EVOLUTIONARY BIOGEOGRAPHY OF AUSTRALIAN RIVERINE TURTLES: *ELSEYA* SPP. AND *EMYDURA MACQUARII KREFFTII*

Thesis submitted by

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STATEMENT OF CONTRIBUTION BY OTHERS

Supervision

Principal supervisors Professor David Blair and Professor Dean Jerry, and co-supervisors Dr Mark Hamann and Dr Kyall Zenger, provided editorial comments on all sections of this thesis and contributed to the original project conception and design.

Co-authorship

Chapter 2 – David Blair, Dean Jerry and Mark Hamann contributed editorially and to the original experimental design.

Chapter 3 – Sharon Farley, under supervision of Arthur Georges, Nancy FitzSimmons, Lachlan Farrington and Col Limpus, generated data for an additional five microsatellite loci that were incorporated into the published version of this chapter. LF also contributed haplotype data used to diagnose the translocated origin of a peripheral population. David Blair and Dean Jerry contributed to the original project design. DB, DJ, AG and NF contributed editorially.

Chapter 4 – Arthur Georges provided sequence data for New Guinean species and the R35 locus. Vimoksalehi Lukoschek assisted with molecular dating analyses. David Blair and Dean Jerry contributed to the original project design. All contributed editorially.

Chapter 5 – David Blair and Dean Jerry contributed editorially and to the project design.

Further assistance

Kyall Zenger performed the clean-up and *de novo* assembly of sequence data used in Chapter 2. Robert Puschendorf provided editorial comments on Chapter 3. Robert Puschendorf and Adella Edwards assisted with GIS applications and figure preparation. Arthur Georges, Ivan Lawler, Jason Schaffer, Alastair Freeman, the Northern Territory Museum, and Eridani Mulder all provided tissue samples for analysis.

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DECLARATION OF ETHICS

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research received animal ethics approval from the JCU Animal Ethics Committee.

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- Erica.

ABSTRACT

Australia supports a highly endemic freshwater fauna. The continent's long isolation, Gondwanan heritage, and present aridity make it of particular interest to freshwater biogeographers. Recent molecular genetic studies of freshwater fishes and macroinvertebrates implicate diverse processes of landform evolution, climatic change and sea level fluctuation in shaping current patterns of biodiversity. These studies indicate a biogeographic complexity for Australian freshwaters that is not yet well understood, especially throughout the geographically complex eastern coastal margin. Australian freshwater turtles are one of the continent's few vertebrate freshwater Gondwanan relics that are still taxonomically and ecologically diverse, and geographically widespread. However, in context of their biogeography they remain poorly studied, despite studies on other continents showing turtles to be particularly suited to illuminating complex evolutionary processes. In this thesis is explored the sensitivity of freshwater turtles as models for biogeographic inference in an Australian context, to seek new insights that may clarify and extend our knowledge of Australian freshwater biogeography. Molecular genetic tools are developed and applied to investigate phylogeographies of turtle species from two Australian genera. The evolutionary history of riverine specialist Australian snapping turtles (genus *Elseya*) is compared to that of the more ecologically generalist and widespread subspecies of *Emydura macquarii*, and in particular that of Krefft's river turtle, *E. m. krefftii*. These taxa were chosen as models because both are primarily riverine and broadly sympatric throughout eastern Australia, but differ significantly in niche breadth, range size, and expected dispersal ability.

To address the lack of suitable genetic resources available for Australian short-necked turtles, Next-Generation shotgun genome sequencing was evaluated as a cost-effective means of developing novel genetic resources in two species of freshwater turtle. Low-coverage Roche 454-sequencing was used to randomly sample genomic sequence data for microsatellite repeats in the study species *Elseya albagula* and *Emydura macquarii krefftii*. Thousands of microsatellite loci suitable for amplification by PCR were found. Of these, 29 loci were developed for high-resolution population genetic analyses in the study species, which also cross-amplified successfully in a range of other Australian short-necked turtle taxa. Further bioinformatic exploration of the genomic sequence datasets enabled reconstruction of near-complete mitochondrial genomes, and characterisation of gene content and repetitive elements. A molecular toolkit of nuclear and mitochondrial markers is presented that provides the foundation for research presented in this thesis, and which will also facilitate future genetic research on Australian freshwater turtles generally.

Drainages within Australia's mid-eastern coastal region (Fitzroy, Burnett and Mary catchments) face considerable urban pressure and contain high freshwater biodiversity and endemism. Mitochondrial (~1.3 kb control region and ND4) and nuclear microsatellite datasets (12 polymorphic loci) were used to investigate genetic structure in the locally endemic white-throated snapping turtle, *Elseya albagula*, to clarify historical biogeography and address pressing conservation issues for this species and this region. Individual drainage basins contained discrete genetic units (average pairwise F_{ST} = 0.15 and Φ_{ST} = 0.75 among drainages), though the degree of divergence among drainages varied. The Fitzroy drainage contained a distinct evolutionary lineage, divergent from a second lineage occurring in both the Burnett and Mary drainages. Genetic data were used to make recommendations regarding recognition of evolutionarily significant units and management units for *E. albagula*. Geological evidence and genetic data for co-distributed freshwater species were consolidated to propose a shared biogeographic history for a diverse regional biota, reflecting historical isolation of the Fitzroy and recent coalescence of the Burnett-Mary drainages during lowered Pleistocene sea levels.

To examine broader-scale evolutionary hypotheses associated with changes to regional riverine connectivity through eustatic sea level change, landform evolution and aridity, a multi-locus molecular approach incorporating mitochondrial (control region, ND4 and 16S) and nuclear (R35 intron) sequences was used to reconstruct phylogenetic relationships and estimate divergence times for all extant *Elseya* species (including undescribed forms) across Australia and New Guinea. The genus *Elseya* was shown to contain four divergent, geographically correlated clades, corresponding to all of New Guinea, southern New Guinea plus northern Australia, north-eastern Australia, and south-eastern Australia. These are estimated to have arisen in the Late-Miocene (between ~5.82-9.7 Ma), and diversified further in the early Pleistocene (between ~2.2-2.43 Ma and 1.36-1.66 Ma), coincident with major phases of aridity and climatic upheaval. Overall, snapping turtles were found to have a long vicariant history in Australia and New Guinea, tied to the disconnection of fluvial habitat through landform evolution, sea level change and ongoing aridification. Major implications of these genetic results for understanding freshwater biodiversity evolution in Australia are discussed, including evidence for periodic connectivity with New Guinea, important regional biogeographic barriers (Lake Carpentaria and the Burdekin-Fitzroy drainage divide), and the location of potential freshwater refugia.

Krefft's river turtle, *Emydura macquarii krefftii*, are common throughout eastern coastal Australia and their extensive longitudinal distribution spans landscape and climatic barriers recently proposed as important in structuring regional freshwater biodiversity. Their evolutionary history in response to climatic oscillations and putative biogeographic barriers was

examined using range-wide sampling (649 individuals representing 18 locations across 11 drainages) and analysis of mitochondrial sequences (~1.3 kb control region and ND4) and nuclear microsatellite (12 polymorphic loci) data. Competing demographic (local persistence versus range expansion) and biogeographic (arid corridor versus drainage divide) hypotheses were considered. Krefft's turtles exhibit significant genetic structure across their range at mitochondrial and microsatellite markers, consistent with isolation across drainage divides. Deep north-south regional divergence (2.2%, ND4 *p*-distance) was consistent with long-term isolation across the Burdekin-Fitzroy drainage divide, not the adjacent Burdekin Gap dry corridor. There was also evidence for rare contemporary overland dispersal across the Burdekin-Fitzroy watershed and for hybridisation with *Emydura tanybaraga* at the northern range limit. Data suggest Krefft's turtles persisted within the arid Burdekin region throughout multiple episodes of Plio-Pleistocene aridity, though very low contemporary genetic diversity indicates this may have been despite potential population bottlenecks.

Overall, riverine turtle species examined in this thesis exhibited strong, geographically correlated, phylogeographic structure. A remarkable degree of genealogical concordance was observed in phylogeographical patterns between turtle taxa, and turtles and other freshwater groups. Though differences in range size and niche breadth were expected to produce disparities in dispersal ability and phylogeographic structure between the two turtle taxa, both exhibited a primary pattern of genetic structure reflecting isolation across drainage divides. Riverine turtles are indeed sensitive models for inferring historical processes influencing freshwater biodiversity in an Australian context. Molecular data presented in this thesis collectively demonstrate the importance of comparing phylogeographic patterns among co-distributed taxa with variable ecological tolerances and dispersal abilities. Furthermore, the current work not only highlights the potential value of further phylogeographic research into ecologically diverse freshwater turtles in Australia, but provides a comprehensive molecular toolkit for doing so.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 – GENERAL INTRODUCTION

Image: An adult male snapping turtle, *Elseya albagula*. Photo by Erica Todd.

CHAPTER 1 – GENERAL INTRODUCTION

The physical structure of riverine environments imposes common constraints on dispersal and population connectivity in aquatic fauna (Meffe & Vrijenhoek 1988; Lowe *et al*. 2006; Hughes *et al*. 2009). For obligate freshwater species, discrete habitat boundaries largely limit dispersal to within the waterway, which is linear and branching. Watersheds separate neighbouring catchments into hydrologically distinct units and act as major barriers to dispersal, thus confining freshwater-limited species to specific drainages for moderate lengths of evolutionary time. Therefore, greatest genetic differentiation is expected at the between-catchment level. However, historical processes such as landform evolution and changes in sea level can result in modifications of the riverine net (Banarescu 1990).

Studying the relationship between historical geographic processes and the distribution of genetic lineages across the landscape in contemporary species can reveal information on species evolution and earth history, and is the purview of the field of phylogeography (Avise 2000). Obligate freshwater species typically show strong phylogeographic structure reflecting historical isolation of drainages (Ward *et al*. 1994; Waters *et al*. 2001). The unique biogeographic constraints experienced by freshwater species also make them intrinsically suited to studies of landscape evolution (Bermingham & Avise 1986).

Phylogeography is necessarily an integrative discipline, combining evidence from molecular genetics with that from landscape history derived from geology, paleontology and climatology. Comparative phylogeography especially, enables examination of hypotheses regarding how abiotic and/or ecological processes drive evolution in a whole community context, allowing greater insight into evolution both of taxa and regions (Bermingham & Moritz 1998). Phylogeography was originally conceived as the bridge (using mitochondrial DNA data) between the disciplines of phylogenetics and population genetics, bringing together macro- and micro-evolutionary thought (Avise *et al*. 1987). Since then, routine incorporation of sensitive nuclear markers such as microsatellites, and sophisticated Bayesian and coalescent analyses into phylogeographic studies has further improved the rigour of biogeographic inference and has made phylogeography a powerful modern discipline within the broader field of molecular ecology (Hickerson *et al*. 2010; Andrew *et al*. 2013).

