T97468	JN715974	JN716136	JN716298
	TP-153-J	WAM T97468	JN715975	JN716137	JN716299
Austrarchaea sp. nov. (WA-2)	TA-154-M	WAM T117055	JN715976	JN716138	JN716300
	TA-155-J	WAM T94089	JN715977	JN716139	JN716301
	TA-156-J[b]	WAM T97466	JN715978	JN716140	JN716302
	TA-157-J[a]	WAM T97466	JN715979	JN716141	N.A.
Austrarchaea sp. nov. (WA-3)	CLG-144-J	WAM T94477	JN715980	JN716142	JN716303
	CLG-145-J[b]	WAM T94477	JN715981	JN716143	JN716304
	CLG-146-J	WAM T114033	JN715982	JN716144	JN716305
Austrarchaea sp. nov. (WA-4)	CN-32-J	WAM T89561	JN715983	JN716145	JN716306
	CN-33-J	WAM T89562	JN715984	JN716146	JN716307
	KV-38-J[b]	WAM T89565	JF909372	JN716147	JN716308

(*continued on next page*)

Table 1 (*continued*)

Species name	Specimen code	Museum reg. no.	GenBank accession numbers		
			COI-COII	COII-ATP6	H3
	KV-39-J	WAM T89565	**JN715985**	**JN716148**	**JN716309**
	CO-158-F	WAM T112584	**JF909373**	**JN716149**	**JN716310**
	CO-159-J	WAM T114034	**JN715986**	**JN716150**	**JN716311**
	GL-160-J	WAM T114035	**JN715987**	**JN716151**	**JN716312**
	TB-161-J	WAM T114036	**JN715988**	**JN716152**	**JN716313**
	CL-163-J	WAM T97464	**JN715989**	**JN716153**	**JN716314**
	CL-164-J	WAM T97464	**JN715990**	**JN716154**	**JN716315**
	CL-165-J	WAM T97464	**JN715991**	**JN716155**	**JN716316**

Full collection data for each specimen can be found in Supplementary material 1. Museum repositories are as follows: AMS, Australian Museum (Sydney); CASENT, California Academy of Sciences (San Francisco); MACN, Museo Argentino de Ciencias Naturales (Buenos Aires); MV, Museum Victoria (Melbourne); QMB, Queensland Museum (Brisbane); SAM, South Australian Museum (Adelaide); WAM, Western Australian Museum (Perth).

[a] Specimens excluded from the 'core' dataset.

[b] Specimens included in the 'partial' dataset (i.e. one specimen per species).

Table 2
Primers used to amplify and sequence mitochondrial (COI, COII, tRNA-K, tRNA-D, ATP8, ATP6) and nuclear (H3) genes for the molecular analyses. Underlined letters denote nucleotide modifications.

Name	Sequence (5′–3′)	Type (Gene)	References
PCR PRIMERS			
ArCO1	CATTTAGCTGGTGCTTCTTCTATT	Forward (COI)	Rix and Harvey (2011)
ArCO1a	CATTTAGCTGGTGCTTCATCTATT	Forward (COI)	Rix and Harvey (2011)
ArCO1c	CATTTGGCTGGGGCGTCATCAATT	Forward (COI)	Rix and Harvey (2011)
ZrCO1	TCTTTACATTTAGCTGGTGCTTCTT	Forward (COI)	Rix and Harvey (2011)
ATP6a	GGASHCCCYDVHGGVACYAAATGAG	Reverse (ATP6)	
ATP6a1	GGWSHYCCYDVHGGVACYAAATGAG	Reverse (ATP6)	
ATP6b	TGVCCDGCHATHATATTAGCVGC	Reverse (ATP6)	
C1-J-1718spF	GGTGGATTTGGTAATTGATTAGTTCC	Forward (COI)	Simon et al. (1994), Rix et al. (2010)
C2-N-3661	CACAAATTTCTGAACATTGACCA	Reverse (COII)	Simon et al. (1994)
C2-N-3661a	CACAAATTTCAGAACATTGACCA	Reverse (COII)	Simon et al. (1994), Rix and Harvey (2011)
C2-N-3661b	CACAAATTTCAGAACATTGACCT	Reverse (COII)	Simon et al. (1994), Rix and Harvey (2011)
C2-N-3661R	TGGTCAATGTTCAGAAATTTGTG	Forward (COII)	Simon et al. (1994)
C2-N-3661aR	TGGTCAATGTTCTGAAATTTGTG	Forward (COII)	Simon et al. (1994)
C2-N-3661bR	AGGTCAATGTTCTGAAATTTGTG	Forward (COII)	Simon et al. (1994)
H3aF	ATGGCTCGTACCAAGCAGACVGC	Forward (H3)	Colgan et al. (1998)
H3aR	ATATCCTTRGGCATRATRGTGAC	Reverse (H3)	Colgan et al. (1998)
H3F1	GTAAAAGTACCGGVGGHAARGC	Forward (H3)	
LCO	GGTCAACAAATCATAAAGATATTGG	Forward (COI)	Folmer et al. (1994)
SEQUENCING/PCR[a] PRIMERS			
SeqF2a	TYCATTATGTWTTAAGAATAGG	Forward (COI)	Rix and Harvey (2011)
SeqF2a1	CATTTYCATTATGTDTTRAGAATRGG	Forward (COI)	Rix and Harvey (2011)
SeqR1	CATCAGGATAATCWGAATAHCG	Reverse (COI)	Rix and Harvey (2011)
SeqR1a	CATCWGGRTARTCHGAATAHCGACG	Reverse (COI)	Rix and Harvey (2011)

[a] Used as PCR primers in certain taxa (see Supplementary material 2 for PCR primer combinations in different taxa).

taxa, including 20 taxa with a proportion of ⩾10% missing data (Table 1). A second analysis – of a pruned 'core' dataset – incorporated 148 taxa, all with <10% missing data (Table 1). Matrices were partitioned into six gene regions, corresponding to COI, COII, tRNAs, ATP8, ATP6 and H3.

Prior to analysis of both the 'complete' and 'core' datasets, PAUP* Version 4.0b10 (Swofford, 2002) and Modeltest Version 3.7 (Posada and Crandall, 1998) were used to choose the appropriate model of nucleotide substitution for each gene partition under an Akaike Information Criterion (AIC) framework. For all six partitions, a general time reversible (GTR), transversional (TVM) (Tavaré, 1986) or two transversion-parameters (K81uf) (Kimura, 1981) model of nucleotide substitution was invoked with the following likelihood settings [Lset nst = 6 rates = gamma]. For each data partition, parameters were estimated independently ([Unlink tratio = (all) pinvar = (all) shape = (all) statefreq = (all) revmat = (all)]), rates were allowed to vary across partitions ([Prset applyto = (all) ratepr = variable]), and four Markov Chain Monte Carlo (MCMC) chains were run for 40 million generations, sampling every 1000 generations, with the first 4,000,000 sampled trees discarded as 'burnin' ([burnin = 4000]). Bayesian log likelihood trace files, burnin times and summary statistics of estimated parameters were visualised using Tracer Version 1.5 (Rambaut and Drummond, 2009) and posterior probabilities were calculated and reported on a 50% majority-rule consensus tree of the post-burnin sample.

To further explore nodal support and the robustness (Giribet, 2003) of clades inferred by the 'complete' and 'core' datasets, a smaller 'partial' dataset was differentially analysed under 12 alternative partitioning strategies and optimality criteria (Table 3). This 'partial' dataset included a subset of 34 taxa from the 'core' dataset, with five outgroups and 29 exemplar species of Archaeidae from Australia (i.e. one specimen per species for taxa with <10% missing data) (Table 1; Fig. 5). Three analyses of the 'partial' dataset explored different gene-partitioning strategies in MrBayes, with separate unpartitioned, gene-partitioned and gene plus

Table 3
Partitions and Bayesian substitution model parameters applied to 12 different analyses of the 34-taxon 'partial' dataset (see Fig. 5).

Analysis	Matrix length	Bayesian partition/s (length)	Substitution model/s (parameters)
FULL ALIGNMENT			
1. Parsimony analysis	2585 bp	N.A.	N.A.
2. Unpartitioned	2585 bp	1× (2585 bp)	GTR + I + G (nst = 6 rates = gamma)
3. Partitioned by gene (6×)	2585 bp	COI (1071 bp) COII (618 bp) tRNAs (117 bp) ATP8 (140 bp) ATP6 (335 bp) H3 (304 bp)	TVM + I + G (nst = 6 rates = gamma) GTR + I + G (nst = 6 rates = gamma) K81uf + I + G (nst = 6 rates = gamma) TrN + I + G (nst = 6 rates = gamma) TVM + I + G (nst = 6 rates = gamma) GTR + I + G (nst = 6 rates = gamma)
4. Partitioned by gene + codon (11×)[a]	2585 bp	COI C.P. 1-2 (714 bp) COI C.P. 3 (357 bp) COII C.P. 1-2 (412 bp) COII C.P. 3 (206 bp) tRNAs (117 bp) ATP8 C.P. 1-2 (94 bp) ATP8 C.P. 3 (46 bp) ATP6 C.P. 1-2 (224 bp) ATP6 C.P. 3 (111 bp) H3 C.P. 1-2 (202 bp) H3 C.P. 3 (102 bp)	TIM + I + G (nst = 6 rates = gamma) TIM + I + G (nst = 6 rates = gamma) GTR + I + G (nst = 6 rates = gamma) K81uf + I + G (nst = 6 rates = gamma) K81uf + I + G (nst = 6 rates = gamma) TrN + I + G (nst = 6 rates = gamma) K81uf + G (nst = 6 rates = gamma) GTR + I + G (nst = 6 rates = gamma) TrN + I + G (nst = 6 rates = gamma) SYM + I (nst = 6 rates = equal) TVM + G (nst = 6 rates = gamma)
CONCATENATED ALIGNMENTS			
5. COI	1071 bp	1× (1071 bp)	TVM + I + G (nst = 6 rates = gamma)
6. COII	618 bp	1× (618 bp)	GTR + I + G (nst = 6 rates = gamma)
7. tRNAs	117 bp	1× (117 bp)	K81uf + I + G (nst = 6 rates = gamma)
8. ATP8	140 bp	1× (140 bp)	TrN + I + G (nst = 6 rates = gamma)
9. ATP6	335 bp	1× (335 bp)	TVM + I + G (nst = 6 rates = gamma)
10. H3	304 bp	1× (304 bp)	GTR + I + G (nst = 6 rates = gamma)
11. COI + COII	1689 bp	1× (1689 bp)	GTR + I + G (nst = 6 rates = gamma)
12. tRNAs + ATP8 + ATP6	592 bp	1× (592 bp)	GTR + I + G (nst = 6 rates = gamma)

[a] Protein-coding genes partitioned according to codon positions 1-2 (C.P. 1-2) versus codon position 3 (C.P. 3).

codon-partitioned analyses of the full (2585 bp) alignment (Table 3). Eight additional Bayesian analyses used concatenated alignments (117–1689 bp in length) to test the phylogenetic signal of different genes or groups of genes across different clades (Table 3). All 11 Bayesian analyses of the 'partial' dataset were run for 40 million generations, with the first 4,000,000 sampled trees discarded as 'burnin' and posterior probabilities calculated and reported on 50% majority-rule consensus trees. A single parsimony analysis of the full (2585 bp) alignment was also executed in PAUP*, using a tree-bisection-reconnection (TBR) search algorithm, with 10,000 replicates and 10 trees held at each step during random stepwise addition. All characters were unordered and equally weighted and gaps were treated as missing data. Parsimony clade support values were estimated using non-parametric bootstrapping (Felsenstein, 1985) in PAUP*, with 1000 pseudoreplicates of a heuristic (TBR) search algorithm incorporating 10 replicates of random stepwise addition of taxa, and 10 trees held at each step. Alignment and Bayesian substitution parameters for all 12 analyses of the 'partial' dataset are summarised in Table 3.