AUSTRALIAN BIOGEOGRAPHY

The island continent of Australia is recognised for its rich and highly endemic biota, whose evolutionary development reflects a long and complex biogeographic history. From temperate Gondwanan origins, Australia's more recent climatic history has been dominated by a progressive, though punctuated transition towards increasingly arid and more capricious

environments. In the Northern Hemisphere, advance and retreat of extensive land-based glaciers during the Pleistocene has been the primary driver of contemporary biogeographic patterns (Hewitt 2000). Australia experienced little glaciation, but instead underwent extreme and widespread aridity during Pleistocene glacial maxima, though the first steps towards aridification began much earlier, in the Mid-to-Late-Miocene (White 1994; Crisp *et al*. 2004; Martin 2006; Byrne *et al*. 2008). Strong patterns of regional endemism seen across the continent for both terrestrial (Cracraft 1991) and aquatic biotas (Unmack 2001) have been hypothesised to reflect longer-term patterns of habitat availability. In recent years, a flurry of phylogeographic research has confirmed this for terrestrial taxa, whereby deepest divergences between genera and infrageneric groups coincide with the onset of desiccation in the Mid-to-Late-Miocene (e.g. Rix & Harvey 2012), while Pleistocene oscillations drove mostly species and population-level diversification (e.g. Byrne 2008).

Recent reviews have summarised current biogeographic knowledge for Australia's mesic (Byrne *et al*. 2011), arid (Byrne *et al*. 2008) and monsoon tropics (Bowman *et al*. 2010) biomes. Collectively, they describe the vicariant diversification of forest-adapted taxa, as original mesic habitats fragmented and withdrew towards the coast, concomitant range expansion and adaptive radiation of more recently evolved arid-zone species, and the integration and diversification of newer colonisers following Australia's recent collision with Asia. However, these appraisals also highlight a lack of detailed phylogeographic studies on diverse freshwater taxa, which represents a significant gap in knowledge of Australian biogeography. Australia's long isolation, Gondwanan heritage, and current aridity make studies of freshwater evolution in this context of considerable biogeographic interest.

AUSTRALIAN FRESHWATER BIOGEOGRAPHY

Australia's aquatic fauna comprises diverse elements that include true freshwater evolutionary relics (lungfish, syncarid crustaceans, chelid turtles), old endemics with Gondwanan affinities (percichthyid fish and parastacid crayfish), and species evolved more recently from marine ancestors (many freshwater fish, some macroinvertebrates) (McDowall 1981; Merrick 2006).

Patterns and processes on a continental scale

A comprehensive assessment of biogeographic patterns for Australian freshwater fishes has been undertaken, based on species distributions (Unmack 2001). This study revealed a striking degree of endemism across the continent and proposed a series of faunal provinces, sub-provinces, and regional relationships that form a comprehensive hypothesis framework for Australian freshwater biogeography suitable for testing with molecular data. Broad-scale patterns of endemism are hypothesised to reflect isolation by aridity and drainage divides

(Unmack 2001). Patterns are considered to have established as early as the Miocene, while the influence of Plio-Pleistocene climatic events seemed minimal. Disjunct distributions of several old endemic freshwater lineages also imply ancient vicariance, consistent with increasing aridity (crayfish, Austin 1995; anurans, Barendse 1984; Roberts & Maxon 1985; turtles, Georges & Thomson 2006). Evidence from dated molecular phylogenies supports this assertion for freshwater crayfish (genus *Cherax*), where divergence among regional centres of endemism in northern, eastern and south-western Australia date to the Miocene epoch (Munasinghe *et al*. 2004).

Unmack's (2001) framework of freshwater faunal provinces is presented in Fig. 1.1. Northern and eastern Australia are considered distinct provinces and support the majority of extant freshwater biodiversity. However, freshwater fish endemism within northern and eastern provinces is low, with a gradation of species ranges evident at the drainage scale (Unmack 2001). Low endemism across northern Australia may be explained by high drainage connectivity during low sea levels and monsoonal flooding across the largely low-relief landscape. However, for eastern Australia, bathymetry suggests drainages remained mostly isolated at times of low sea level. Low regional endemism indicates an absence of distinct barriers to freshwater dispersal in this region, and longitudinal variation in climate from tropical to more temperate conditions is considered responsible for current species' distributions (Unmack 2001). For northern Australia, molecular evidence from several freshwater species supports recent (Plio-Pleistocene) population connectivity, particularly via paleo-Lake Carpentaria (Alacs 2008; de Bruyn *et al*. 2004; Cook *et al*. 2012), which united rivers throughout the Gulf of Carpentaria with southern New Guinea. However, within the Eastern Province, molecular evidence largely denies faunal breaks for freshwater taxa and instead reveals a biogeographic complexity for the region that warrants further attention.

Molecular evidence for biogeographic complexity of Australia's eastern margin

The eastern margin was formed by uplift through accretionary processes of the Great Dividing Range (GDR), which parallels the entire length of Australia's east coast (~3,500 km) in a north-south alignment. The GDR represents the watershed boundary between east coastal and inland drainage systems and has been responsible for sheltering the east coast from widespread aridity. In an otherwise geologically quiescent continent, development of the eastern margin reflects multifarious processes of landform development including uplift, erosion, volcanism, scarp retreat, and sea level fluctuation (Griffin & McDougall 1975; Chappell *et al*. 1996; Jones 2006; Vasconcelos *et al*. 2008), which have produced complex drainage patterns (Hodgkinson *et al*. 2007). Quaternary climate cycles are known to have driven repeated contraction and expansion of closed forest habitats (VanDerWal *et al*. 2009), and presumably, freshwater habitats also.

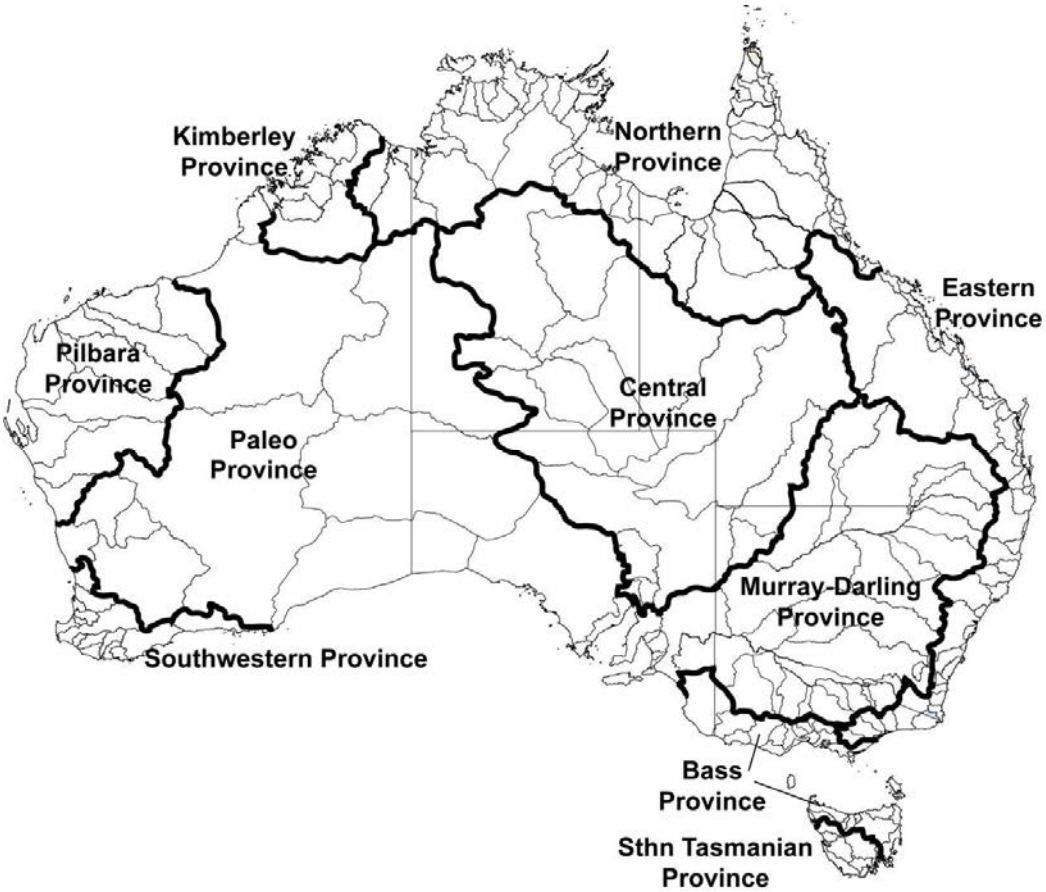

Fig. 1.1 Map showing biogeographic provinces in Australia based on freshwater fish species endemism (Unmack 2001). Bold lines delineate faunal provinces, fine lines delineate drainage boundaries within provinces.

Geological and climatic complexity within the eastern margin is reflected in the complex patterns of genetic structure described for increasing numbers of obligate freshwater taxa, including cryptic local diversity and deep lineage disjunctions at higher geographical scales. For example, recent genetic studies of several widespread freshwater species, including fishes (*Pseudomogil signifier*, Wong *et al*. 2004; *Tandanus* catfish, Jerry 2008) and platypus (*Ornithorhynchus anatinus*, Gongora *et al*. 2012), reveal deep north-south lineage disjunction that implicates a major biogeographic barrier disrupting freshwater connectivity in the vicinity of the Burdekin drainage. The genetic break is consistent with an historical arid corridor previously recognised as a vicariant barrier for terrestrial taxa (Cracraft 1991; Chapple *et al*. 2011). However, sampling within the Burdekin drainage was limited by its depauperate ichthyofauna and the exact biogeographic nature of the disjunction remains speculative.

In north-eastern Australia, several freshwater fishes exhibit a pattern of genetic structure inconsistent with contemporary stream architecture, suggesting historical drainage rearrangement (Hurwood & Hughes 1998; McGlashan & Hughes 2000). Regional volcanism (until as recently as 10,000 ya, Stephenson *et al*. 1980) is one possible explanation. Genetic lineage structure in a range of freshwater crustaceans and fishes inhabiting near-shore sand islands of south-eastern Australia largely pre-dates geological formation of their habitat, while island-mainland divergence within lineages is consistent with Pleistocene sea level rise (Page & Hughes 2007; Page *et al*. 2012). Other studies report a high degree of incongruence across multiple co-distributed taxa (e.g. gudgeons, Thacker *et al*. 2007, 2008). Incongruence suggests a complex history of inter-drainage dispersal and vicariance, but also that dispersal in freshwater taxa can be very idiosyncratic, depending largely upon individual species' ecology. Indeed, freshwater species with more specialised ecologies have been shown to be highly genetically structured within drainage basins (Hughes *et al*. 2012), while others with much more generalist habitat requirements lack significant phylogeographic structure despite extensive multi-drainage distributions (McGlashan & Hughes 2001a; Bostock *et al*. 2006).

Collectively, phylogeographic work to date on freshwater species within the eastern margin reveals a biogeographic complexity that was unexpected from species distribution data alone. Multifarious processes of landscape development and climate seem responsible for current patterns of freshwater biodiversity, though ecological tolerances of individual species are also important. Previous work is largely based on mitochondrial and/or allozyme markers and many studies lack fine-scale geographic sampling. Although these markers were previously considered most ideal for phylogeographical studies of animal populations (Avise *et al*. 1987; Avise 1991), microsatellites have more recently become a powerful adjunct marker capable of inferring finer-scale patterns of diversity and divergence (Estoup *et al*. 1998; Andrew *et al*. 2013). Further detailed phylogeographic studies of diverse freshwater groups are needed to

clarify several aspects of the region's biogeography highlighted by previous research. The following are considered priorities for future research:

- Dated molecular phylogenies of widespread freshwater groups with currently disjunct distributions, to establish whether patterns of divergence reflect longer-term environmental change or more recent Pleistocene climatic influences.
- Further investigation of the role of Pleistocene climatic cycles on freshwater species' distributions, particularly for those species that are currently widespread.
- Examination of hypotheses related to specific biogeographic features underlying regional genetic breaks identified from previous research.
- Further investigation of the role of species ecology in producing discordant phylogeographic patterns in response to the same underlying landscape.
- Molecular studies of understudied taxonomic groups, such as many aquatic invertebrates and turtles.