2.6. Divergence date estimation

To estimate nodal divergence dates for Australian species of Archaeidae, a relaxed Bayesian molecular clock analysis (Drummond et al., 2006) was performed using BEAST Version 1.6.1 (Drummond and Rambaut, 2007). Prior to analysis, the 'partial' dataset (see above) was expanded to include an additional outgroup species from the infraorder Mygalomorphae (*Haplopelma schmidti* von Wirth, 1991), with sequences obtained from GenBank (Accession No. AY309259; Qiu et al., 2005). This modified, re-rooted 'partial' dataset (of 35 taxa) was concatenated to include only the mitochondrial partitions COI, COII, tRNA-K, tRNA-D, ATP8 and ATP6 (2287 bp in total). As no recent (i.e. Tertiary) external calibration points exist for Australian Archaeidae, a widely-used prior (arthropod) substitution rate of 0.0115 was applied according to the Brower (1994) metric of 2.3% pairwise sequence divergence per million years for mitochondrial loci. This rate metric has been widely used in phylogenetic studies of araneomorph spiders (e.g. Hedin, 2001; Chang et al., 2007; Framenau et al., 2010; Vink and Dupérré, 2010), hence its application here, although a slightly higher rate may be characteristic of mygalomorph taxa (see Bond et al., 2001; Arnedo and Ferrández, 2007; Cooper et al., 2011). Two older external calibrations of Jurassic and early Cretaceous age – corresponding to the earliest known fossil Archaeidae (see Selden et al., 2008) and the putative separation of Madagascar/India from East Antarctica (see Boyer et al., 2007) – were also considered and tested using the modified 'partial' dataset (Fig. 7), but both resulted in gross overestimates of clade divergence, presumably due to site saturation and the inherent credibility error associated with calibrating very deep (e.g. Mesozoic) nodes using mitochondrial data (DeSalle et al., 1987; Juan et al., 1995) (Fig. 7). The BEAST input (.xml) file was created using BEAUti Version 1.6.1 (part of the BEAST software package), applying a relaxed uncorrelated lognormal molecular clock with Yule speciation process and general time reversible (GTR plus gamma; see Table 3) site heterogeneity model. Monophyly of the ingroup (Araneomorphae), superfamily Palpimanoidea and family Archaeidae were enforced, and Bayesian MCMC simulations were run for 40 million generations, sampling every 1000 generations with the first 4,000,000 generations discarded as 'burnin'. Trace files and summary statistics of estimated parameters were visualised using Tracer Version 1.5, with the final calibrated chronogram

and node estimates visualised and edited using FigTree Version 1.2.1 (Rambaut, 2009).

2.7. Biogeographic inferences and population genetics

Biogeographic inferences were made using a combination of phylogenetic and population genetic methods, with the aim of developing explicit, testable vicariant biogeographic hypotheses for the diversification of Australian Archaeidae (see Discussion, below). ArcMap Version 9.3.1 (ESRI Inc.) with Virtual Earth (Microsoft Corporation) was used to create 'area chronograms', to reconcile inferred clades and divergence dates with the known distributions of Australian archaeid taxa (Figs. 8–10). For closely related south-western Australian populations, and to further analyse population genetic structure within *Austrarchaea mainae* Platnick, 1991b, nested clade analyses of COI haplotype data were used to infer unrooted statistical parsimony networks in TCS Version 1.21 (Clement et al., 2000). Uncorrected pairwise and mean inter-group sequence divergences for all taxa were calculated using MEGA Version 5 (Tamura et al., 2011).

3. Results

3.1. Sequence statistics

The final aligned matrix of the 'complete' dataset consisted of 168 specimens, six data partitions and was 2591 nucleotides in length, including 1258 (49%) parsimony-informative (PI) sites. The final aligned matrix of the 'core' dataset was 2585 nucleotides in length (slightly shorter than the 'complete' matrix due to an autapomorphic two amino acid insertion in ATP8 in *Zearchaea* sp. 1), with 148 specimens, six data partitions and 1208 (47%) parsimony-informative sites. For both the 'complete' and 'core' datasets, the COI data partition was 1071 nucleotides in length (39–42% PI); the COII data partition was 618 nucleotides in length (53–54% PI); the tRNA (K/D) data partition was 117 nucleotides in length (37–38% PI); the ATP8 data partition was 140–146 nucleotides in length (77–78% PI); the ATP6 data partition was 335 nucleotides in length (62–64% PI); and the H3 data partition was 304 nucleotides in length (33–34% PI) (Table 3). The mean base composition across the entire 'core' matrix was as follows: A = 0.279; C = 0.110; G = 0.185; T = 0.425.

3.2. Phylogenetic analyses

Gene-partitioned Bayesian analyses of the 'complete' and 'core' datasets produced largely identical topologies, with strong support for a monophyletic clade of Australian Archaeidae and three major endemic lineages, including a north-eastern Queensland lineage, a mid-eastern Australian lineage and southern Australian lineage (Figs. 2–4). One enigmatic species from the Gibraltar Range National Park in northern New South Wales, *Austrarchaea monteithi* Rix and Harvey, 2011, was inferred as a sister-species to all other taxa in mid-eastern Australia (Figs. 2 and 3). Monophyletic clades congruent with previously described species (see Rix and Harvey, 2011) plus nine newly-inferred (undescribed) species were all supported by >95% posterior probability (PP) values in both analyses (Figs. 3 and 4).

Differential analysis of the 'partial' dataset was used to further test the phylogenetic interrelationships among species – using a one taxon per species approach – highlighting the varying robustness (Giribet, 2003) of clades under alternative partitioning strategies and optimality criteria (Fig. 5). Results were consistent with Bayesian analyses of the 'complete' and 'core' datasets, in that nodes inferred with >95% PP support in the 'complete' and 'core' analyses (Figs. 2–4), were also inferred and supported by similarly high PP and/or bootstrap values in at least three or four of the 12 individual analyses of the 'partial' dataset (Fig. 5). Clades poorly supported in the 'complete' and 'core' analyses were also poorly resolved in analyses of the 'partial' dataset, with strong (>95%) clade support inferred by at most two – and usually none – of the 12 individual analyses (Fig. 5). Several alternative clades and topologies were inferred in different analyses of the 'partial' dataset, but these were rarely supported by strong PP or bootstrap values. One exception, however, was the inferred but very poorly supported sister-group relationship between the Palpimanidae and the family Archaeidae (Fig. 2): in five of the twelve individual analyses of the 'partial' dataset, an alternative Mecysmaucheniidae + Archaeidae clade was inferred with 85–99% PP support, a result also replicated in the calibrated BEAST analysis of the mitochondrial data (Fig. 7).

Five ambiguous, poorly supported nodes inferred from the 'complete' dataset were collapsed following differential analysis of the 'partial' dataset (Fig. 5), leaving an otherwise well-resolved hypothesis for the phylogeny of Archaeidae from southern and eastern Australia (Fig. 6). Four monophyletic, well-supported regional clades were inferred within the mid-eastern Australian lineage, along with three equally well-supported regional clades in the southern Australian lineage (Fig. 6). Two species from mid-eastern Australia (*A. milledgei* Rix and Harvey, 2011 and *A. helenae* Rix and Harvey, 2011) could not be assigned to any of the four mid-eastern clades with confidence.

3.3. Divergence date estimation

Divergence date estimates from a relaxed molecular clock analysis of the mitochondrial data reveal that the three major lineages of Australian Archaeidae shared a common ancestor in the very early Tertiary, with the southern Australian lineage diverging from all other eastern Australian taxa in the Paleocene or early Eocene, well before the final separation of Australia from East Antarctica (Crisp et al., 2004) (95% highest posterior density [HPD] 69–46 million years ago [mya]) (Fig. 7). The north-eastern Queensland lineage diverged in the Eocene (95% HPD 51–34 mya), with *A. monteithi* diverging from all other mid-eastern Australian taxa in the Oligocene or earlier (95% HPD 39–26 mya) (Figs. 7 and 8). The diverse clade of mid-eastern Australian species shared a common ancestor at the beginning of the Miocene (95% HPD 27–20 mya) (Figs. 7–9).

Although divergence date estimates are not available for most species in the north-eastern Queensland clade, speciation events in southern and south-eastern Australian taxa are strongly correlated with the Miocene epoch (23–5.3 mya). Major regional clades appear to have diverged in the very early Miocene, potentially in response to disjunctions in the distribution of closed rainforests (Figs. 8–10), with subsequent *in-situ* speciation resulting from the climatic fragmentation of mesic habitats throughout Australia (see Section 4, below). Northern taxa in the south-eastern Queensland, Border Ranges and subtropical New South Wales clades appear to have diverged first, in the early-mid Miocene (Figs. 8 and 9), with southern and temperate taxa diverging slightly later, in the mid-late Miocene (Figs. 8 and 10). Species within each of the two endemic Western Australian clades appear to have diverged most recently, with inferred dates for all five species falling within the late Miocene (95% HPD 10–4 mya) (Fig. 10).

3.4. Population genetics

Mean inter-specific, uncorrected pairwise sequence divergences among COI haplotypes ranged from 3.3% to 6.8% in the Western Australian Stirling Range clade, to 9.2–11.4% and 7.7–14% in the

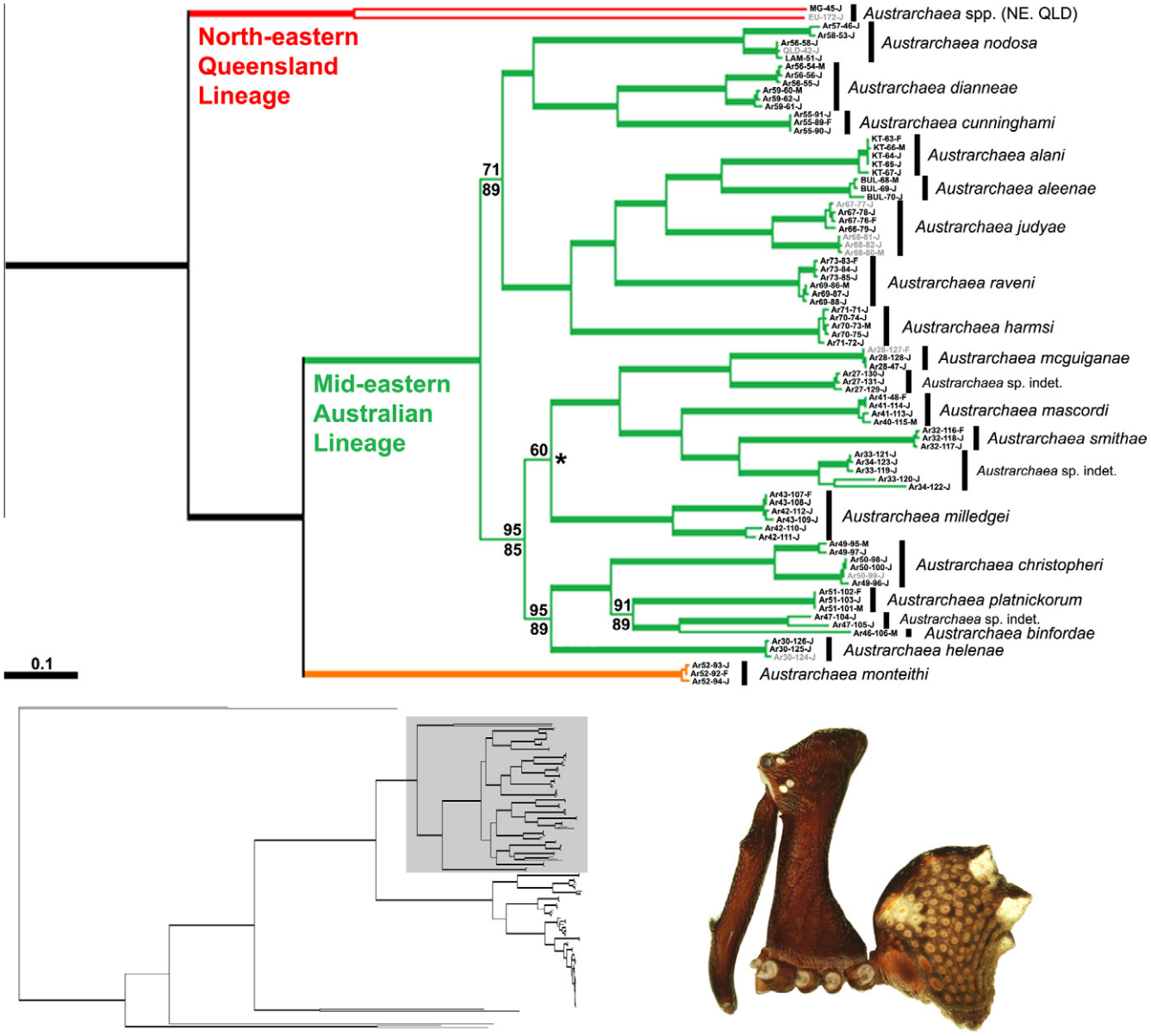

Fig. 3. Bayesian majority-rule consensus subtree for eastern Australian Archaeidae, resulting from a combined, gene-partitioned phylogenetic analysis of the 'complete' dataset (168 taxa; 2591 bp; 40 million generations) (see inset; Fig. 2). This tree was largely identical (except where highlighted*) to that resulting from a combined, gene-partitioned analysis of the 'core' dataset with <10% missing data (148 taxa; 2585 bp; 40 million generations). Clades with >95% posterior probability support are denoted by thickened branches, with lower individual clade support values shown above nodes (for the 'complete' dataset) or below nodes (for the 'core' dataset). Grey taxon labels denote specimens excluded from the 'core' dataset. The inset illustration shows *Austrarchaea aleenae* Rix and Harvey, 2011 from the Bulburin National Park, Queensland.

Border Ranges and Victorian-South Australian clades, respectively. Intra-specific pairwise divergences for COI varied from 0% to 6% (highest among populations of *A. milledgei* from the Barrington Tops), with most population-level divergences less than 4%. Nested clade analyses of COI haplotypes from Western Australian taxa revealed unconnected haplotype networks congruent with monophyletic clades inferred for the six endemic Western Australian species (Fig. 4); for *A. mainae*, three unconnected haplotype networks were inferred for three main population lineages, from Walpole-Nornalup National Park, Bremer Bay and from the Greater Albany/Porongurup region, respectively (Figs. 4 and 10). *Austrarchaea mainae* was known from only a single locality prior to its re-discovery in 2007, and remains a listed, threatened species in Western Australia (discussed in Rix and Harvey, 2008). The total linear distribution for the main Greater Albany population of this species now totals ~70 km, with the two outlying, genetically-isolated populations from Bremer Bay and Walpole extending the total known coastal range of *A. mainae* to over 200 km.

4. Discussion

4.1. Phylogeny

The current study presents the first comprehensive hypothesis of phylogenetic interrelationships among Australian assassin spiders, extending and further testing the preliminary hypothesis of Rix and Harvey (2011), and highlighting the diversity, antiquity and endemism of the family within Australia. Molecular phylogenetic results provide strong evidence for the monophyly of all Australian Archaeidae and for the presence of three major endemic lineages, each with non-overlapping distributions in north-eastern

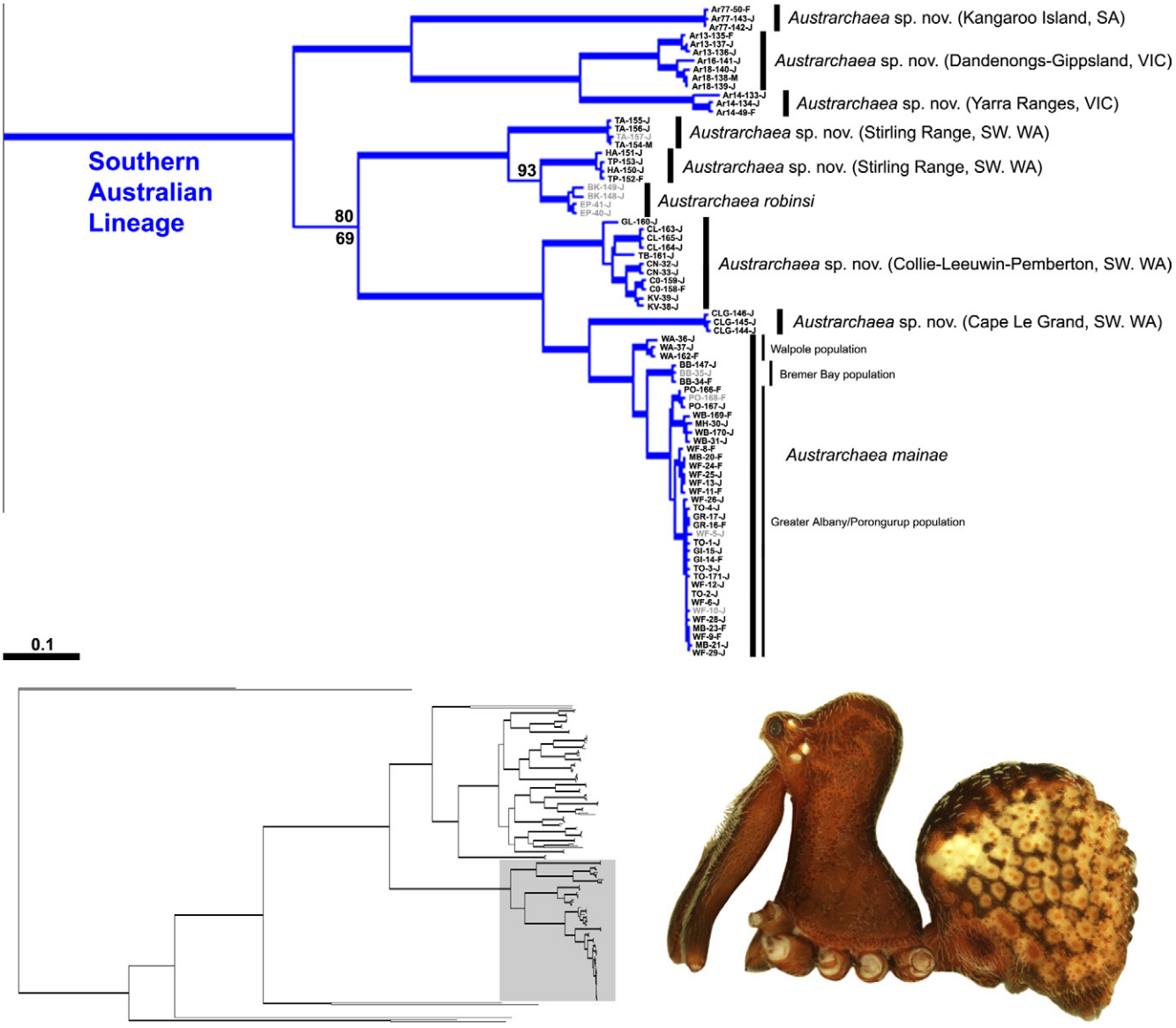

Fig. 4. Bayesian majority-rule consensus subtree for southern Australian Archaeidae, resulting from a combined, gene-partitioned phylogenetic analysis of the 'complete' dataset (168 taxa; 2591 bp; 40 million generations) (see inset; Fig. 2). This tree was largely identical to that resulting from a combined, gene-partitioned analysis of the 'core' dataset with <10% missing data (148 taxa; 2585 bp; 40 million generations). Clades with >95% posterior probability support are denoted by thickened branches, with lower individual clade support values shown above nodes (for the 'complete' dataset) or below nodes (for the 'core' dataset). Grey taxon labels denote specimens excluded from the 'core' dataset. The inset illustration shows *Austrarchaea* sp. nov. (VIC-2) from the Yarra Ranges National Park, Victoria.

Queensland, mid-eastern Australia and southern Australia, respectively (Fig. 2). The north-eastern Queensland lineage, although not fully sampled for this study, includes *Austrarchaea daviesae* Forster and Platnick, 1984 and numerous undescribed species (Fig. 1G) from the rainforests of the Queensland 'Wet Tropics', between Cooktown and Mackay. The mid-eastern Australian lineage includes at least 18 described species (Fig. 1A, E and F) from the rainforests of south-eastern Queensland and eastern New South Wales (Rix and Harvey, 2011). The southern Australian lineage, including *A. hickmani* (Butler, 1929), *A. mainae*, *A. robinsi* Harvey, 2002a and at least eight undescribed species (Figs. 1B–D), is widely distributed across Victoria, South Australia and south-western Western Australia. One additional enigmatic species – *Austrarchaea monteithi* from the Gibraltar Range National Park in northern New South Wales – was found to be a sister taxon to the mid-eastern Australian clade and not closely-related to any other known archaeid species in Australia, a result replicated by Rix and Harvey (2011). *Austrarchaea monteithi* is the only known member of a fourth, otherwise extinct lineage of Australian Archaeidae, although further field work is required in the Washpool, Nymboida, Richmond Range, Yabbra, Guy Fawkes River and Nymboi-Binderay National Parks to determine if related species occur in nearby mountainous regions. *Austrarchaea monteithi* itself is currently known from only five specimens collected in 1980 and 2010, and while seemingly quite common at the Gibraltar Range National Park in 2010 (M. Rix, pers. obs.), the overall distribution, abundance and ecology of this species remain very poorly understood (see Rix and Harvey, 2011).

4.2. Biogeography of the Australian mesic zone

The phylogeographic results of the current study provide compelling evidence for a relictual, highly endemic Australian assassin spider fauna, with a biogeographic history intimately linked to the Tertiary contraction and fragmentation of Australia's mesic biomes (Crisp et al., 2004; Byrne et al., 2008). All known Australian species,

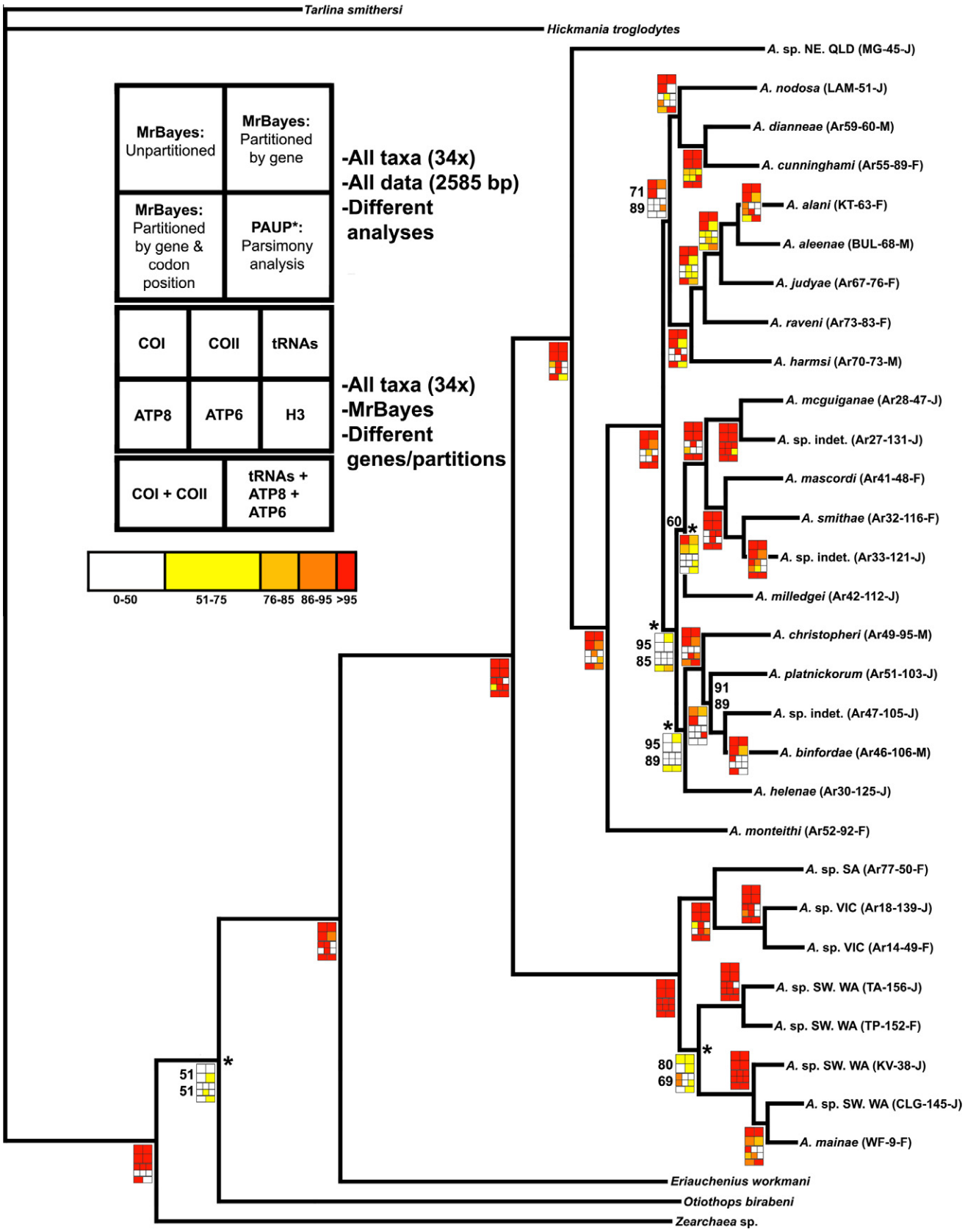

Fig. 5. Summary cladogram comparing nodal support, following differential analysis of the 'partial' dataset (34 taxa) under alternative partitioning strategies and optimality criteria. Posterior probability and parsimony bootstrap support values (0–100) for each clade and each analysis are denoted by coloured boxes (see inset), with node numbers denoting posterior probabilities ⩽95% inferred from Bayesian analyses of the 'complete' (above nodes) and 'core' (below nodes) datasets (see Figs. 2–4). Note the presence of five ambiguous nodes, highlighted (*) and collapsed in the final consensus topology (see Fig. 6).