TURTLES

Model organisms for phylogeography

Freshwater and terrestrial turtles were used as early phylogeographic models in studies of Northern Hemisphere communities, where they provide fine examples of genealogical concordance across species and with other faunal groups (Walker & Avise 1998; Weisrock & Janzen 2000). The longevity, low vagility and site fidelity of most turtle species mean they typically exhibit strong phylogeographic signatures that are retained over extended timescales. Turtles often show high levels of cryptic lineage diversity that are closely tied to the underlying geological history of a region (Roman *et al*. 1999; Beheregaray *et al*. 2003; Fritz *et al*. 2006). As a group, turtles have proven sensitive phylogeographic models for inferring how historical evolutionary forces shape current patterns of diversity. However, turtles are less well studied on the southern continents, where biogeography generally remains less well understood (Beheregaray 2008).

Australian turtle diversity

The extant turtle fauna of Australia and New Guinea is a diverse and highly endemic assemblage of freshwater species belonging almost exclusively to the family Chelidae (side-necked turtles, sub-order Pleurodira). Chelid turtles occur elsewhere only in South America, though with unique genus-level diversity, and so have undisputed Gondwanan origins. Seven extant Australian genera are recognised with about 26 species, though several more species await formal description (Georges & Thomson 2010). Turtles are important ecological

components of Australian freshwater communities, yet very little is known about the basic biology of most species. In particular, there is poor comprehension of evolutionary relationships and intra-specific patterns of genetic diversity.

Zoogeographic hypotheses for Australian turtles have been presented (Burbidge *et al*. 1974; Georges & Thomson 2006). Extant species diversity is concentrated within tropical and sub-tropical habitats along Australia's northern and eastern coastlines where there is still perennial drainage (Fig. 1.2). The fossil record also indicates living Australian chelids are relics of a much more diverse and widespread turtle fauna of wetter prehistoric times. In south-eastern Australia, low turtle species diversity (Fig. 1.2), except for a few taxa also found further northwards, may reflect recent reinvasions following regionally extreme cold and drought during the most recent glacial maximum (12,000-18,000 ya) (Georges & Thomson 2006). Zoogeographic hypotheses relating to Pleistocene contractions and invasions of turtle populations in south-eastern Australia are currently being tested with molecular genetic data for snake-necked *Chelodina* spp., as part of a PhD study by Kate Hodges at the University of Canberra. In northern Australia and southern New Guinea, distributions of many turtle taxa may be explained by periodic freshwater connectivity via Lake Carpentaria, as well as rivers crossing the Arafura Sill to the west (Georges & Thomson 2006). Snake-necked turtles (*Chelodina rugosa*) have a genetic signature consistent with connectivity via Lake Carpentaria (Alacs 2008), though other turtle taxa in the region remain to be studied genetically. Most biogeographic hypotheses for Australian turtles remain to be tested with molecular genetic data and regional biogeographic patterns for turtles are largely speculative.

The long evolutionary history *in situ*, extant diversity and high local endemism of Australian chelid turtles make them ideal study organisms for exploring competing theories about freshwater biogeography in Australia. Those species that are primarily riverine should be especially sensitive to historical changes to fluvial connectivity across the landscape and so should make sensitive model taxa to examine freshwater biogeography.

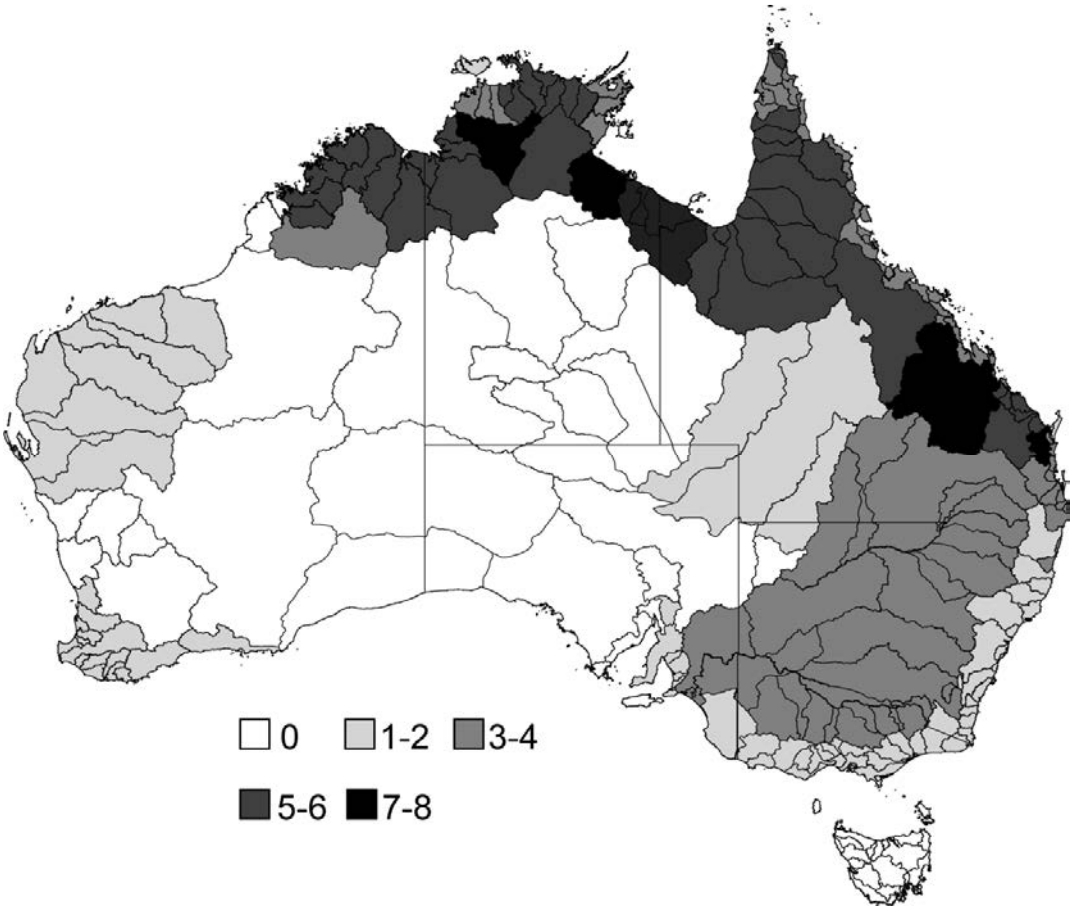

Fig 1.2 A map of Australian drainage basins showing the distribution of turtle species richness across the continent. Shading indicates numbers of species present per drainage basin, highlighting concentration of turtle species richness along the tropical and sub-tropical northern and eastern coastlines. Modified from Georges and Thomson (2006).

Study species

Species from two genera of riverine turtles (*Elseya* spp. and *Emydura* as represented by *E. macquarii krefftii*), which differ significantly in aspects of their ecology (geographic range and degree of habitat specificity) and taxonomic scale (genus versus subspecies) were used as model taxa to investigate biogeographic processes shaping biodiversity in northern and eastern Australia. Though these taxa are largely sympatric throughout much of eastern Australia (Fig. 1.3), ecological differences between them are expected to engender differences in dispersal potential. As dispersal potential generally negatively correlates with genetic population structure and positively correlates with range size (Bohonak 1999; Holt 2003), the two turtle taxa may be expected to differ in their measurable phylogeographic structure and in their evolutionary response to a common landscape.

Australian snapping turtles - Elseya *spp.*

Australian snapping turtles (genus *Elseya*) are large-bodied riverine specialist species with a patchy distribution across northern and eastern Australia and New Guinea (Fig. 1.3). Their reliance upon permanent (perennial) riverine habitat reflects a remarkable ecophysiological adaptation enabling them to respire aquatically via highly vascularised cloacal bursae (Mathie & Franklin 2006). The genus constitutes a series of at least six electrophoretically distinct allopatric taxa previously regarded as a single widespread species, *E. dentata* (Georges & Adams 1996). Phylogenetic relationships within the genus have not been studied in detail (Le *et al*. 2013) but may be expected to reflect historical fluvial connections across the landscape rather than terrestrial dispersal. Their extensive but patchy distribution and reliance upon permanent riverine habitat means that, as a group, *Elseya* spp. have the potential to reveal both broad and fine-scale vicariant biogeographic patterns at varying temporal scales. Narrow habitat requirements coupled with restricted geographical ranges of most *Elseya* species also mean they are potentially highly sensitive to changes in habitat and flow regimes associated with river regulation, and most are considered high priorities for conservation (Thomson *et al*. 2006).

Krefft's river turtle - Emydura macquarii krefftii

There are four subspecies within the *Emydura macquarii* complex, defined by largely non-overlapping geographical ranges and minor morphological differences (Georges & Thomson 2010). Krefft's turtle is the northernmost of these. The subspecies is common throughout coastal-flowing drainages from the Mary River in the south, to the Normanby River in the north; an extensive longitudinal distribution spanning over 1600 km of coastline (Fig. 1.3). Like most *Emydura* species, *E. m. krefftii* is considered an ecological generalist because of its broad