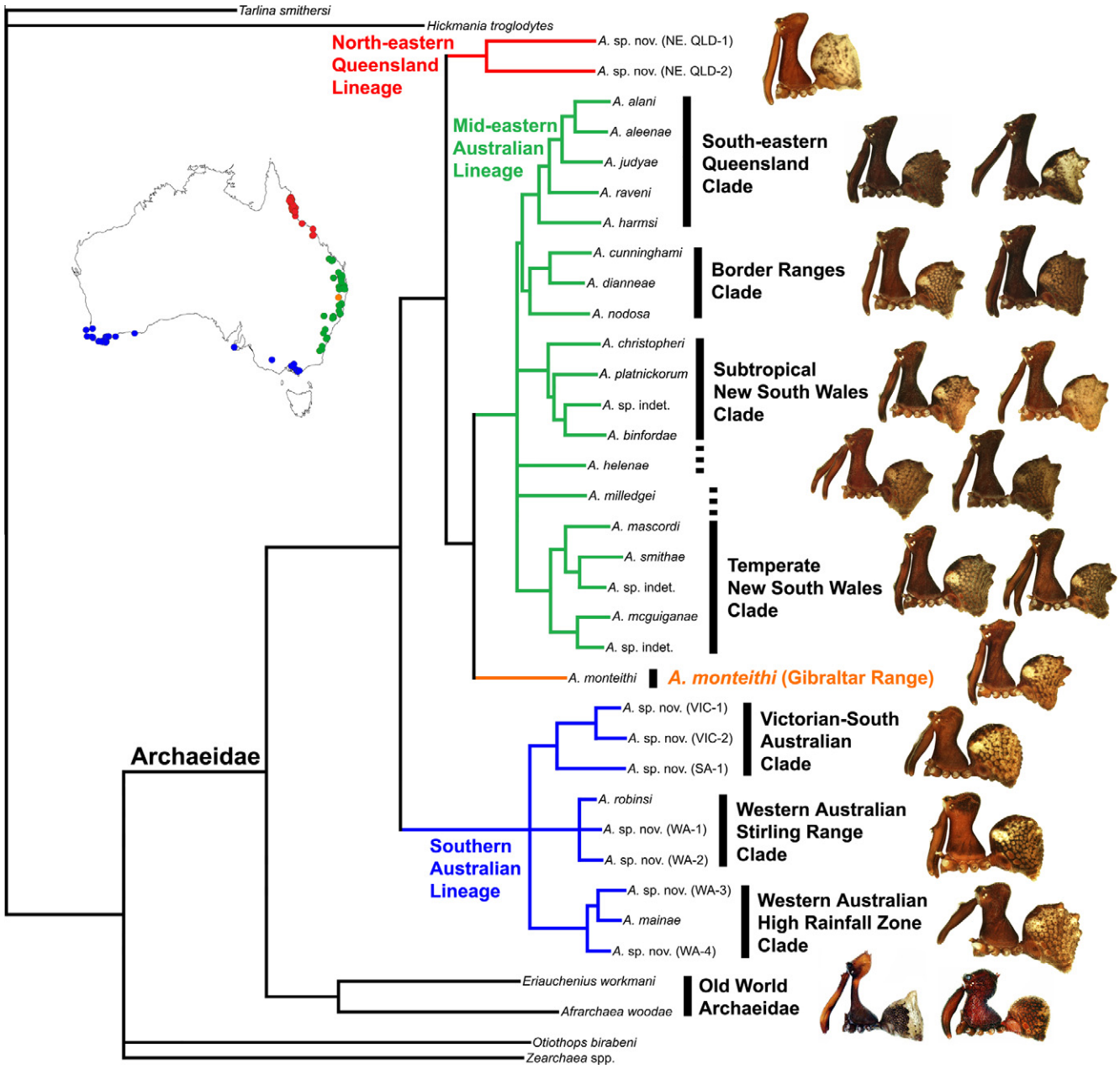

Fig. 6. Summary cladogram showing inferred phylogenetic relationships among species of Archaeidae from southern and south-eastern Australia. Major Australian lineages and regional clades are coloured and labelled according to their distribution (see inset; Figs. 2–4), with lateral profile images of representative taxa also illustrated. Images of Old World Archaeidae (*Eriauchenius workmani*, *Afrarchaea woodae*) courtesy of H. Wood, used with permission.

both described and undescribed, are short-range endemic taxa (see Harvey, 2002b; Harvey et al., 2011), with serially allopatric distributions throughout the east and extreme south-west of mainland Australia (Figs. 8–10) (Rix and Harvey, 2011). Divergence date estimation using mitochondrial data suggests that Australian species in all major lineages diverged during the Miocene (23–5.3 mya) (Figs. 7–10), the last in Western Australia during the late Miocene (~10–4 mya) (Fig. 10). Deeper phylogenetic divergences, concordant with biogeographic barriers in central-eastern Queensland and south-eastern Australia, suggest that the major lineages of Australian Archaeidae diverged in the early Tertiary (~69–34 mya), prior to the final rifting of Australia from East Antarctica (Figs. 7–9). The notable absence of Archaeidae from Tasmania and the Australian Alps (Rix and Harvey, 2011), combined with the extinction of all assassin spiders across the Northern Hemisphere (see Penney, 2003), indicates that the family may be highly susceptible to glaciation and cold climate fluctuations in temperate latitudes.

To more closely explore inferred biogeographic patterns for Archaeidae in the Australian mesic zone, clade divergence dates and area cladograms are reconciled (see Figs. 8–10) and patterns discussed in relation to other sympatric taxa and to known geological events. Given their antiquity, diversity, extreme endemism and widespread distribution throughout the Australian mesic zone, assassin spiders are eminently suitable for testing historical biogeographic hypotheses. Furthermore, with all Australian species exhibiting extremely small, almost exclusively non-overlapping distributions (Rix and Harvey, 2011), assassin spiders have the potential to reveal fine-scale vicariant biogeographic patterns across widely varying temporal scales, including patterns potentially

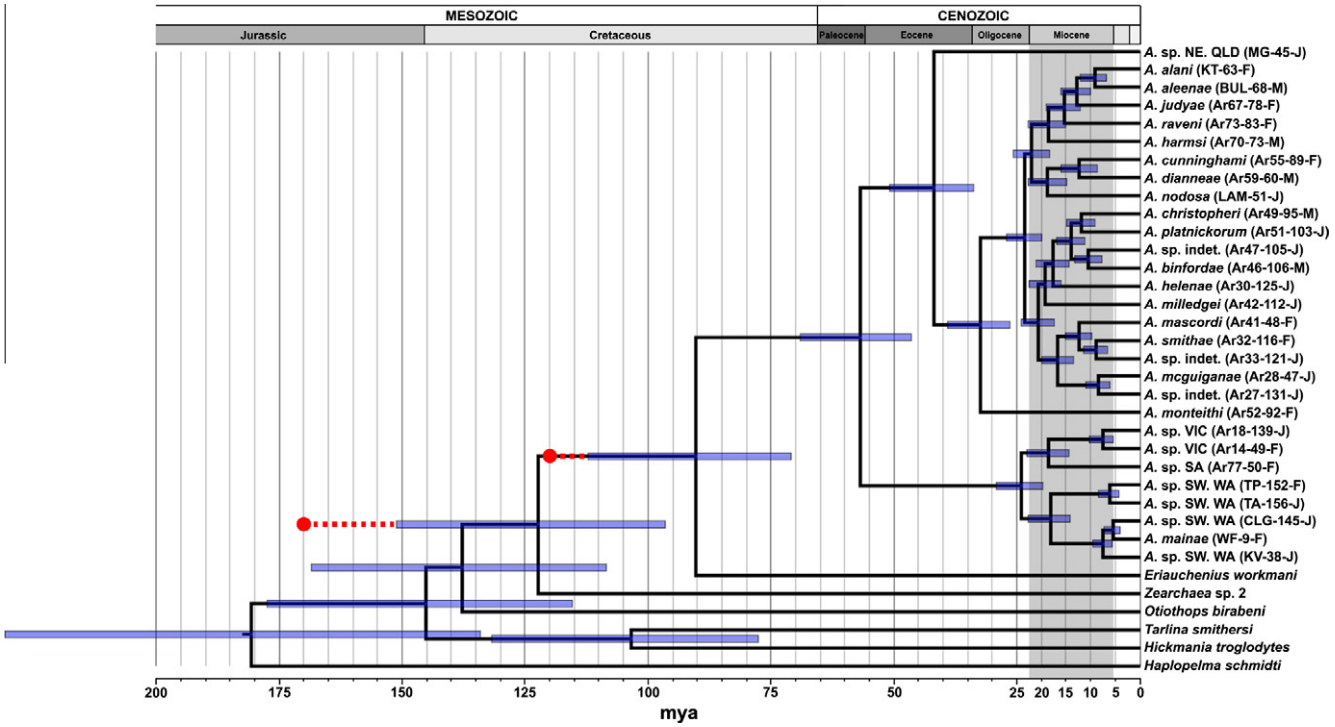

Fig. 7. Chronogram and topology for Australian Archaeidae, resulting from an unpartitioned BEAST analysis of the modified (re-rooted) 'partial' dataset (35 taxa; 2287 bp; Yule process tree prior; 40 million generations). Mitochondrial sequence data were calibrated using a relaxed uncorrelated lognormal molecular clock, applying a prior (arthropod) substitution rate of 0.0115 (after Brower, 1994). Horizontal bars represent 95% credibility estimates for node ages; dots denote potential minimum-age fossil calibration points for selected nodes (see text for details). Note the Miocene epoch (shaded grey), during which most Australian species diverged, and the significant credibility error associated with inferring deep (Mesozoic) nodes from mitochondrial data.

obscured or undetectable in other taxa. Crisp et al. (2004) recognised the need for rigorous (and numerous) molecular phylogenetic studies and associated chronograms to better understand patterns of speciation and divergence in a complex Australian context, and the following discussion draws heavily on previous phylogenetic research, using a comparative phylogeographic approach.

4.2.1. Early Tertiary divergences

The inferred phylogeny for the Australian Archaeidae reveals three major monophyletic lineages (Figs. 2 and 6), each of which diverged well before the subsequent and largely synchronous diversification of species-clades (Fig. 7). These deep phylogenetic splits between major lineages – one broadly concordant with the Australian alpine zone in south-eastern Australia and another with a dry corridor in central eastern Queensland (the St. Lawrence Gap) (Figs. 8 and 9) – appear to have had their origins in the early Tertiary period (Paleocene and Eocene, respectively), well before the final rifting and separation of south-eastern Australia from East Antarctica ~40–35 mya (Li and Powell, 2001; McLoughlin, 2001; Crisp et al., 2004). A third major divergence, possibly of Oligocene age, between the mid-eastern Australian lineage and a lineage represented only by *A. monteithi*, is difficult to interpret biogeographically given the absence or possible extinction of other species in the relictual *A. monteithi* clade.

The St. Lawrence Gap, between Gladstone and Mackay in central eastern Queensland (Fig. 9), has long been recognised as a distinctive overlap zone between rainforest floristic regions (Webb and Tracey, 1981) and a key biogeographic barrier separating tropical and subtropical rainforest taxa (see Moussalli et al., 2005; Baker et al., 2008). Webb and Tracey (1981) hypothesised that the tropical and subtropical/warm-temperate floristic regions of eastern Queensland approximate "core areas" near where ancient widespread floras from Gondwana evolved under different climatic, edaphic or topographic conditions. They further recognised the St. Lawrence Gap (or the "St. Lawrence–Gladstone dry corridor") as an important climatic barrier due to the regional absence of coastal and subcoastal highlands, and postulated that the ancient topographic stability of this central Queensland coast would have led to the independent evolution of rainforests north and south of the dry corridor. Numerous phylogeographic studies of eastern rainforest taxa have confirmed the significance of the St. Lawrence Gap as an historical barrier to gene flow, although results are equivocal as to its relative importance compared to the Burdekin Gap, a second dry corridor situated further north between Bowen and Townsville (see Hoskin et al., 2003; Joseph et al., 1993; Schäuble and Moritz, 2001; Hugall et al., 2003; O'Connor and Moritz, 2003; Moussalli et al., 2005). Certainly, the Eocene divergence date estimate of 51–34 mya inferred for Archaeidae in the current study (Fig. 7), combined with the subsequent divergence of tropical north-eastern species from the Eungella and Daintree National Parks (Figs. 2 and 3), suggests that the rainforests of central eastern Queensland may have been effectively isolated on either side of the St. Lawrence Gap before extensive aridification in the Miocene and earlier than the formation of the Burdekin Gap.

A second major divergence between southern and eastern Australian lineages, roughly concordant with the mountainous Australian alpine zone in southern New South Wales and eastern Victoria (Fig. 8), has few analogues among Australian mesic zone taxa. The great inferred age of this divergence (~69–46 mya) suggests that there was a significant – and presumably vicariant – biogeographic barrier in south-eastern Australia in the Paleocene or early Eocene, which fundamentally divided the south-eastern closed rainforests. Chapple et al. (2005), in an analysis of skinks of the genus *Egernia*, recovered a geographically similar phylogenetic break in eastern Victoria, but this was inferred as being of a more recent age, possibly of late Miocene or Pliocene origin. As

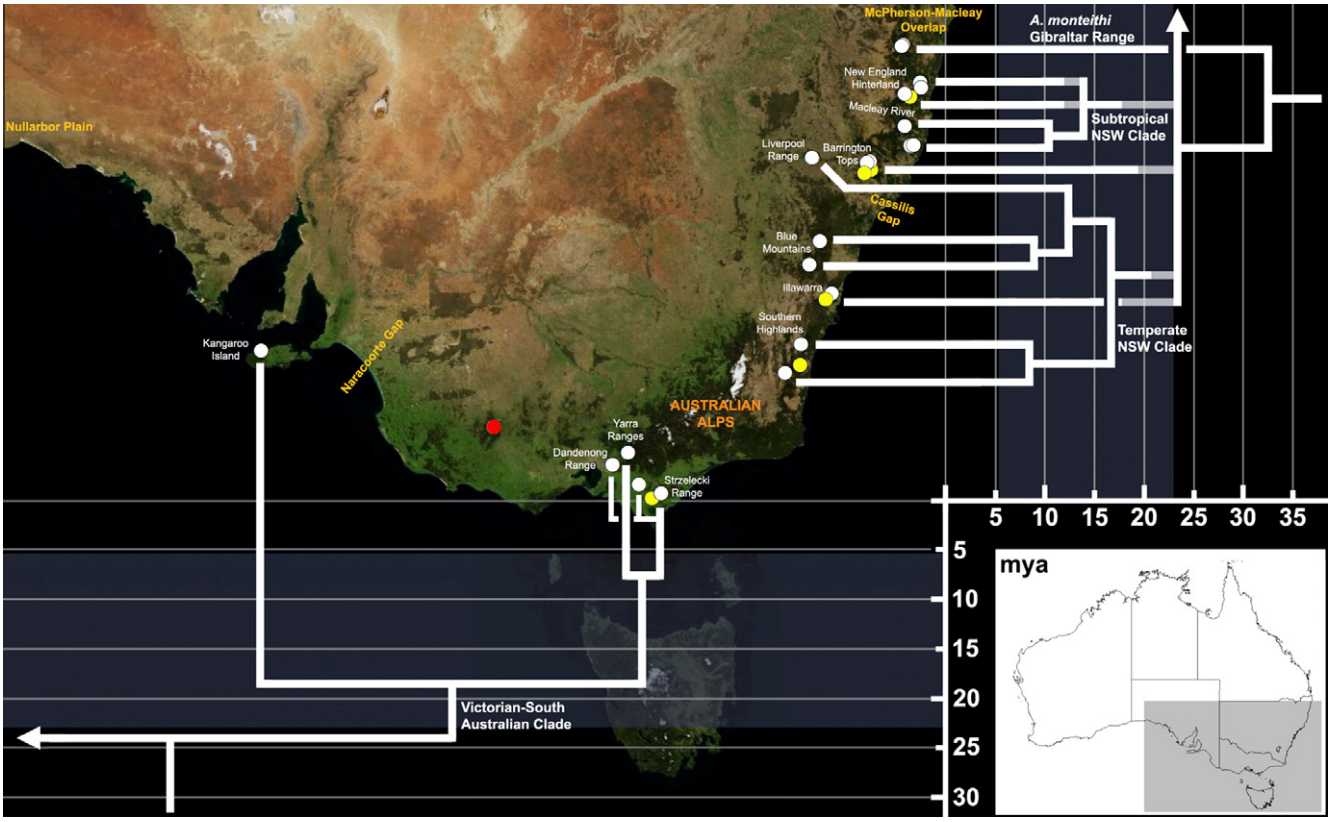

Fig. 8. Area chronogram for Archaeidae from temperate south-eastern Australia, showing mean divergence estimates inferred from an unpartitioned BEAST analysis of the modified (re-rooted) 'partial' dataset (35 taxa; 2287 bp; 40 million generations) (see Fig. 7). Locality dots denote known collection records of Archaeidae; yellow dots denote populations from which genotyped specimens were unavailable for sequencing; red dots denote populations from which recognised species were unavailable for sequencing. The Miocene epoch (23–5.3 mya) is shaded grey.

highlighted by Chapple et al. (2005), eastern Victoria has not traditionally been recognised as a significant biogeographic barrier and few obvious explanations are apparent. Webb and Tracey (1981) recognised a floristic provincial separation east of the Errinundra Plateau, to differentiate the uniformly wet cool-temperate rainforests of Victoria and Tasmania, but most faunistic studies fail to recover a concordant pattern (e.g. see Cracraft, 1991; Moussalli et al., 2005; Lucky, 2011). In the case of Archaeidae, the inferred Paleocene vicariance may only be explicable according to the biology and apparent sensitivity of these spiders to glacial or otherwise alpine conditions. Certainly, within Australia, assassin spiders have never been collected in alpine or sub-alpine habitats, nor have any populations ever been found in Tasmania, which has a cold-temperate climate and was subject to widespread glaciation during the Pleistocene (Ollier, 1986). Volcanic lava flows and fault-block uplift of the southern New South Wales and Victorian highlands commenced in the late Cretaceous and continued throughout the Tertiary (Wellman and McDougall, 1974; Wellman, 1982; Green and Osborne, 1994), creating elevated highlands by early Tertiary times (Ollier, 1986). However, whether these south-eastern highlands were cold enough in the Paleocene to act as a climatic barrier to assassin spiders is unclear, given the apparently warm–wet conditions of the period (Ollier, 1986; Adam, 1992). A second hypothesis could be that volcanism itself was responsible for broadly separating closed rainforests in Victoria and southern New South Wales, given the extensive lava fields that were active in the region during mountain-building phases (see Wellman and McDougall, 1974). The Australian Alps remain a significant physical and climatic barrier to closed wet forests in south-eastern Australia (see Webb and Tracey, 1981; Adam, 1992) and further studies are required to test whether other mesic zone taxa may have been similarly affected by their early Tertiary formation.

4.2.2. Early Miocene divergences

The onset of the Miocene epoch ~23 mya saw the beginning of a profoundly transformative phase in the climatic and evolutionary history of the Australian continent, and one that would have a particularly important influence on mesic biomes. During the Oligocene (34–23 mya), Australia completely separated from East Antarctica and started rapidly moving north, forming the Southern Ocean and initiating the development of the Antarctic Circumpolar Current (ACC) (Crisp et al., 2004; Byrne et al., 2008). The intensification of the ACC steepened latitudinal temperature gradients, resulting in global cooling and the widespread glaciation of Antarctica (Crisp et al., 2004; Byrne et al., 2008). Australia continued to drift further north into warmer, subtropical latitudes throughout the Oligocene and, by the onset of the Miocene, the Australian climate had started to become drier and more seasonal, although closed forests were still widespread across southern Australia (Adam, 1992; Crisp et al., 2004; Byrne et al., 2008). Initial fragmentation of the Australian mesic zone in the early Miocene was followed by ongoing contraction and aridification in the mid-late Miocene, as *Nothofagus* and other rainforest communities were rapidly replaced by sclerophyllous taxa, especially in inland regions (Adam, 1992; Crisp et al., 2004; Byrne et al., 2008). By the late Miocene, the rainforests of the eastern Great Dividing Range were probably already segregated into regional forest blocks separated by more xeric, lowland communities (Adam, 1992).

Although a number of studies have focussed on the influence of relatively recent, Plio-Pleistocene divergences in shaping the

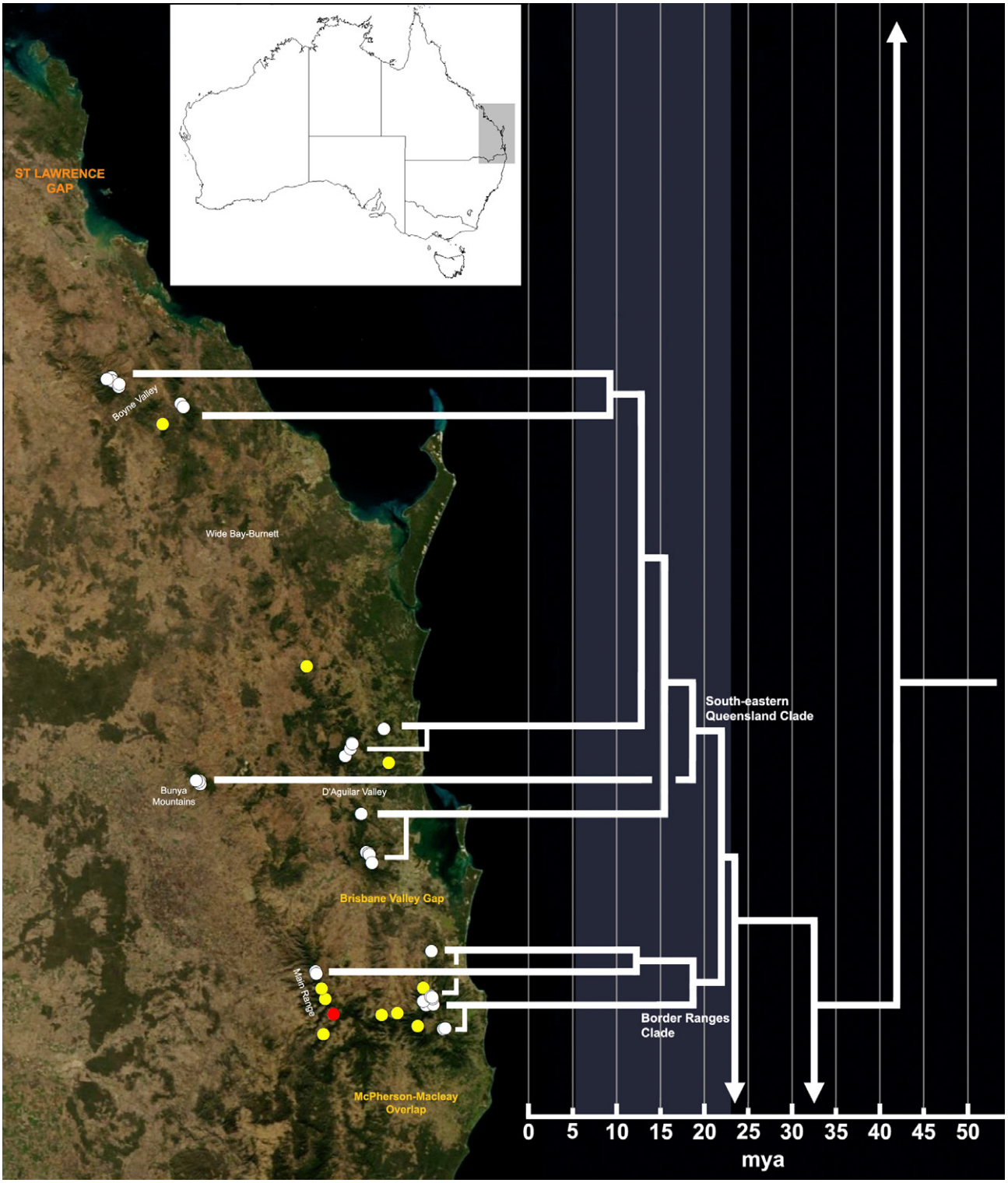

Fig. 9. Area chronogram for Archaeidae from subtropical south-eastern Queensland, showing mean divergence estimates inferred from a calibrated, unpartitioned BEAST analysis of the modified (re-rooted) 'partial' dataset (35 taxa; 2287 bp; 40 million generations) (see Fig. 7). Locality dots denote known collection records of Archaeidae; yellow dots denote populations from which genotyped specimens were unavailable for sequencing; red dots denote populations from which recognised species were unavailable for sequencing. The Miocene epoch (23–5.3 mya) is shaded grey.

Australian rainforest biota (e.g. Schneider and Moritz, 1999; Ponniah and Hughes, 2004, 2006; Chapple et al., 2005; Sota et al., 2005; Baker et al., 2008), the current study adds to a growing list of analyses to highlight the importance of potentially older, Miocene processes in driving the diversification of rainforest taxa (Moritz et al., 2000; Lucky, 2011). Phylogenetic results for archaeid species in the mid-eastern and southern Australian lineages reveal the presence of seven regional clades (Fig. 6), each of which appear to have diverged in the very late Oligocene or early Miocene (Figs. 7–10), prior to the main diversification of species lineages in the