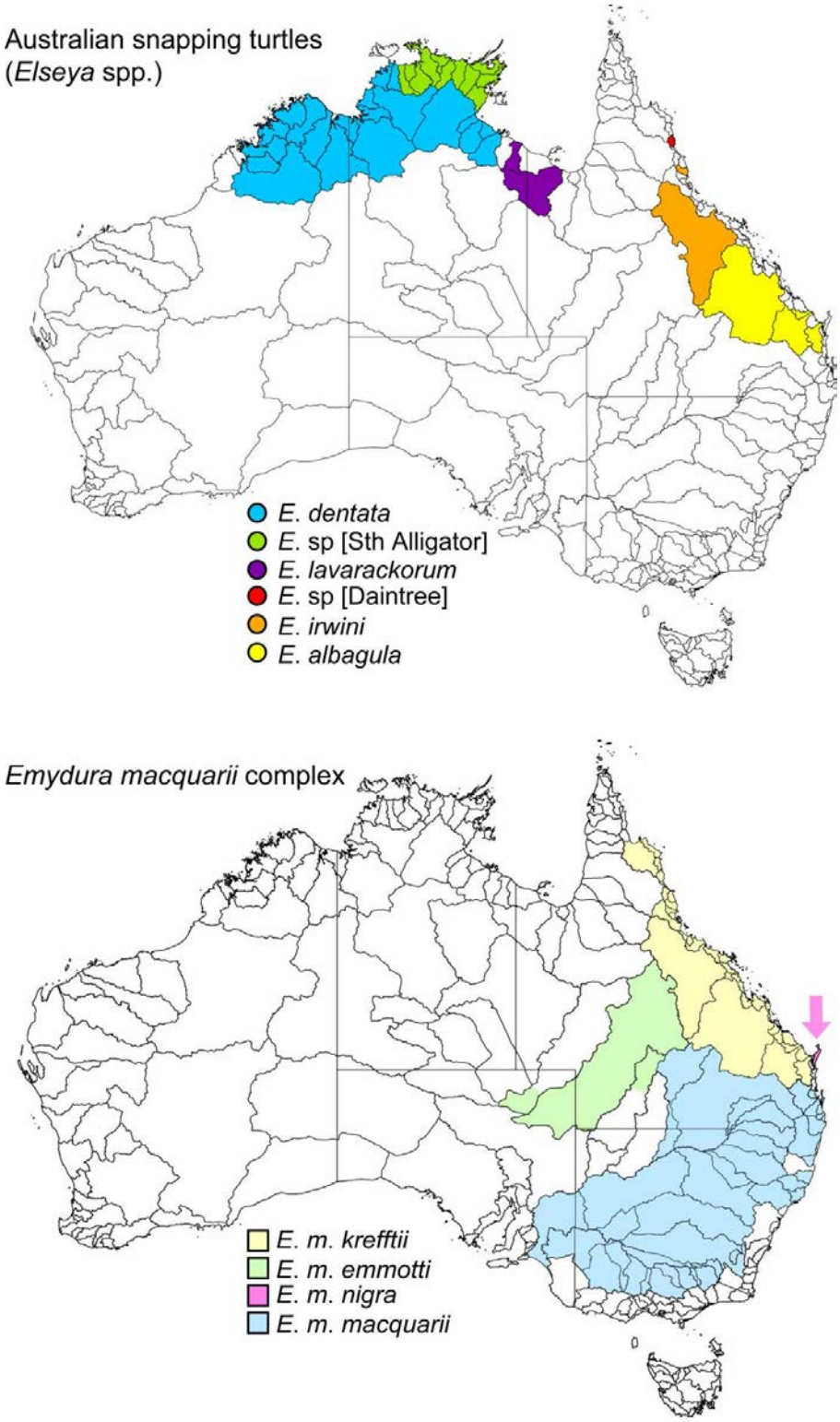

Fig. 1.3 Maps showing the distribution of Australian snapping turtles (genus *Elseya*) (top) and subspecies of the *Emydura macquarii* complex (bottom), indicating their broadly overlapping distributions in eastern Australia. The pink arrow highlights the narrow range of *E. m. nigra* on Frazer Is.

opportunistic diet and capacity to occupy rivers as well as larger permanent waterbodies of the floodplain (Georges & Thomson 2006). In contrast to the genus *Elseya*, electrophoretic surveys show all *Emydura* species to be very closely related genetically (Georges & Adams 1996), indicating they may represent a relatively recent radiation.

THESIS AIMS AND STRUCTURE

The overarching objectives of this thesis are twofold: 1) to provide new insights that will clarify and extend our knowledge of the evolutionary development of Australia's unique freshwater biodiversity from the perspective of a faunal group with a long evolutionary history *in situ*, and in so doing, 2) to evaluate turtles as model taxa for inferring freshwater biogeographic processes in an Australian context. Specific research objectives are introduced in each of four data chapters, outlined below, followed by a synopsis that marries these in terms of the overall thesis objectives. Though research presented in this thesis is entirely my own work, papers resulting from Chapters 2 to 5 will be, or have been, published as co-authored manuscripts, and a 'Statement of contribution by others' is given outlining others' involvement.

Chapter 2 addresses the lack of suitable genetic resources previously available for Australian turtles. The objective was to develop a molecular genetic toolkit that could be applied in work reported in later chapters, and which would also facilitate future genetic study of Australian turtle species. An emerging genomic sequencing approach was applied to develop microsatellite and mitochondrial markers in each of two focal taxa, *Elseya albagula* and *Emydura macquarii krefftii*. The microsatellite development component of Chapter 2 has been published in *Conservation Genetics Resources.*

Chapter 3 describes the application of microsatellite and mitochondrial data to uncover contemporary and historical influences on population genetic structure and connectivity in the southern snapping turtle, *Elseya albagula*, across its endemic distribution in the Fitzroy, Burnett and Mary Rivers in mid-eastern coastal Australia. The objective of this chapter was to clarify historical biogeography and address pressing conservation issues for this species and this region. Work presented in Chapter 3 has been published in *Zoological Journal of the Linnean Society*.

Chapter 4 describes a biogeographic history and timeline for the evolution of the genus *Elseya* across Australia and New Guinea, using mitochondrial and nuclear sequence data. The objective was to examine broader-scale evolutionary hypotheses associated with regional changes to fluvial connectivity through advancing aridity, sea level fluctuations and landform evolution. Work presented in Chapter has been published in *Journal of Biogeography*.

In Chapter 5, mitochondrial and microsatellite datasets were applied to investigate range-wide population genetic structure and diversity in Krefft's river turtles, *Emydura macquarii krefftii*. The objective was to address specific hypotheses related to landscape and climatic barriers previously proposed as important in structuring east coast freshwater communities, and to compare genetic patterns described for *Elseya* spp. across the same region. Work presented in Chapter 5 is currently under review in *Ecology and Evolution*.

In Chapter 6 is presented a synopsis of major research findings described in each of the previous chapters, which outlines how research presented in this thesis overall contributes to our understanding of freshwater biogeography in Australia.

CHAPTER 2 – LOW-COVERAGE GENOMIC SEQUENCING FACILITATES EFFICIENT GENETIC MARKER DISCOVERY IN TWO AUSTRALIAN FRESHWATER TURTLES

The microsatellite development component of this chapter has been published, as follows:

Todd, E., Blair, D., Hamann, M. & Jerry, D. (2011) Twenty-nine microsatellite markers for two Australian freshwater turtles, *Elseya albagula* and *Emydura macquarii krefftii*: development from 454-sequence data and utility in related taxa. *Conservation Genetics Resources*, **3**, 449-456.

Image: Typical riverine habitat of *Emydura macquarii krefftii* and *Elseya albagula*, in the upstream Connors River within the Fitzroy drainage basin. Photo by Erica Todd.

CHAPTER 2 – LOW-COVERAGE GENOMIC SEQUENCING FACILITATES EFFICIENT GENETIC MARKER DISCOVERY IN TWO AUSTRALIAN FRESHWATER TURTLES

SUMMARY

Low-coverage shotgun genome sequencing has emerged as a simple and cost-effective means of isolating large numbers of microsatellite markers in previously unstudied species, with several benefits over traditional protocols based on Sanger sequencing of enriched genomic libraries. The utility of this new approach is evaluated in turtles, a highly threatened yet poorly represented vertebrate clade. Roche 454-sequencing was used to randomly sample 36.6 and 46.2 Mbp of genomic sequence data for microsatellite repeats in the Australian freshwater turtle species *Elseya albagula* (n = 108,198 reads) and *Emydura macquarii krefftii* (n = 135,332 reads), respectively. Stringent search parameters identified microsatellite loci in 1.3% of all reads, with thousands of loci isolated in total, of which hundreds were found to be suitable for amplification by PCR. Of these, 29 loci were developed for high-resolution population genetic analyses in the study species, which also cross-amplified successfully in a range of other Australian short-necked turtle taxa. Further bioinformatic exploration of the sequence data enabled isolation of mitochondrial sequences and characterisation of gene content and repetitive elements. Results not only demonstrate the utility of the shotgun sequencing approach for genetic marker development in turtles, but the value of small-scale genome survey sequencing for future genomic research. It lays the foundation for later chapters, which apply microsatellite and mitochondrial markers to infer population and evolutionary histories for *Elseya* spp. and *E. m. krefftii*.

INTRODUCTION

The recent emergence of ultra-high-throughput 'next-generation' genomic sequencing technologies has revolutionised life sciences research (Hudson 2008; Mardis 2008; Schuster 2008). Billions of bases of genome-wide sequence information can now be produced as short (80-450 bp) sequence reads, rapidly and inexpensively, compared with traditional Sanger technology. Apart from enabling whole-genome studies, novel research applications are also being developed (Wegley *et al.* 2007; Abdelkrim *et al.* 2009; Futschik & Schlötterer 2010). Low-coverage shotgun sequencing, producing in the order of just 10's-100's of thousands of sequence reads, and representing just a small fraction of the genome, is yielding biologically useful information and finding particular utility in the development of genomic resources (Rasmussen & Noor 2009). This is perhaps best demonstrated by the proliferation of studies now using low-coverage shotgun sequencing as an approach to microsatellite marker discovery

(Schoebel *et al*. 2013), first demonstrated by Abdelkrim *et al.* (2009) in the New Zealand blue duck, *Hymenolaimus malacorhynchos*.

Microsatellites are among the most widely-applied genetic markers with diverse applications in conservation biology, population genetics, genome mapping, and evolutionary studies, among others (Goldstein & Schlötterer 1999). Their high per-locus information content, genome-wide ubiquitous distribution and simple co-dominant pattern of Mendelian inheritance make them particularly powerful and popular genetic markers for fine-scale questions concerning population genetic structure, gene flow, and mating systems (Selkoe & Toonen 2006). However, the need to isolate a new panel of microsatellite markers *de novo* for each new study species, due to variable and often limited cross-species transferability of existing markers (Barbará *et al.* 2007), has been a major drawback to their use. Traditional microsatellite isolation protocols involve time-consuming and costly processes of enrichment, cloning and Sanger sequencing, and are prone to low return for a substantial input of effort (Zane *et al.* 2002). The expense, labour and inefficiency of enrichment protocols previously restricted the accessibility of these powerful markers for researchers in small laboratories working with non-model taxa.

The longer average read lengths (currently 350-450 bp) provided by Roche's 454 Genome Sequencer platform now makes it theoretically possible to randomly sample potentially thousands of microsatellite loci in a genome-wide fashion, while providing ample flanking sequence for PCR primer design. Small-scale sequencing projects for this specific purpose have already proven successful in a growing number of diverse taxa, including previously unstudied species of conservation value (e.g. Abdelkrim *et al.* 2009; Boomer & Stow 2010; Csencsics *et al.* 2010). An additional bonus of this approach is the wealth of genomic sequence information produced as a by-product, a novelty that seems seldom utilised or fully explored. Small-scale sequencing projects may be useful for developing further genomic resources, including near-complete mitochondrial genomes and gene-associated markers (Rasmussen & Noor 2009).

Chelonians are threatened globally by anthropogenic activities such as overharvesting and habitat modification (Bour 2008; Buhlmann *et al.* 2009). This is also true for the Australian freshwater turtle fauna (Pleurodira: Chelidae), which form an evolutionarily distinct, yet poorly researched group of high conservation value (Buhlmann *et al.* 2009). However, limited or no population-level genetic data are available for most species as few high-resolution genetic tools exist for the group. Though a panel of microsatellite markers was recently developed for the Australian snake-necked turtles, genus *Chelodina*, using traditional approaches (Alacs *et al.* 2009), none have previously been available for the six Australian short-necked turtle genera.

The aim of this chapter was to utilise low-coverage 454 shotgun genome sequencing for microsatellite discovery in two species of Australian freshwater turtle, *Elseya albagula* (*Ea*) and *Emydura macquarii krefftii* (*Ek*), and to characterise a subset as markers for high-resolution population genetic studies in these species. A further aim was to report on a series of analyses investigating content of the 454 data, including mitochondrial genome reconstruction, characterisation of repetitive elements, and analysis of functional (gene) content.

METHODS

Sample preparation and 454-sequencing

Animals of both species were sampled from the lower Fitzroy River in coastal Queensland, Australia (23°11'19.02"S, 150°24'10.13"E), to provide DNA for 454-sequencing. Total genomic DNA (gDNA) was extracted from skin biopsies (stored in 100% ethanol at -20 °C prior to use) using a DNeasy spin-column tissue extraction kit (Qiagen) according to manufacturer's instructions, including RNAse digestion. For each species, extractions from six individuals were pooled and concentrated to > 300 ng/µL gDNA. Quality and quantity of gDNA was assessed by spectrophotometric absorbance and electrophoresis through a 0.8% agarose gel.