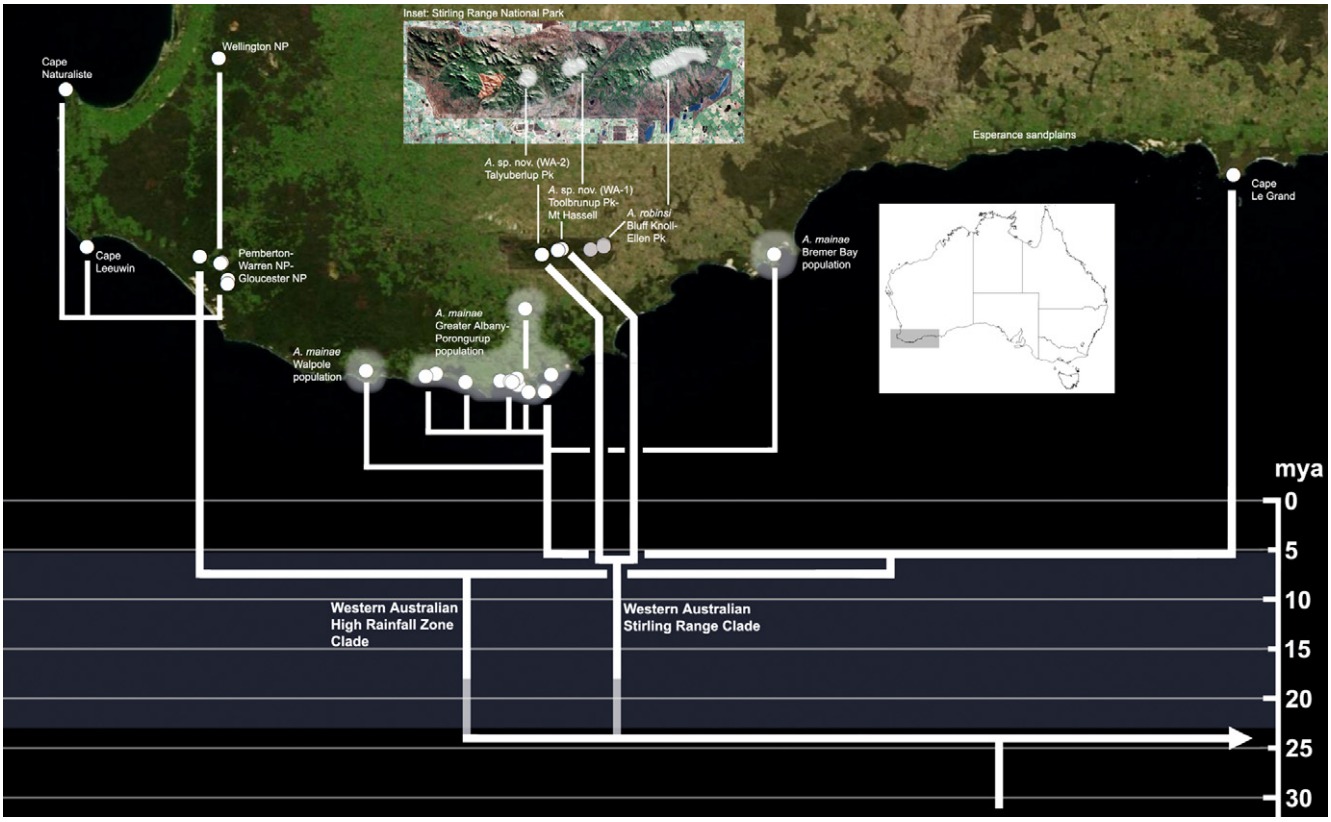

Fig. 10. Area chronogram for Archaeidae from temperate south-western Western Australia, showing mean divergence estimates inferred from a calibrated, unpartitioned BEAST analysis of the modified (re-rooted) 'partial' dataset (35 taxa; 2287 bp; 40 million generations) (see Fig. 7). Locality dots denote known collection records of Archaeidae; grey dots denote populations included only in the 'complete' dataset. Note the three population lineages of *Austrarchaea mainae* (denoted by transparent shading), inferred using both phylogenetic and nested clade analyses, and the distributions of three species in the Stirling Range National Park (see inset). The Miocene epoch (23-5.3 mya) is shaded grey.

mid-late Miocene (Fig. 7). Given the largely non-overlapping distributions of taxa in these regional clades, it is suggested that each divergence may be the phylogenetic signature of an early Miocene disjunction in the distribution of closed rainforests, testable in comparison to other taxa. The oldest of these divergences – separating Western Australian Archaeidae from species in south-eastern Australia – is concordant with the Nullarbor Plain, a vast desert region extending from west of the Eyre Peninsula in South Australia to south-western Western Australia (Fig. 8). The Nullarbor Plain has long been recognised as one of the most important xeric barriers in temperate Australia (see Burbidge, 1960; Cracraft, 1986; Hopper and Gioia, 2004) and the inferred divergence age estimate of ~24 mya (95% HPD 29–20 mya) corresponds closely to the formation of the Plain as a result of marine inundations in the early Miocene (Cracraft, 1986; Crisp et al., 2004; Hopper and Gioia, 2004) and to calibrated molecular divergences inferred for other taxa (e.g. Barendse, 1984; Jennings et al., 2003; Crisp et al., 2004; Morgan et al., 2007; Schultz et al., 2009).

Deep phylogenetic divergences among eastern Australian Archaeidae provide evidence for at least four major early Miocene barriers in south-eastern Australia (Figs. 8 and 9). The oldest of these divergences separate subtropical clades in south-eastern Queensland and north-eastern New South Wales, and are concordant with the 'Brisbane Valley Gap' and the McPherson–Macleay Overlap (Figs. 8 and 9), both of early Miocene age (95% HPD 27–18 mya). The McPherson–Macleay Overlap (see Colgan et al., 2009) has long been recognised as both an ecological overlap zone and a biogeographic barrier for rainforest taxa (Lucky, 2011), and in the current study it is also recognised as such, given the endemic distribution of the Border Ranges clade along the Main and McPherson Ranges (Fig. 9). North of the Border Ranges, the Brisbane Valley lowlands separate a second diverse clade of south-eastern Queensland Archaeidae, and south of the Border Ranges, the McPherson–Macleay Overlap zone separates Queensland species from two endemic clades of New South Wales taxa (Figs. 8 and 9). The Border Ranges were uplifted in the very early Miocene due to the eruption of hot-spot shield volcanoes between 25 and 22 mya, and were subsequently eroded to form the mountainous topography seen today (Willmott, 2004). Numerous comparative phylogenetic studies have explored the biogeographic significance of this region, and the results here add to a growing number of analyses highlighting biogeographic disjunctions in extreme south-eastern Queensland and north-eastern New South Wales (Burbidge, 1960; Ward, 1980; Crisp et al., 1995; Crisp et al., 2001; Schäuble and Moritz, 2001; Knowles et al., 2004; Moussalli et al., 2005; Sota et al., 2005; Ponniah and Hughes, 2006; Couper et al., 2008; Colgan et al., 2009; Ladiges et al., 2011; Lucky, 2011). Further south, the Cassilis/Hunter Valley Gap (Fig. 8) (see Cotton, 1949; Forster et al., 1987) was found to divide two major clades of Archaeidae from eastern New South Wales, in a line broadly concordant with the lowland Hunter River Valley. Like the McPherson–Macleay Overlap, the Hunter Valley region has long been recognised as an important biogeographic barrier in south-eastern Australia, for multiple plant and animal taxa (Cracraft, 1991; Adam, 1992; Moussalli et al., 2005; Hugall and Stanisic, 2011; Ladiges et al., 2011). The results of the current study clarify the inferred early Miocene age of this vicariance (95% HPD 23–16 mya), and also draw attention to the possible western boundary of this Gap east of the Liverpool Range (see below). A fourth biogeographic barrier, roughly

concordant with sclerophyllous lowlands in the Naracoorte region of far south-eastern South Australia (the 'Naracoorte Gap' or Mallee Divide) (Fig. 8), separates Victorian and South Australian archaeid species in the southern Australian lineage (95% HPD 23–14 mya). Relictual mesic habitats in South Australia are now largely confined to the Mount Lofty Ranges, Fleurieu Peninsula and Kangaroo Island, and a biogeographic barrier located between these habitats and the western extension of the Great Dividing Range has also been inferred for other temperate taxa (Ford, 1987; Cracraft, 1991; Jennings et al., 2003).

4.2.3. Miocene diversification of the Australian Archaeidae

After the separation of major archaeid clades in the early Tertiary, species lineages in Australia appear to have diverged throughout the Miocene (Figs. 7–10). Divergence date estimates suggest that taxa in more tropical, northerly regions diverged first, in the early-mid Miocene (Figs. 8 and 9), with temperate species diverging slightly later, in the mid-late Miocene (Figs. 8 and 10). Inferred area chronograms are concordant with a model of serial allopatric speciation due to the fragmentation of refugial habitats, and the results of the current study provide considerable insights into the mode, tempo and fine-scale pattern of archaeid speciation across mesic Australia.

In south-eastern Queensland, assassin spider populations north of the Brisbane Valley and south of the St. Lawrence Gap appear to have been isolated according to the geography and proximity of montane rainforest blocks, with the initial separation of isolated inland populations (at the Bunya Mountains) followed by the successive separation of coastal highland populations across the D'Aguilar Valley, the Wide Bay-Burnett lowlands and the Boyne Valley gaps (Fig. 9). The Bunya Mountains are the remains of a broad shield volcano that erupted in the very early Miocene (~24–23 mya; Willmott, 2004) and the inferred divergence age estimate of ~19 mya (95% HPD 23–15 mya) for the separation of *Austrarchaea harmsi* Rix and Harvey, 2011 from other south-eastern Queensland taxa is consistent with the geology of the region and with the likely eastward retreat of rainforests during the Miocene (Martin, 1987; Adam, 1992). Further south, the inferred age of the Border Ranges clade (~19 mya; 95% HPD 23–15 mya) is congruent with the inferred divergence age estimate for *A. harmsi*, presumably also reflecting the volcanic formation of the Main and McPherson Ranges ~25–22 mya (Willmott, 2004). Interestingly, the inferred divergence age estimate of ~12 mya (95% HPD 16–9 mya), for the separation of *A. cunninghami* Rix and Harvey, 2011 from *A. dianneae* Rix and Harvey, 2011 (Figs. 7 and 9), is concordant with the formation of the eastern Main Range escarpment and Fassifern Valley ~15–10 mya, due to the progressive erosion of the Main Range crest throughout the early Miocene (Willmott, 2004).

In New South Wales, the relatively small number of populations known for each archaeid species (see Fig. 8) renders the interpretation of fine-scale biogeographic barriers more difficult, although several key patterns are apparent. North of the Cassilis Gap, in the subtropical rainforests of the New England hinterland and Port Macquarie lowlands, species divergences are broadly concordant with a successive north–south separation of rainforests south of the Dorrigo Plateau, with an inferred divergence age estimate of ~14 mya (95% HPD 17–11 mya) for the separation of clades (and rainforests) either side of the Macleay River Valley (Figs. 7 and 8). Two species from north and south of the Cassilis Gap – *A. milledgei* from the Barrington Tops and *A. helenae* from the Illawarra Escarpment, respectively – could not be assigned with confidence to either of the two regional New South Wales clades (Fig. 6), although results from Bayesian analysis of the 'complete' dataset suggest that they may be sister-species to the temperate and subtropical clades respectively, each having been separated either side of the Cassilis Gap (Figs. 3 and 8). The unexpected yet strongly supported inclusion of *A. mascordi* Rix and Harvey, 2011 in the temperate New South Wales clade (Fig. 6) suggests that the isolated mesic forests of the Liverpool Range (see Adam, 1992) have greater biogeographic affinities to the *south* of the Cassilis Gap (Fig. 8), implying the primacy of the northern arm of the Hunter River (north of Scone) in defining the Gap's imprecise western boundary. South of the Cassilis Gap, broad divergences between populations of Archaeidae in the Liverpool Range, Blue Mountains and Southern Highlands are of mid-late Miocene age (95% HPD 20–10 mya), with evidence for later Miocene speciation events in the Greater Blue Mountains and Southern Highlands (95% HPD 11–6 mya) (Fig. 8).

For regional clades in the southern Australian lineage, species divergences are of mostly late Miocene age (Figs. 7, 8 and 10), suggesting that the fragmentation of temperate habitats in south-western Western Australia and Victoria may have occurred significantly later than in lower latitudes in mid-eastern Australia. In eastern Victoria, archaeids are known only from the highland mesic forests of the Dandenong, Yarra and Strzelecki Ranges, with populations in the Central Highlands (i.e. Yarra Ranges National Park) inferred as having diverged from the Dandenong and Strzelecki Range populations ~8 mya (95% HPD 10–5 mya) (Fig. 8). In Western Australia, six species of Archaeidae are found in the extreme south of the State, between Cape Naturaliste and Cape Le Grand (Fig. 10), with divergence date estimates suggesting that all Western Australian species diverged synchronously in the late Miocene ~9–5 mya (95% HPD 10–4 mya). Of these six species, two (*A. mainae* and *A.* sp. nov. WA-4; Fig. 1B) have relatively broad, non-overlapping distributions in the wet sclerophyll forests and coastal heathlands between Wellington National Park and Bremer Bay (Fig. 10), closely tracking the boundaries of the southern high rainfall province (see Hopper and Gioia, 2004; Cooper et al., 2011). One additional species in the Western Australian high rainfall zone clade (*A.* sp. nov. WA-3) is endemic to the isolated mesic heathlands of Cape Le Grand National Park, east of the Esperance sandplains (Fig. 10). Further inland, three species lineages are inferred for allopatric, montane populations in the Stirling Range National Park, each restricted to high altitude mesic heathlands separated by open, xeric lowlands (Fig. 10). *Austrarchaea robinsi* is restricted to the Stirling Range's 'Eastern Massif', between Bluff Knoll and Ellen Peak (see Rix et al., 2009); *A.* sp. nov. (WA-1) occurs on the Toolbrunup Peak/Mount Hassell uplands west of Chester Pass; and *A.* sp. nov. (WA-2) is known only from the summit of Talyuberlup Peak (Fig. 10). Few phylogeographic studies have been undertaken on montane taxa in the Stirling Range National Park, but a recent analysis of trapdoor spiders in the genus *Moggridgea* showed a pattern of allopatric divergence strikingly congruent with that inferred for species of *Austrarchaea* (see Cooper et al., 2011). Both groups of spiders are restricted to highly localised, mesic microhabitats on the Talyuberlup, Toolbrunup and Eastern Massif uplands, and all three populations have apparently been isolated for millions of years, the oldest divergences separating Talyuberlup Peak from the uplands further east. In species of *Austrarchaea* – as in *Moggridgea* – these deep Stirling Range vicariances have resulted in the reciprocal monophyly of both mitochondrial and nuclear markers (see Cooper et al., 2011), with species of *Austrarchaea* further distinguishable based on morphology (M. Rix, unpublished data). That assassin spiders occur in the Stirling Range National Park at all is testament to their great antiquity and remarkable ability to persist in mesic refugia, and further studies are required to determine distributional boundaries and the possible conservation implications of their surviving on the Range's highest summits.