Samples were barcoded to facilitate multiplexed sequencing of three pools (two turtle species plus a lobster species for an unrelated study) over a half plate of shotgun pyrosequencing, performed on Roche 454 GS-FLX instrumentation using Titanium chemistry at the Australian Genome Research Facility, Brisbane. Approximately 5 µg of gDNA per species was used in library preparation following Roche protocols. Pyrosequencing methodology is described in detail elsewhere (Margulies *et al.* 2005). Briefly, a single-stranded DNA library is constructed for each sample by randomly shearing gDNA and ligating short adapter sequences to the fragment ends. Individual fragments are isolated by binding to individual beads, which are then captured in droplets of emulsion oil and their associated fragments clonally amplified by emulsion PCR. Amplified fragments are denatured before beads are deposited into individual wells of a fibre optic slide, where fragments are sequenced using modified pyrosequencing technology (Ronaghi *et al.* 1996), which uses immobilised ATP sulphurylase and firefly luciferase to generate light from free pyrophosphate.

Prior to analysis, raw sequences were filtered using SEQCLEAN software (http://compbio.dfci.harvard.edu/tgi/software/) to remove poor-quality reads, reads < 60 bp, sequencing adapters, and poly A/T tails. To remove redundancy within the datasets and identify potential contiguous sequence regions, a *de novo* assembly was performed for each species separtely with repeat-masking in MIRA 3.2 (http://sourceforge.net/projects/mira-assembler/). In this way, a dataset containing assembled contigs plus all remaining singleton reads was

generated for each species, hereafter referred to as the non-redundant (NR) datasets. NR datasets were used in mitochondrial genome reconstruction and in functional and repetitive content analyses.

Microsatellite marker development

Microsatellite discovery and PCR primer design

Microsatellite discovery through to PCR primer design was completed using a published PERL script (QDD1, Meglécz *et al.* 2010) incorporating PRIMER3 software (Rozen & Skaletsky 2000) for primer design. Filtered unassembled sequence reads were screened for microsatellite repeats consisting of di- to hexabase motifs of at least 7, 6, 5, 5 and 5 repeats, respectively. Stringent parameters used in sequence sub-selection and primer design included the following restrictions: i) nanosatellite-free flanking regions of at least 20 nucleotides (nanosatellite being a monobase repetition of > 4 nucleotides or a di- to hexabase repetition > 2); ii) amplicon length 100-350 bp; iii) optimal primer GC content 50% (range 40-60%); iv) optimal primer melting temperature T_M 60 °C (range 57-63 °C) with maximum 3 °C difference between paired primers; v) optimal primer length 20 bp (range 18-27 bp); vi) at least 1 consecutive G/C at 3′ end of primers (GC clamp); and vii) low global alignment and self or pairwise complementarity scores. Perfect and compound/interrupted repeats were initially considered.

Primer pairs for 25 loci per species were synthesised for empirical testing. Loci with perfect repeats, greater numbers of repeat units, and low primer complementarity scores were prioritised. For each selected locus, sequence quality within primer sites was confirmed by aligning base calls with their corresponding quality scores (analogous to Phred scores) in an Excel spreadsheet. Basic local alignment search (BLAST) was used to identify potential redundancy and genomic multi-copies not removed by QDD1 (all-against-all search of loci with primers) and potential non-specific primer interactions (primer sequence against raw sequence database). Finally, online nucleotide BLAST (BLASTn) searches of each locus against the GenBank non-redundant database confirmed no associations with known gene regions or transposable elements, and no contamination of sequence data with human or microbial DNA.

Characterisation

Initial screening of 25 candidate loci per species for amplification success and specificity was performed using a cost-effective indirect fluorescent labelling technique (Shimizu *et al.* 2002), and eight individuals per species sampled from across their geographic range. Individual amplifications were performed using the Type-it microsatellite multiplex PCR kit (Qiagen) in 10 µL reactions, containing 20-50 ng template, 1x Type-it Master Mix (Qiagen) and 0.2 µM each primer (forward and reverse). Indirectly labelled reactions contained a tailed forward

primer and a reporter primer (5′ labelled with fluorescent dye modification HEX, TET or FAM) at a 1:4 ratio (total = 0.2 µM). PCR cycling conditions were as follows: initial 5 min denaturation at 95 °C, followed by 28 cycles of 95 °C for 30 sec (denaturation)/58 °C for 90 sec (annealing)/72 °C for 30 sec (extension), with a final extension 30 min at 60 °C. Loci exhibiting reliable amplification of a single product of expected size following visualisation by gel electrophoresis (1.5% agarose), were genotyped to assess polymorphism (n = 20 loci for *Ea*, 23 for *Ek*). PCR products were column purified through Sephadex G-50 resin and size separated on a MegaBACE 1000 capillary sequencer (Amersham Biosciences) using a 400 bp DNA ladder as internal size standard. Alleles were identified using FRAGMENT PROFILER 1.2 software (Amersham Biosciences).

Subsequently, directly-labelled forward primers (HEX, TET or FAM) were synthesised for up to 15 loci per species (n = 14 for *Ea*, 15 for *Ek*) that produced polymorphic and interpretable peak profiles, to allow downstream PCR multiplexing of up to six loci. Selected markers were characterised in the respective species in directly labelled singleplex reactions using 48 individuals sampled from the lower Fitzroy River (see above). Genomic DNA was extracted from tissues using a modified salting-out protocol (Sambrook & Russell 2001). Approximately 2 mm^3 of tissue was digested overnight at 55 °C in 500 µL lysis buffer (50 mM Tris, 20 mM EDTA, 400 mM NaCl, 0.5% SDS) containing 0.15 mg Proteinase K. DNA was salt-extracted and precipitated with 142 µL 5 M NaCl and 550 µL 100% EtOH, washed with 70% EtOH and re-suspended in 50 µL molecular grade water. PCR conditions were as described above, except that primer concentration for locus Ekref10 was increased three-fold (0.6 µM). Multiplex PCR combinations were designed *in silico*, using information on allele size ranges and dye labelling, with the aid of MULTIPLEX MANAGER 1.0 software (Holleley & Geerts 2009), and subsequently tested under identical PCR conditions.

Basic summary statistics for each locus (number of alleles, observed and expected heterozygosities) were calculated in GENALEX 6.4 (Peakall & Smouse 2006), which was also used to test for deviations from Hardy-Weinberg expectations (HWE). Polymorphic information content (PIC) was calculated in CERVUS 3.0 (Kalinowski *et al.* 2007) and potential linkage disequilibrium between pairs of loci was investigated using GENEPOP 4.0.10 (Rousset 2008). MICRO-CHECKER 2.2.3 (van Oosterhout *et al.* 2004) was used to screen for potential null alleles and scoring error.

Microsatellite markers were also tested for amplification success and polymorphism in five to eight individuals from a range of other Australian short-necked turtle taxa. Markers developed in *E. albagula* were tested in congeneric species *E. irwini* (Burdekin R and Johnstone R populations) and *E. lavarackorum*. Markers developed in *E. m. krefftii* were tested in related

sub-species *E. m. macquarii*, *E. m. emmotti* and *E. m. nigra*, as well as congeneric species *E. subglobosa worrelli*, *E. tanybaraga* and *E. victoriae*. All markers were tested in *Elusor macrurus*, *Rheodytes leukops* and *Myuchelys latisternum*, and were also tested between the two focal species. Positive controls containing DNA from the original species were included in each experiment.

Further exploration of 454 data

Mitochondrial genome reconstruction

As the mitochondrial genome occurs in high copy-number per cell and is co-purified during gDNA extraction, a proportion of sequence reads can be assumed to be of mitochondrial origin. To identify such reads, sequence similarity searches (stand-alone BLASTn) were performed for each NR dataset against the only complete mitochondrial genome sequence then available for pleurodire turtles, that of the pelomedusid, *Pelomedusa subrufa* (GenBank ref. AF039066). Complete mitochondrial genomes have since become available for a South American and an Australian snake-neck chelid (Wang *et al.* 2012). Sequences returning a significant hit to the *P. subrufa* genome were manually aligned in BIOEDIT 7.0.5 (available from http://www.mbio.ncsu.edu/bioedit/bioedit.html) to create a consensus sequence for each species, using the *P. subrufa* genome as a reference. The mitochondrial origin of identified fragments was confirmed via online BLASTn against the GenBank non-redundant database.

Functional (gene) content

Potential functional content within the NR datasets was investigated using the Gene Ontology interface, Blast2GO (Conesa *et al.* 2005). Gene Ontology (GO) provides a standardised categorisation system for gene products based on three ontologies: biological process, cellular component, and molecular function. Blast2GO uses sequence similarity (BLAST) searches to find homologous sequences in published databases, and extracts GO terms associated with each hit before returning an evaluated GO annotation for each query sequence. In this way, consistent gene annotations are assigned to a set of sequences. Here, as sequence data is genomic in origin, and therefore contains functional sequence interspersed with non-functional introns, Blast2GO was run using the BLASTx option. This translates nucleotide sequences into protein for comparison against the (more conservative) GenBank protein databases. An E-value cut-off of E-6 was specified, and due to the large volume of sequence data, only large contigs (> 500 bp) were queried (n = 2,284 for *Ea*; n = 3,063 for *Ek*).

To investigate sequence content further, including non-functional sequence, the Blast2GO platform was also used to query large contigs directly against the GenBank non-redundant nucleotide database (BLASTn).

Repetitive elements

To characterise the repetitive content of the sequence data, each NR dataset was screened for interspersed repeats and low complexity regions using the protein similarity option in REPEATMASKER 3.3 (available from http://www.repeatmasker.org). Sequences are compared to a (conserved) database of transposable element encoded proteins, making this approach more suitable for datasets from non-model species for which no reference repeat library is available. However, copies of non-coding transposable elements like SINEs and long terminal repeats of retroviral-like elements are not detected with this approach.

RESULTS

454-sequencing and *de novo* assembly

A half plate of 454-sequencing with three pools produced 365,937 total sequence reads. Summary data for the two turtle species are presented and compared with similar published studies (years 2009-2010) in Table 2.1. Post clean-up, slightly more genomic information was obtained for *Ek*: 46.2 Mbp of DNA sequence data (135,332 sequence reads, 342 bp average length, range 60-1160 bp), compared to *Ea*: 36.6 Mbp (108,198 reads, 339 bp average length, range 60-769 bp). This sequencing effort is equivalent to approximately 1.3-1.6% genome coverage per species (assuming an average chelonian haploid genome of 2836.2 Mbp, http://www.genomesize.com). GC content of each dataset was 43% and within the range commonly reported for reptiles. Raw sequence reads have been deposited into NCBI's Sequence Read Archive (Accession number SRR899958 for *E. albagula* and SRR899968 for *E. m. krefftii*).

De novo assembly statistics for *Ek* and *Ea* datasets are presented in Table 2.2. Assembly of the *Ek* dataset produced 10,593 contigs of mean length 480.5 bp, range 102-11,247 bp. Reads longer than 500 bp numbered 3,063 (28.9%). The *Ea* assembly produced 7,879 contigs of mean length 487.0 bp, range 101-4,464 bp. There were 2,284 (29.0%) contigs > 500 bp. The NR datasets for *Ek* and *Ea* respectively, contained 120,853 sequences or 42.2 Mbp, and 97,664 sequences or 33.8 Mbp. Table 2.3 summarises proportions of non-redundant, mitochondrial and identified coding and repetitive sequence reads (see later section) contained in the overall dataset of each species.

Table 2.1 Results summary for studies reporting on the identification of microsatellite loci from 454-sequence data, published 2009-2010, including the current study.

Study	Organism	Run size	Mbp	No. reads	Av. length (bp)	Microsatellite search parameters	% reads with repeats	% loci with primers	Dominant motif
Current study	Freshwater turtles					di-hexamer[4]			
	(*Emydura m. krefftii*)	1/6 plate[1]	46.2	135,332	342	≥ 7, 6, 5, 5, 5 repeats	1.3%	20.6%	Di (>70%)
	(*Elseya albagula*)	1/6 plate[1]	36.6	108,198	339		1.4%	17.3%	Di (>70%)
Castoe *et al.* (2010)	Copperhead snake (*Agkistrodon contortrix*)	≥ 3/8 plate	26.9	128,773	215	di-tetramer ≥ 6, 4, 3 repeats	11.3%	31.2%	Tetra (52.6%)
Csencsics *et al.* (2010)	Dwarf bulrush (*Typha minima*)	1/16 plate[1]	26.2	76,692	341	di-tetramer ≥ 8, 10, 6 repeats	0.4%	32.6%	Tri (53.1%)
Perry & Rowe (2010)	Water strider (*Gerris incognitus*)	1/4 plate[1]	61.5	182,912	369	di-decamer[4] ≥ 5 repeats	16.8%	nr	Di (88.5%)
Saarinen & Austin (2010)	Okaloosa darter (*Etheostoma okaloosae*)	1/8 plate[1]	29.0	82,463	352	di-tetramer ≥ 6, 4, 4 repeats	9.0%	21.6%	Di (71.7%)
Abdelkrim *et al.* (2009)	Blue duck (*Hymenolaimus malacorhynchos*)	1/16 plate	4.1	17,215	243	di-tetramer ≥ 8 repeats	1.3%	10.4%	Tri (46.3%)
Allentoft *et al.* (2009)	Heavy-footed moa[2] (*Pachyornis elephantopus*)	1/4 plate	nr	79,796	112	di-tetramer nr	0.2%	3.6%	nr
Rasmussen & Noor (2009)	Scuttle fly (*Megaselia scalaris*)	1/4 plate	nr	129,080	231	di-trimer ≥ 10 repeats	nr	nr	nr
Vanpe *et al.* (2009)	Short-beaked echidna (*Tachyglossus aculeatus*)	12 runs[3]	nr	885,433	nr	di-tetramer ≥ 7 repeats	0.84%	58.6%	Di (81.1%)
Tangphatsornruang *et al.* (2009)	Mungbean (*Vigna radiate*)	nr	100.5	470,024	216	di-octamer ≥ 10, 7, 5, 4, 4, 4, 4 repeats	0.31%	nr	Di (59.5%)

[1]Study employed newer 'Titanium' sequencing chemistry. [2]Ancient DNA from an extinct species used for sequencing. [3]Study accessed existing 454-sequence data from GenBank. [4]Search included interrupted and compound repeats. nr, not reported.

Table 2.2 Summary statistics for *de novo* sequence assembly of 454 reads from *Emydura macquarii krefftii* (*Ek*) and *Elseya albagula* (*Ea*).

	Assembly statistics				***Contig statistics***		
	No. of contigs (no. > 500 bp)	**No. of reads assembled (%)**	**Singleton reads**	**Non-redundant dataset (Mbp)**	**Length range (bp)**	**Mean length (bp)**	**Reads per contig (mean)**
Ek	10,593	25,072	110,260	42.2	102-11,247	480.5	2-232
	(3,063)	(18.5)					(2.4)
Ea	7,879	18,413	89,785	33.8	101-4,464	478.0	2-65
	(2,284)	(17.0)					(2.3)

Table 2.3 Number and proportion of different types of sequence reads within 454-sequence datasets of *Emydura macquarii krefftii* (*Ek*) and *Elseya albagula* (*Ea*).

	Ek		***Ea***	
Sequence type	**No. of reads**	**%**	**No. of reads**	**%**
All reads	135,332	100	108,198	100
Non-redundant sequences	120,853	89.30	97,664	90.26
Predicted repetitive sequences	12,361	9.13	9,094	8.40
Predicted coding sequences[1]	1,445	1.07	1,141	1.05
Mitochondrial sequences	75	0.06	67	0.06

[1]Refers to the number of sequences contained in large contigs (> 500 bp) that could be assigned a functional GO annotation in the Blast2GO analysis and will be an underestimate of true functional content because only large contigs were screened and because some genes will not have homologs in the databases.

Microsatellite development

Marker discovery and PCR primer design

For *Ek*, 1,955 microsatellite loci matching the search criteria were isolated from 1,824 repeat-containing sequences. Slightly fewer microsatellites were returned for *Ea*: 1,590 loci within 1,484 repeat-containing sequences. However, the proportion of total sequences with repeats was comparable between species, being 1.3% and 1.4% respectively. Representation by each motif class is shown in Fig. 2.1 and was similar between species, with dinucleotide motifs dominating (> 70%, both species). Among motif classes (Fig. 2.2), AC repeats were the most common dinucleotide motif, AAT repeats were the most common trinucleotide motif, and AAAC, AAAG, AAAT and AGAT were the most commonly observed tetranucleotide motifs. Penta- and hexanucleotides were sporadically represented, with the exception of 45 instances of an AACCCC repeat in *Ek*.

PCR primer pairs could be designed for 261 perfect and 114 compound/interrupted *Ek* repeats, and 175 perfect and 82 compound/interrupted *Ea* repeats. Loci with primers represented 20.6% (*Ek*) and 17.3% (*Ea*) of sequences containing microsatellites and 0.3% (*Ek*) and 0.2% (*Ea*) of total sequence reads (Fig. 2.1). Perfect microsatellite repeats with primers represented 14.3% (*Ek*) and 11.8% (*Ea*) of microsatellite-containing sequences (Table 2.4). Although the majority of perfect loci with primers designed contained dinucleotide repeats, trinucleotides had the greatest proportion of primers designed, followed by tetra- and pentanucleotides, then dinucleotides, and hexanucleotides (Table 2.4, see also Fig. 2.1). Appendix 2A contains details of primer pairs designed for all perfect microsatellite repeats, including a unique sequence ID for each read, repeat motif, number of tandem repeats, forward and reverse primer sequences (with unique names) and whether or not the primers have been empirically tested.

Microsatellite characterisation

Of the 25 loci empirically tested in each species, 16 *Ea* loci and 19 *Ek* loci produced interpretable peak profiles. However, two *Ea* loci were monomorphic, and were not considered further. Characteristics of the 29 loci developed for population genetic analyses (14 in *Ea*, 15 in *Ek*), are summarised in Table 2.5, including multiplex PCR combinations. All loci were found to be in HWE and no significant linkage disequilibrium was detected after FDR correction (Benjamini & Hochberg 1995). However, null alleles were suggested at locus Ealb07, Ekref09 and Ekref15 due to homozygote excess. Evidence of an insertion/deletion within the flanking region of Ekref13 was detected in the form of two alleles differing by a single base pair.

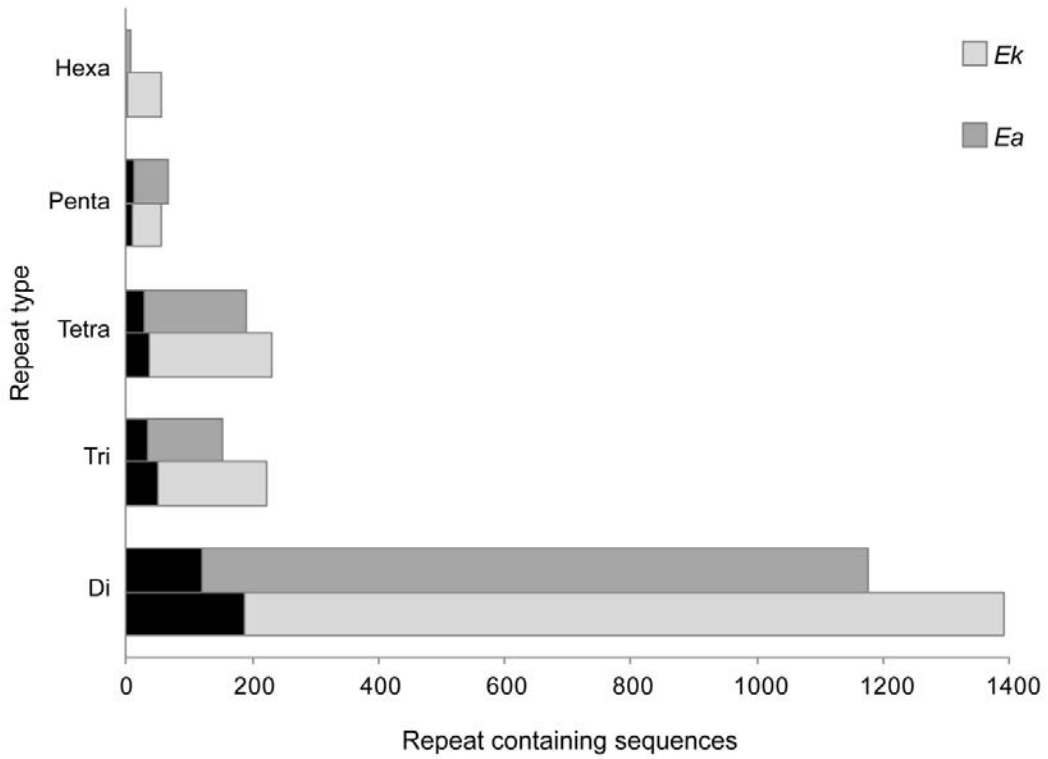

Fig. 2.1 Numbers of identified microsatellite loci (grey), and the proportion of loci suitable for PCR primer design (black), in 454-sequence reads randomly sampled from the genomes of two freshwater turtle species, *Emydura macquarii krefftii* (n = 135,332 reads) and *Elseya albagula* (n = 108,198 reads).