5. Conclusion

The Australian Archaeidae clearly offer great potential for testing historical biogeographic processes in temperate and eastern Australia, as evidenced by their distribution and diversity and their deep phylogenetic structuring at the population and species levels. Old relictual and short-range endemic taxa are especially amenable to biogeographic study (Harvey, 2002b; Harvey et al., 2011) and the Archaeidae are a useful group for better understanding the biology and biogeography of the Australian mesic zone. Indeed, given their endemism and serial allopatry, their dependence on refugial microhabitats and persistence throughout the Cenozoic, species of *Austrarchaea* have the potential to reveal evolutionary patterns at widely varying temporal and spatial scales, in both tropical and temperate systems. Taxonomic revisions of the Australian species are currently underway (see Rix and Harvey, 2011) and these, once completed, will provide a valuable framework for future systematic research on these remarkable spiders.

Acknowledgments

This research was made possible by an Australian Biological Resources Study (ABRS) taxonomy research grant to M. Harvey and M. Rix (Grant No. 209-08), with critical initial support provided by Roy Teale (Biota Environmental Sciences) and significant additional funding provided by Cliffs Natural Resources and Verve Energy. Numerous friends, family and colleagues assisted in the collection of assassin spiders in the field, including Greta Binford, Sarah Comer, Alan Danks, Karen Edward, Danilo Harms, Stephanie Harms, Lara Lopardo, Graham Milledge, Melinda Moir, Janet Newell, Ricardo Ott, Alan Rix, Dale Roberts, Beverley Roberts, Helen Smith, Roy Teale, Julianne Waldock, Janine Wojcieszek and Hannah Wood. Special thanks are due to Danilo Harms (University of Western Australia) for his invaluable assistance to M.G.R. during extensive field work throughout south-eastern Australia in March–May 2010, to Alan Rix for his efforts in accessing the Bulburin and Kroombit Tops National Parks in October 2010, and to Janine Wojcieszek for the many hours spent patiently waiting for M.G.R. to collect assassin spiders in Western Australia. Thanks also to Christine Lambkin and Robert Raven (Queensland Museum) for their significant eleventh-hour assistance with permits in Queensland, and to Michael Sharp (New South Wales Parks and Wildlife Service) for helping M.G.R. access the eastern Coolah Tops National Park in April 2010. Specimens were collected under permit throughout Queensland (Permit No. WITK5498008), New South Wales (Permit Nos. S13035, XX48918), Victoria (Permit No. 10005193), South Australia (Permit No. W25803) and Western Australia (Permit Nos. SF005357, SF005814, SF006247, SF006821). The laboratory and molecular components of this research were made possible by facilities in the School of Animal Biology at the University of Western Australia, and special thanks are due to Janine Wojcieszek for her invaluable help in processing molecular samples. Finally, thanks to Hannah Wood and Charles Griswold (California Academy of Sciences) for their ongoing support, discussion and collaboration, and special thanks to Hannah for providing data on Australian specimens housed at the CAS, and for sending DNA extractions of Malagasy and African archaeid taxa for use in molecular analyses. Cor Vink and an anonymous reviewer provided helpful comments on an earlier draft of the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.10.009.

References

Adam, P., 1992. Australian Rainforests. Oxford University Press, New York.
Arnedo, M., Ferrández, M.-A., 2007. Mitochondrial markers reveal deep population subdivision in the European protected spider *Macrothele calpeiana* (Walckenaer, 1805) (Araneae, Hexathelidae). Conservation Genetics 8, 1147–1162.
Baker, C.H., Graham, G.C., Scott, K.D., Cameron, S.L., Yeates, D.K., Merritt, D.J., 2008. Distribution and phylogenetic relationships of Australian glow-worms *Arachnocampa* (Diptera, Keroplatidae). Molecular Phylogenetics and Evolution 48, 506–514.
Barendse, W., 1984. Speciation in the genus *Crinia* (Anura: Myobatrachidae) in southern Australia: a phylogenetic analysis of allozyme data supporting endemic speciation in southwestern Australia. Evolution 38, 1238–1250.
Bell, R.C., Parra, J.L., Tonione, M., Hoskin, C.J., Mackenzie, J.B., Williams, S.E., Moritz, C., 2010. Patterns of persistence and isolation indicate resilience to climate change in montane rainforest lizards. Molecular Ecology 19, 2531–2544.
Bond, J.E., Hedin, M.C., Ramírez, M.G., Opell, B.D., 2001. Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*. Molecular Ecology 10, 899–910.
Boyer, S.L., Clouse, R.M., Benavides, L.R., Sharma, P., Schwendinger, P.J., Karunarathna, I., Giribet, G., 2007. Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. Journal of Biogeography 34, 2070–2085.
Boyer, S.L., Giribet, G., 2007. A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. Cladistics 23, 337–361.
Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences of the United States of America 91, 6491–6495.
Burbidge, N.T., 1960. The phytogeography of the Australian region. Australian Journal of Botany 8, 75–211.
Butler, L.S.G., 1929. Studies in Australian spiders, No. 1. Proceedings of the Royal Society of Victoria 42, 41–52.
Byrne, M., Yeates, D.K., Joseph, L., Kearney, M., Bowler, J., Williams, M.A.J., Cooper, S., Donnellan, S.C., Keogh, J.S., Leys, R., Melville, J., Murphy, D.J., Porch, N., Wyrwoll, K.-H., 2008. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. Molecular Ecology 17, 4398–4417.
Chang, J., Song, D., Zhou, K., 2007. Incongruous nuclear and mitochondrial phylogeographic patterns in two sympatric lineages of the wolf spider *Pardosa astrigera* (Araneae: Lycosidae) from China. Molecular Phylogenetics and Evolution 42, 104–121.
Chapple, D., Keogh, J.S., Hutchinson, M., 2005. Substantial genetic substructuring in southeastern and alpine Australia revealed by molecular phylogeography of the *Egernia whitii* (Lacertilia: Scincidae) species group. Molecular Ecology 14, 1279–1292.
Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9, 1657–1659.
Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46, 419–437.
Colgan, D.J., O'Meally, D., Sadlier, R.A., 2009. Phylogeographic patterns in reptiles on the New England Tablelands at the south-western boundary of the McPherson Macleay Overlap. Australian Journal of Zoology 57, 317–328.
Cooper, S.J.B., Harvey, M.S., Saint, K.M., Main, B.Y., 2011. Deep phylogeographic structuring of populations of the trapdoor spider *Moggridgea tingle* (Migidae) from southwestern Australia: evidence for long-term refugia within refugia. Molecular Ecology 20, 3219–3236.
Cotton, C.A., 1949. A review of tectonic relief in Australia. The Journal of Geology 57, 280–296.
Couper, P.J., Sadlier, R.A., Shea, G.M., Wilmer, J.W., 2008. A reassessment of *Saltuarius swaini* (Lacertilia: Diplodactylidae) in southeastern Queensland and New South Wales; two new taxa, phylogeny, biogeography and conservation. Records of the Australian Museum 60, 87–118.
Cracraft, J., 1986. Origin and evolution of continental biotas: speciation and historical congruence within the Australian avifauna. Evolution 40, 977–996.
Cracraft, J., 1991. Patterns of diversification within continental biotas: hierarchical congruence among the areas of endemism of Australian vertebrates. Australian Systematic Botany 4, 211–227.
Crews, S.C., Puente-Rolón, A.R., Rutstein, E., Gillespie, R.G., 2010. A comparison of populations of island and adjacent mainland species of Caribbean *Selenops* (Araneae: Selenopidae) spiders. Molecular Phylogenetics and Evolution 54, 970–983.
Crisp, M., Cook, L., Steane, D., 2004. Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 359, 1551–1571.
Crisp, M.D., Laffan, S., Linder, H.P., Monro, A., 2001. Endemism in the Australian flora. Journal of Biogeography 28, 183–198.
Crisp, M.D., Linder, H.P., Weston, P.H., 1995. Cladistic biogeography of plants in Australia and New Guinea: congruent pattern reveals two endemic tropical tracks. Systematic Biology 44, 457–473.

DeSalle, R., Freedman, T., Prager, E.M., Wilson, A.C., 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. Journal of Molecular Evolution 26, 157–164.

Dunlop, J.A., Penney, D., Jekel, D., 2011. A summary list of fossil spiders and their relatives. In: Platnick, N.I. (Ed.), The World Spider Catalog, Version 11.5. American Museum of Natural History, New York, <http://research.amnh.org/entomology/spiders/catalog/index.html>.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology 4, 699–710.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7, 214.

Eskov, K.Y., Golovatch, S.I., 1986. On the origin of trans-Pacific disjunctions. Zoologische Jahrbuecher für Systematik 113, 265–285.

Felsenstein, J., 1985. Confidence limits on phylogenies – an approach using the bootstrap. Evolution 39, 783–791.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3, 294–299.

Ford, J., 1987. Minor isolates and minor geographical barriers in avian speciation in continental Australia. Emu 87, 90–102.

Forster, R.R., 1956. A new spider of the genus *Archaea* from Australia. Memoirs of the Queensland Museum 13, 151–154.

Forster, R.R., Platnick, N.I., 1984. A review of the archaeid spiders and their relatives, with notes on the limits of the superfamily Palpimanoidea (Arachnida, Araneae). Bulletin of the American Museum of Natural History 178, 1–106.

Forster, R.R., Platnick, N.I., Gray, M.R., 1987. A review of the spider superfamilies Hypochiloidea and Austrochiloidea (Araneae, Araneomorphae). Bulletin of the American Museum of Natural History 185, 1–116.

Framenau, V.W., Dupérré, N., Blackledge, T.A., Vink, C.J., 2010. Systematics of the new Australasian orb-weaving spider genus *Backobourkia* (Araneae: Araneidae: Araneinae). Arthropod Systematics & Phylogeny 68, 79–111.

Giribet, G., 2003. Stability in phylogenetic formulations and its relationship to nodal support. Systematic Biology 52, 554–564.

Green, K., Osborne, W., 1994. Wildlife of the Australian Snow-Country. Reed Books, Chatswood, NSW.

Griswold, C.E., 2003. Araneae, spiders. In: Goodman, S., Benstead, J. (Eds.), The Natural History of Madagascar. University of Chicago Press, Chicago, IL, pp. 579–587.

Griswold, C.E., Ramírez, M.J., Coddington, J.A., Platnick, N.I., 2005. Atlas of phylogenetic data for entelegyne spiders (Araneae: Araneomorphae: Entelegynae) with comments on their phylogeny. Proceedings of the California Academy of Sciences 56 (Suppl. II), 1–324.

Harvey, M.S., 2002a. A new species of *Austrarchaea* (Araneae: Archaeidae) from Western Australia. Records of the Western Australian Museum 21, 35–37.

Harvey, M.S., 2002b. Short-range endemism among the Australian fauna: some examples from non-marine environments. Invertebrate Systematics 16, 555–570.

Harvey, M.S., Rix, M.G., Framenau, V.W., Hamilton, Z.R., Johnson, M.S., Teale, R.J., Humphreys, G., Humphreys, W.F., 2011. Protecting the innocent: studying short-range endemic taxa enhances conservation outcomes. Invertebrate Systematics 25, 1–10.

Hedin, M.C., 2001. Molecular insights into species phylogeny, biogeography, and morphological stasis in the ancient spider genus *Hypochilus* (Araneae: Hypochilidae). Molecular Phylogenetics and Evolution 18, 238–251.

Hewitt, J., 1919. Description of new South African spiders and a solifuge of the genus *Chelypus*. Records of the Albany Museum 3, 196–215.

Higgins, E.T., Petterd, W.F., 1883. Description of a new cave-inhabiting spider, together with notes on mammalian remains from a recently discovered cave in the Chudleigh district. Papers and Proceedings of the Royal Society of Tasmania 1882, 191–192.

Hopper, S.D., Gioia, P., 2004. The Southwest Australian Floristic Region: evolution and conservation of a global hot spot of biodiversity. Annual Review of Ecology, Evolution and Systematics 35, 623–650.

Hopper, S.D., Harvey, M.S., Chappill, J.A., Main, A.R., Main, B.Y., 1996. The Western Australian biota as Gondwanan heritage – a review. In: Hopper, S.D., Chappill, J.A., Harvey, M.S., George, A.S. (Eds.), Gondwanan Heritage. Past Present and Future of the Western Australian Biota. Surrey Beatty & Sons, Chipping Norton, pp. 1–46.

Hoskin, C.J., Couper, P.J., Schneider, C.J., 2003. A new species of *Phyllurus* (Lacertilia: Gekkonidae) and a revised phylogeny and key for the Australian leaf-tailed geckos. Australian Journal of Zoology 51, 153–164.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Hugall, A., Stanisic, J., Moritz, C., 2003. The case of the *Sphaerospira* lineage and history of Queensland rainforests. In: Lydeard, C., Lindberg, D. (Eds.), Molecular Systematics and Phylogeography of Mollusks. Smithsonian Institution Press, Washington, DC, pp. 270–301.