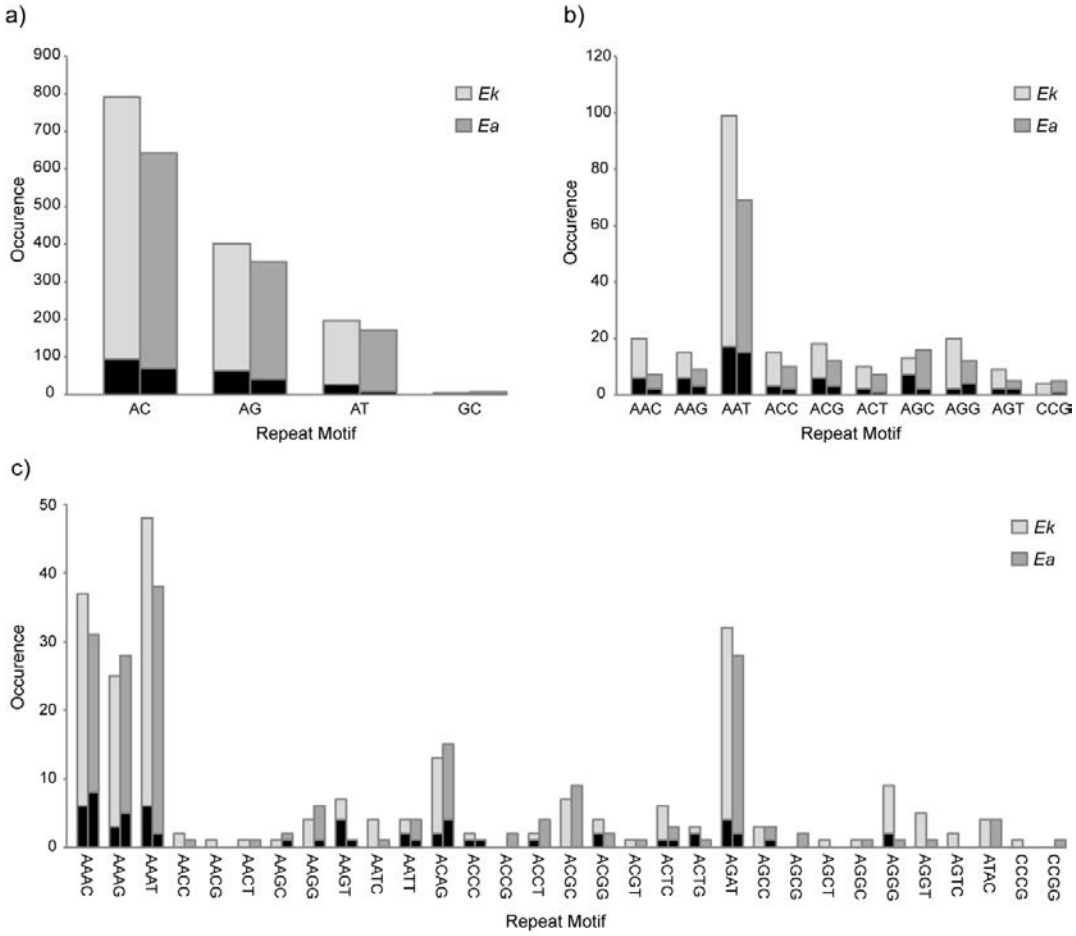

Fig. 2.2 Observed counts of identified microsatellite loci (grey), and the proportion of loci suitable for PCR primer design (black), for different repeat motifs of a) dinucleotide repeats, b) trinucleotide repeats and c) tetranucleotide repeats.

Table 2.4 Numbers of microsatellites identified, and those with primers designed, for perfect repeats isolated from 454-sequence data obtained from two turtle species, *Emydura m. krefftii* (*Ek*) and *Elseya albagula* (*Ea*).

	No. repeats	**Repeat-containing sequences**		**Perfect loci with primers**		**% perfect loci with primers**	
		Ek	*Ea*	*Ek*	*Ea*	*Ek*	*Ea*
Dinucleotide	All	1392	1175	168	104	12.1	8.8
	(≥ 7)	505	443	39	32	7.7	7.2
	≥ 10	74	31	0	0	-	-
	≥ 20						
Trinucleotide	All	223	152	46	31	20.6	20.4
	(≥ 6)	20	8	3	1	15	12.5
	≥ 10	1	0	0	0	-	-
	≥ 20						
Tetranucleotide	All	230	189	36	28	15.6	14.8
	(≥ 5)	3	12	0	0	-	-
	≥ 10	0	0	0	0	-	-
	≥ 20						
Pentanucleotide	All	55	66	9	12	16.4	18.2
	(≥ 5)	7	3	1	0	14.3	-
	≥ 10	0	0	0	0	-	-
	≥ 20						
Hexanucleotide	All	55	8	2	0	3.6	-
	(≥ 5)	10	1	0	0	-	-
	≥ 10	1	0	0	0	-	-
	≥ 20						
TOTAL		**1955**	**1590**	**261**	**175**	**13.3**	**11.0**

Table 2.5 Details of 14 *Elseya albagula* and 15 *Emydura m. krefftii* microsatellite loci developed from 454 shotgun sequence data.

Locus	Primer sequence 5′ to 3′	Repeat motif	Primer conc. (µM)	T_A (°C)	N_A	Allele size range (bp)	H_O	H_E	*p* HWE	PIC	GenBank no.
Elseya albagula											
Ealb02[aB]	F: [6FAM]GTTAATTCTTTCCAAGCCTGC R: AAACAATAGGCCTAATCACAG	$(TAAAA)_7$	0.2	58	5	87-106[1]	0.729	0.639	0.077	0.586	HQ690746
Ealb05[aB]	F: [HEX]CCAGCACATTTGTTCGTTC R: TCTCATTCGATTTACAAGAGAC	$(TTTC)_8$	0.2	58	7	82-119[1]	0.792	0.755	0.150	0.715	HQ690747
Ealb06[aC]	F: [HEX]GTATGAGCCATGATCCCATTG R: CCCTGTTTAGTGCATCTCCC	$(CAAA)_7$	0.2	58	2	100-104	0.354	0.317	0.220	0.267	HQ690748
Ealb07[aC]	F: [6FAM]TGAATGATAACAGATGTCTGGC R: AGCATGAGATTCGTGTCTGG	$(ATAG)_8$	0.2	58	4	216-232	0.563	0.731	0.424	0.682	HQ690749
Ealb09[aA]	F: [TET]ATAGAACTGACCCTTGATGCG R: CTCCTCTGCCCAGCTAACAC	$(ATT)_{10}$	0.2	58	4	174-183	0.625	0.592	0.442	0.518	HQ690750
Ealb10[aA]	F: [HEX]CTCATCAATGGTGTGGTTCAC R: CAGCAGAGTGGCCTTTACTACC	$(AGG)_9$	0.2	58	3	198-204	0.553	0.520	0.476	0.451	HQ690751
Ealb14[aC]	F: [TET]CACTTCCAGAATCCTCTGCC R: TGATAGTGGATGACTTCAGGG	$(TTC)_8$	0.2	58	3	124-130	0.396	0.393	0.553	0.358	HQ690752
Ealb15[aA]	F: [6FAM]GGTTCACTGATGTTGTTGAAACTG R: TGGTTCCCATTGCCTAAGAG	$(AC)_{15}$	0.2	58	5	170-182	0.542	0.606	0.562	0.536	HQ690753
Ealb17[aA]	F: [HEX]GGGCGATGTAGTACGTGTGG R: TGTGTACTTCTTGTAGGGTTAAAGAGC	$(AC)_{12}$	0.2	58	3	98-102	0.521	0.463	0.707	0.413	HQ690754
Ealb18[aA]	F: [6FAM]TCCATTCTTCTTTGTGAACCG R: GACCTGGCGTCGTTGTATG	$(AC)_{12}$	0.2	58	8	136-150	0.854	0.828	0.822	0.804	HQ690755
Ealb19[aC]	F: [6FAM]TCCCTCTGCACAAAGTGCC R: GTGCTAGGAACTGCCTGTGG	$(GA)_{11}$	0.2	58	2	89-99	0.271	0.342	0.872	0.283	HQ690756
Ealb20[aA]	F: [TET]AGCCACTGGAGGTGATTGTC R: CCACTGAGACACCATTGAGC	$(CT)_{11}$	0.2	58	5	113-125	0.563	0.553	0.903	0.457	HQ690757
Ealb24[aA]	F: [HEX]CTGGAATTTGATCCAGAGTTTGC R: ACTGTACCAGAAGCACTCC	$(ATT)_5G(TAA)_{10}$	0.2	58	5	212-239	0.625	0.571	0.965	0.517	HQ690758
Ealb25[aA]	F: [TET]ACAAGATGTCCCTCACCCTG R: AGAAATCTCAGCTTTGAGCCC	$(CT)_5(CA)_{10}$	0.2	58	5	141-149	0.583	0.634	0.968	0.588	HQ690759
Emydura macquarii krefftii											
Ekref04[kB]	F: [HEX]CCTGATTTATTTCCTACGCTCAG R: GGTGACAAGGTTGGTACAAGAAC	$(TTCTA)_8$	0.2	58	7	146-176	0.854	0.807	0.030	0.780	HQ690760

Locus	Primer sequence 5′ to 3′	Repeat motif	Primer conc. (µM)	T_A (°C)	N_A	Allele size range (bp)	H_O	H_E	*p* HWE	PIC	GenBank no.
Ekref06[kD]	F: [HEX]GCCCTGTCTTTCCAATTCAG R: GCCAGTTTCTATGTTTGCAGC	$(GTTT)_9$	0.2	58	3	141-154[1]	0.191	0.177	0.193	0.169	HQ690761
Ekref07[kC]	F: [TET]AGCACTGTTAAGACTTCCTACG R: TGGAGGCATCCTTGTGACC	$(AGAT)_8$	0.2	58	9	328-368	0.813	0.753	0.241	0.727	HQ690762
Ekref08[kC]	F: [HEX]GTTCTGGGTGAGGGTGTGG R: TGTCCCAAAGAACAAGGCTC	$(TGCC)_7$	0.2	58	8	166-194	0.833	0.773	0.472	0.739	HQ690763
Ekref09[kD]	F: [6FAM]TGGCCTATCCTAGAGGAGGTG R: GAGTCATCCCATGTTCCAATTC	$(GGAG)_7$	0.2	58	5	139-155	0.563	0.696	0.657	0.644	HQ690764
Ekref10[kC]	F: [TET]ATGCTGCTGAAGCAGGTGTC R: ATGCTCGTTGAGGCTGTAGG	$(CAG)_{13}$	0.6	58	12	141-185[1]	0.875	0.875	0.684	0.862	HQ690765
Ekref12[kD]	F: [TET]AGGCCACCCAGTTTACACC R: TCTTTCAATGAGCTCCACCTG	$(ATT)_{11}$	0.2	58	8	161-189[1]	0.792	0.768	0.730	0.735	HQ690766
Ekref13[kA]	F: [HEX]GCTGAATGGCAATGTAACCC R: GCATTTCAAAGAGACTGCCC	$(TAA)_{10}$	0.2	58	4	120-129[2]	0.660	0.649	0.739	0.594	HQ690767
Ekref14[kA]	F: [TET]AGAGCCTAGAAGGAATGGGC R: CAAGGGAAGTGAAACAGTGG	$(GCT)_9$	0.2	58	6	94-109	0.830	0.828	0.741	0.804	HQ690768
Ekref15[kD]	F: [HEX]GCTTTCTCAGACGGGAGGC R: AGGGCAGATAGCTACCACAG	$(AAT)_9$	0.2	58	6	276-291	0.478	0.638	0.815	0.566	HQ690769
Ekref17[kB]	F: [6FAM]TTCAAATGCACCTTCACTGC R: CGCAGTCACACTCTCACACC	$(CA)_{15}$	0.2	58	15	132-162	0.813	0.857	0.824	0.843	HQ690770
Ekref18[kC]	F: [6FAM]GAGCATTCATGCGTGGAAC R: GCAGAGAAATGAGGAAAGGATG	$(GT)_{13}$	0.2	58	7	153-167	0.729	0.759	0.913	0.722	HQ690771
Ekref20[kB]	F: [TET]AATTGTCAGTGCAGAAGGGTG R: ACAAAGGACACAGTCCCTGC	$(AC)_{12}$	0.2	58	9	133-156[1]	0.804	0.744	0.941	0.708	HQ690772
Ekref21[kA]	F: [6FAM]GAGTGTTCCGCAGCATATTG R: TGAAGGATGCACAACCCAC	$(GT)_{11}$	0.2	58	6	154-170	0.638	0.616	0.971	0.579	HQ690773
Ekref22[kC]	F: [HEX]AGAGAAGTGGCTTCGGTGTG R: GCTGAAAGATGAGGTGTGGG	$(CA)_{11}$	0.2	58	10	326-354	0.787	0.822	0.976	0.802	HQ690774

[a A,B,C] *E. albagula* multiplex, [k A,B,C,D] *E. m. krefftii* multiplex. T_A, annealing temperature; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *p* HWE, Hardy-Weinberg Equilibrium significance value after FDR correction; PIC, polymorphic information content. [1]Locus exhibits slight allele size compression/expansion in parts of size range. [2]Locus has allele of unexpected length (121 bp) due to suspected insertion/deletion.