Hugall, A.F., Foster, R., Hutchinson, M., Lee, M.S.Y., 2008. Phylogeny of Australasian agamid lizards based on nuclear and mitochondrial genes: implications for morphological evolution and biogeography. Biological Journal of the Linnean Society 93, 343–358.

Hugall, A.F., Stanisic, J., 2011. Beyond the prolegomenon: a molecular phylogeny of the Australian camaenid land snail radiation. Zoological Journal of the Linnean Society 161, 531–572.

Jennings, W.B., Pianka, E.R., Donnellan, S., 2003. Systematics of the lizard family Pygopodidae with implications for the diversification of Australian temperate biotas. Systematic Biology 52, 757–780.

Joseph, L., Moritz, C., 1994. Mitochondrial DNA phylogeography of birds in eastern Australian rainforests: first fragments. Australian Journal of Zoology 42, 385–403.

Joseph, L., Moritz, C., Hugall, A., 1993. A mitochondrial DNA perspective on the historical biogeography of mideastern Queensland rainforest birds. Memoirs of the Queensland Museum 34, 201–214.

Joseph, L., Moritz, C., Hugall, A., 1995. Molecular support for vicariance as a source of diversity in rainforest. Proceedings of the Royal Society of London Series B 260, 177–182.

Juan, C., Oromí, P., Hewitt, G.M., 1995. Mitochondrial DNA phylogeny and sequential colonization of Canary Islands by darkling beetles of the genus *Pimelia*. Proceedings of the Royal Society of London Series B 261, 173–180.

Kimura, M., 1981. Estimation of evolutionary distances between homologous nucleotide sequences. Proceedings of the National Academy of Sciences of the United States of America 78, 454–458.

Koch, C.L., Berendt, G.C., 1854. Die im Bernstein befindlichen Crustaceen, Myriapoden, Arachniden und Apteren der Vorweld. In: Berendt, G.C. (Ed.), Die im Bernstein befindlichen Organischen Reste der Vorweld, Berlin, pp. 1–124.

Knowles, R., Mahony, M., Armstrong, J., Donnellan, S., 2004. Systematics of sphagnum frogs of the genus *Philoria* (Anura: Myobatrachidae) in eastern Australia, with the description of two new species. Records of the Australian Museum 56, 57–74.

Ladiges, P., Parra-O., C., Gibbs, A., Udovicic, F., Nelson, G., Bayly, M., 2011. Historical biogeographical patterns in continental Australia: congruence among areas of endemism of two major clades of eucalypts. Cladistics 27, 29–41.

Legendre, R., 1961. Études sur les *Archaea* (Aranéides). II. La capture des proies et la prise de nourriture. Bulletin de la Société zoologique de France 86, 316–319.

Li, Z.X., Powell, C.M., 2001. An outline of the palaeogeographic evolution of the Australasian region since the beginning of the Neoproterozoic. Earth-Science Reviews 53, 237–277.

Lotz, L.N., 1996. Afrotropical Archaeidae (Araneae): 1. New species of *Afrarchaea* with notes on *Afrarchaea godfreyi* (Hewitt, 1919). Navorsinge van die Nasionale Museum, Bloemfontein 12, 141–160.

Lotz, L.N., 2003. Afrotropical Archaeidae: 2. New species of the genera *Archaea* and *Afrarchaea* (Arachnida: Araneae). Navorsinge van die Nasionale Museum, Bloemfontein 19, 221–240.

Lotz, L.N., 2006. Afrotropical Archaeidae: 3. The female of *Eriauchenius cornutus* and new species of *Afrarchaea* (Arachnida: Araneae) from South Africa. Navorsinge van die Nasionale Museum, Bloemfontein 22, 113–127.

Lucky, A., 2011. Molecular phylogeny and biogeography of the spider ants, genus *Leptomyrmex* Mayr (Hymenoptera: Formicidae). Molecular Phylogenetics and Evolution 59, 281–292.

Martin, H.A., 1987. Cainozoic history of the vegetation and climate of the Lachlan River region, New South Wales. Proceedings of the Linnean Society of New South Wales 109, 213–257.

McLoughlin, S., 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. Australian Journal of Botany 49, 271–300.

Morgan, M.J., Roberts, J.D., Keogh, J.S., 2007. Molecular phylogenetic dating supports an ancient endemic speciation model in Australia's biodiversity hotspot. Molecular Phylogenetics and Evolution 44, 371–385.

Moritz, C., Patton, J.L., Schneider, C.J., Smith, T.B., 2000. Diversification of rainforest faunas: an integrated molecular approach. Annual Review of Ecology and Systematics 31, 533–563.

Moussalli, A., Hugall, A.F., Moritz, C., 2005. A mitochondrial phylogeny of the rainforest skink genus *Saproscincus*, Wells and Wellington (1984). Molecular Phylogenetics and Evolution 34, 190–202.

O'Connor, D., Moritz, C., 2003. A molecular phylogeny of the Australian skink genera *Eulamprus*, *Gnypetoscincus* and *Nangura*. Australian Journal of Zoology 51, 317–330.

Ollier, C.D., 1986. The origin of alpine landforms in Australasia. In: Barlow, B.A. (Ed.), Flora and Fauna of Alpine Australasia. Ages and Origins. CSIRO, Melbourne, pp. 3–26.

Penney, D., 2003. *Afrarchaea grimaldii*, a new species of Archaeidae (Araneae) in Cretaceous Burmese amber. Journal of Arachnology 31, 122–130.

Pickard-Cambridge, O., 1881. On some new genera and species of Araneidea. Proceedings of the Zoological Society of London 1881, 765–775.

Platnick, N.I., 1991a. On Malagasy *Archaea* (Araneae, Archaeidae). Journal of African Zoology 105, 135–140.

Platnick, N.I., 1991b. On Western Australian *Austrarchaea* (Araneae, Archaeidae). Bulletin of the British Arachnological Society 8, 259–261.

Platnick, N.I., 2011. The World Spider Catalog, Version 12.0. American Museum of Natural History, New York. <http://research.amnh.org/entomology/spiders/catalog/index.html>.

Pola, M., Gosliner, T.M., 2010. The first molecular phylogeny of cladobranchian opisthobranchs (Mollusca, Gastropoda, Nudibranchia). Molecular Phylogenetics and Evolution 56, 931–941.

Ponniah, M., Hughes, J.M., 2004. The evolution of Queensland spiny mountain crayfish of the genus *Euastacus*. I. Testing vicariance and dispersal with interspecific mitochondrial DNA. Evolution 58, 1071–1085.

Ponniah, M., Hughes, J.M., 2006. The evolution of Queensland spiny mountain crayfish of the genus *Euastacus*. II. Investigating simultaneous vicariance with intraspecific genetic data. Marine and Freshwater Research 57, 349–362.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Qiu, Y., Song, D., Zhou, K., Sun, H., 2005. The mitochondrial sequences of *Heptathela hangzhouensis* and *Ornithoctonus huwena* reveal unique gene arrangements and atypical tRNAs. Journal of Molecular Evolution 60, 57–71.
Rambaut, A., 2009. FigTree Version 1.3.1. <http://tree.bio.ed.ac.uk/software/figtree/>.
Rambaut, A., Drummond, A.J., 2009. Tracer Version 1.5. <http://beast.bio.ed.ac.uk/Tracer>.
Rix, M.G., Harvey, M.S., 2008. A survey of populations of Main's Assassin Spider (*Austrarchaea mainae*) near Albany. Unpublished report to Verve Energy and the Water Corporation, Western Australian Museum, Perth, pp. 1–9.
Rix, M.G., Harvey, M.S., 2010. The first pararchaeid spider (Araneae: Pararchaeidae) from New Caledonia, with a discussion on spinneret spigots and egg sac morphology in *Ozarchaea*. Zootaxa 2412, 27–40.
Rix, M.G., Harvey, M.S., 2011. Australian Assassins, Part I: a review of the assassin spiders (Araneae: Archaeidae) of mid-eastern Australia. ZooKeys 123, 1–100.
Rix, M.G., Harvey, M.S., Roberts, J.D., 2008. Molecular phylogenetics of the spider family Micropholcommatidae (Arachnida: Araneae) using nuclear rRNA genes (18S and 28S). Molecular Phylogenetics and Evolution 46, 1031–1048.
Rix, M.G., Harvey, M.S., Roberts, J.D., 2010. A revision of the textricellin spider genus *Raveniella* (Araneae: Araneoidea: Micropholcommatidae): exploring patterns of phylogeny and biogeography in an Australian biodiversity hotspot. Invertebrate Systematics 24, 209–237.
Rix, M.G., Roberts, J.D., Harvey, M.S., 2009. The spider families Synotaxidae and Malkaridae (Arachnida: Araneae: Araneoidea) in Western Australia. Records of the Western Australian Museum 25, 295–304.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Schäuble, C.S., Moritz, C., 2001. Comparative phylogeography of two open forest frogs from eastern Australia. Biological Journal of the Linnean Society 74, 157–170.
Schneider, C.J., Cunningham, M., Moritz, C., 1998. Comparative phylogeography and the history of endemic vertebrates in the Wet Tropics rainforests of Australia. Molecular Ecology 7, 487–498.
Schneider, C., Moritz, C., 1999. Rainforest refugia and evolution in Australia's Wet Tropics. Proceedings of the Royal Society of London Series B 266, 191–196.
Schultz, M.B., Smith, S.A., Horwitz, P., Richardson, A.M.M., Crandall, K.A., Austin, C.M., 2009. Evolution underground: a molecular phylogenetic investigation of Australian burrowing freshwater crayfish (Decapoda: Parastacidae) with particular focus on *Engaeus* Erichson. Molecular Phylogenetics and Evolution 50, 580–598.
Selden, P.A., Diying, H., Dong, R., 2008. Palpimanoid spiders from the Jurassic of China. Journal of Arachnology 36, 306–321.
Selden, P.A., Penney, D., 2010. Fossil spiders. Biological Reviews 85, 171–206.
Sharma, P., Giribet, G., 2009. Sandokanid phylogeny based on eight molecular markers – the evolution of a southeast Asian endemic family of Laniatores (Arachnida, Opiliones). Molecular Phylogenetics and Evolution 52, 432–447.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87, 651–701.
Sota, T., Takami, Y., Monteith, G.B., Moore, B.P., 2005. Phylogeny and character evolution of endemic Australian carabid beetles of the genus *Pamborus* based on mitochondrial and nuclear gene sequences. Molecular Phylogenetics and Evolution 36, 391–404.
Specht, R.L., 1981. Major vegetation formations in Australia. In: Keast, A. (Ed.), Ecological Biogeography of Australia. Dr. W. Junk bv Publishers, The Hague, pp. 165–297.
Swofford, D.L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28, 2731–2739.
Tavaré, S., 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences 17, 57–86.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface. flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25, 4876–4882.
Vink, C.J., Dupérré, N., 2010. Pisauridae (Arachnida: Araneae). Fauna of New Zealand 64, 1–60.
Ward, P.S., 1980. A systematic revision of the *Rhytidoponera impressa* group (Hymenoptera: Formicidae) in Australia and New Guinea. Australian Journal of Zoology 28, 475–498.
Webb, L.J., Tracey, J.G., 1981. Australian rainforests: patterns and change. In: Keast, A. (Ed.), Ecological Biogeography of Australia. Dr. W. Junk bv Publishers, The Hague, pp. 607–694.
Wellman, P., 1982. The southeastern highlands: a geological and environmental history from 100 million years ago to the present. Victorian Naturalist 99, 110–120.
Wellman, P., McDougall, I., 1974. Cainozoic igneous activity in eastern Australia. Tectonophysics 23, 49–65.
Willmott, W. 2004. Rocks and Landscapes of the National Parks of Southern Queensland. Geological Society of Australia Queensland Division, Brisbane.
Wirth, V.v., 1991. Eine neue Vogelspinnenart aus Vietnam *Haplopelma schmidti* sp. n. (Araneae: Theraphosidae: Ornithoctoninae). Arachnologischer Anzeiger 18, 6–11.
Wood, H., 2008. A revision of the assassin spiders of the *Eriauchenius* gracilicollis group, a clade of spiders endemic to Madagascar (Araneae: Archaeidae). Zoological Journal of the Linnean Society 152, 255–296.
Wood, H.M., Griswold, C.E., Spicer, G.S., 2007. Phylogenetic relationships within an endemic group of Malagasy 'assassin spiders' (Araneae, Archaeidae): ancestral character reconstruction, convergent evolution and biogeography. Molecular Phylogenetics and Evolution 45, 612–619.
Yeates, D.K., Bouchard, P., Monteith, G.B., 2002. Patterns and levels of endemism in the Australian Wet Tropics rainforest: evidence from flightless insects. Invertebrate Systematics 16, 605–619.
Yoshizawa, K., Johnson, K.P., 2010. How stable is the "Polyphyly of Lice" hypothesis (Insecta: Psocodea)? A comparison of phylogenetic signal in multiple genes. Molecular Phylogenetics and Evolution 55, 939–951.