Ek markers had higher allelic richness (mean N_A = 7.7 ± 0.80, range 3-15) than *Ea* markers (mean N_A = 4.4 ± 0.46, range 2-8). However, levels of expected heterozygosity were high for both marker suites (*Ek* mean H_E = 0.718 ± 0.044, range 0.177-0.875; *Ea* mean H_E = 0.567 ± 0.040, range 0.317-0.827), indicating both will be useful for studies of population genetic structure, connectivity and mating systems in these taxa.

Details of cross-species amplification of the 29 markers are presented in Tables 2.6 and 2.7 for the *Ea* and *Ek* marker suites, respectively. Allele lengths were typically very similar across species and comparable with those reported in the original taxa. Cross-amplification success was high for both marker suites, although levels of polymorphism were variable. Transferability was highest for the *Ek* markers among the six *Emydura* species tested (9-14 of 15 loci were polymorphic), indicating these markers are likely to be useful for population-level studies across the genus *Emydura*. Conversely, transferability of *Ea* markers was low among the *Elseya* species tested (4-5 of 14 loci were polymorphic), suggesting they may have limited utility for population studies in congeneric species. Although *Ea* markers transferred well within *Ek* (10/14 loci polymorphic), the inverse was less successful (4/15 loci polymorphic). Between the two marker suites, several loci amplified successfully and were polymorphic in *Elusor macrurus* (19/29 loci polymorphic) and *Rheodytes leukops* (10/29 loci polymorphic). Only six of the 29 loci were polymorphic in *Myuchelys latisternum*.

Further exploration of 454 data

Mitochondrial genome assembly

Based on alignment with *P. subrufa*, over 70% of the coding portion of the mitochondrial genome was recovered in each species, plus 880 bp of the *Ek* control region. A further 820 bp of the *Ea* control region was identified through the Blast2GO BLASTn analysis of large contigs (see below). Overall, 75 *Ek* reads and 67 *Ea* reads were found to be mitochondrial in origin, accounting for 0.06% of total sequence reads in each species (Table 2.2). The majority of these were assembled within contigs. In total, a 12.2 kb consensus and an 11.3 kb consensus sequence was generated for the mitochondrial genomes of *Ek* and *Ea*, respectively. These are presented, with reference to the *P. subrufa* mitochondrial genome, in Fig. 2.3 and 2.4. Gaps remaining in the assembly were all within the realms of conventional PCR. Sequencing of these gaps has since enabled full coverage of both genomes and an annotated genome sequence for each species is being prepared for publication (E. V. Todd *et al*. unpubl. data). Sequence information for reads and contigs of mitochondrial origin is presented in Appendix 2B.

Table 2.6 Cross-species amplification success of 14 microsatellite markers developed for *Elseya albagula* from 454-sequence data.

Locus	Success	N_A	Size range	Success	N_A	Size range	Success	N_A	Size range	Success	N_A	Size range
	Elseya irwini			*Elseya irwini* [Johnstone R]			*Elseya lavarackorum*			*Emydura m. krefftii*		
Ealb02	8/8	1	109	8/8	2	114-119	8/8	1	109	8/8	3	94-104
Ealb05	8/8	1	102	8/8	1	102	8/8	1	102	8/8	5	106-127[1]
Ealb06	8/8	1	122	8/8	1	122	8/8	1	115	8/8	4	110-126
Ealb07	8/8	1	234	8/8	1	242	8/8	1	234	8/8	6	230-258
Ealb09	8/8	1	194	8/8	2	194-197	8/8	1	195	8/8	1	182
Ealb10	8/8	1	215	8/8	1	215	8/8	1	215	8/8	2	209-215
Ealb14	8/8	1	141	8/8	1	141	8/8	1	141	8/8	1	138
Ealb15	8/8	3	203-209	8/8	2	191-193	8/8	2	189-197	8/8	1	181
Ealb17	8/8	1	109	8/8	1	109	8/8	1	109	8/8	2	111
Ealb18	8/8	3	169-179	8/8	6	159-175	8/8	6	150-168	0/8	-	-
Ealb19	8/8	2	108-116	8/8	1	116	8/8	2	116-120	8/8	3	119-125
Ealb20	8/8	1	123	8/8	1	123	8/8	2	117-123	8/8	2	109-119
Ealb24	8/8	2	226-232	8/8	3	226-232	8/8	3	229-241	8/8	3	224-235[1]
Ealb25	0/8	-	-	0/8	-	-	0/8	-	-	8/8	3	150-156
	Elusor macrurus			*Rheodytes leukops*			*Myuchelys latisternum*					
Ealb02	6/6	1	99	8/8	1	95	8/8	2	99-104			
Ealb05	0/6	-	-	8/8	13	127-292[1]	0/8	-	-			
Ealb06	6/6	2	110-114	8/8	1	122	8/8	2	114-118			
Ealb07	6/6	2	229-241	8/8	5	230-254	0/8	-	-			
Ealb09	6/6	2	182-189[1]	8/8	1	186	8/8	2	183-186			
Ealb10	6/6	1	215	8/8	1	221	8/8	1	215			
Ealb14	6/6	2	138-144	8/8	1	138	8/8	1	135			
Ealb15	6/6	2	170-182	8/8	3	185-189	8/8	1	181			
Ealb17	6/6	2	109-111	8/8	1	110	8/8	1	109			
Ealb18	6/6	1	150	0/8	-	-	8/8	1	149			
Ealb19	6/6	3	114-118	8/8	1	112	0/8	-	-			
Ealb20	6/6	2	119-121	8/8	2	119-121	8/8	1	123			
Ealb24	6/6	3	223-238	0/8	-	-	8/8	1	213			
Ealb25	6/6	2	148-150	8/8	1	148	8/8	1	148			

N_A, number of alleles. [1]Locus exhibits slight allele size compression/expansion across range. Species designations follow Georges and Thomson (2010).

Table 2.7 Cross-species amplification success of 15 microsatellite markers developed for *Emydura macquarii krefftii* from 454-sequence data.

Locus	Success	N_A	Size range	Success	N_A	Size range	Success	N_A	Size range	Success	N_A	Size range	Success	N_A	Size range
	Emydura m. emmotti			*Emydura m. macquarii*			*Emydura m. nigra*			*Emydura subglobosa worrelli*			*Emydura tanybaraga*		
Ekref04	8/8	3	162-172	8/8	3	173-183	8/8	6	162-188[1]	8/8	3	152-167	4/5	1	157
Ekref06	8/8	1	158	8/8	5	158-179[1]	8/8	3	154-162	8/8	3	163-184[1]	5/5	2	154-157[3]
Ekref07	8/8	1	345	8/8	1	345	8/8	1	345	8/8	1	345	5/5	1	345
Ekref08	8/8	5	187-215	8/8	4	187-203	8/8	4	187-199	8/8	2	193-194[2]	5/5	2	193-194[2]
Ekref09	8/8	3	157-165	8/8	2	157-161[1]	8/8	4	156-169[1]	8/8	1	165	5/5	1	165
Ekref10	8/8	4	167-187[1]	8/8	8	150-182[1]	8/8	6	170-187[1]	8/8	1	153	5/5	3	153-159
Ekref12	8/8	1	190	8/8	5	184-199[1]	8/8	3	184-196	8/8	2	184-187	5/5	2	178-190
Ekref13	8/8	1	141	8/8	2	144-147	8/8	3	142-148	8/8	3	141-147	5/5	2	141-144
Ekref14	8/8	1	111	8/8	2	108-117	8/8	1	111	8/8	1	111	5/5	1	115
Ekref15	8/8	1	298	8/8	2	292-298	8/8	3	295-301	8/8	1	293	5/5	2	299-301[3]
Ekref17	8/8	5	177-187	8/8	8	160-183[1]	8/8	4	156-181[1]	8/8	7	164-191[1]	5/5	2	182-186
Ekref18	8/8	3	177-190[1]	8/8	3	177-181	8/8	3	177-183	8/8	1	173	5/5	1	171
Ekref20	8/8	2	155-169	8/8	4	145-157	8/8	4	149-159	8/8	3	157-161	5/5	2	163-171
Ekref21	8/8	2	178-186	8/8	5	180-200	8/8	3	178-190	8/8	5	180-199[1]	5/5	3	184-194
Ekref22	8/8	3	354-364	8/8	6	350-361[1]	8/8	3	352-357[2]	8/8	5	355-365	5/5	4	348-360
	Emydura victoriae			*Elusor macrurus*			*Rheodytes leukops*			*Myuchelys latisternum*			*Elseya albagula*		
Ekref04	6/6	2	162-172	6/6	3	173-183	8/8	5	167-188[1]	8/8	4	156-172[1]	8/8	1	167
Ekref06	6/6	3	167-179	1/6	1	162	0/8	-	-	8/8	1	146	8/8	2	150-154
Ekref07	6/6	1	345	6/6	2	345-357	8/8	8	353-397[1]	8/8	2	357-364[1]	8/8	3	361-373
Ekref08	6/6	4	194-214	6/6	3	189-199[3]	8/8	1	196	0/8	-	-	8/8	1	176
Ekref09	6/6	1	165	1/6	1	169	8/8	1	160	8/8	1	165	8/8	1	171
Ekref10	6/6	3	153-173[1]	6/6	2	147-167[3]	8/8	1	147	8/8	1	156	8/8	1	150
Ekref12	6/6	2	187-190	6/6	1	183	8/8	4	193-211	8/8	1	177	8/8	2	183-189
Ekref13	6/6	5	141-153	6/6	2	141-144	8/8	2	128-144[1]	8/8	1	132	8/8	1	138
Ekref14	6/6	4	124-133	6/6	2	108-111	8/8	1	105	8/8	1	105	8/8	1	105
Ekref15	6/6	2	298-307	6/6	4	289-301	8/8	2	292-295	8/8	5	295-313	8/8	1	292
Ekref17	6/6	5	178-197[1]	1/6	1	156	8/8	14	183-290[1]	8/8	1	149	8/8	8	156-185[1]
Ekref18	6/6	2	173-177	6/6	5	171-187	8/8	1	175	0/8	-	-	8/8	1	177
Ekref20	6/6	1	146	6/6	4	153-201	8/8	1	173	8/8	-	Many peaks	8/8	1	114
Ekref21	6/6	2	188-190	6/6	1	179	0/8	-	-	0/8	-	-	0/8	-	-
Ekref22	6/6	3	352-370	1/6	1	357	8/8	1	319	0/8	-	-	0/8	-	-

N_A, number of alleles. [1]Locus exhibits slight allele size compression/expansion across range. [2]Locus has allele of unexpected length (121 bp) due to expected insertion/deletion. [3]Allele spacing does not match motif size in original species. Species designations follow Georges and Thomson (2010).

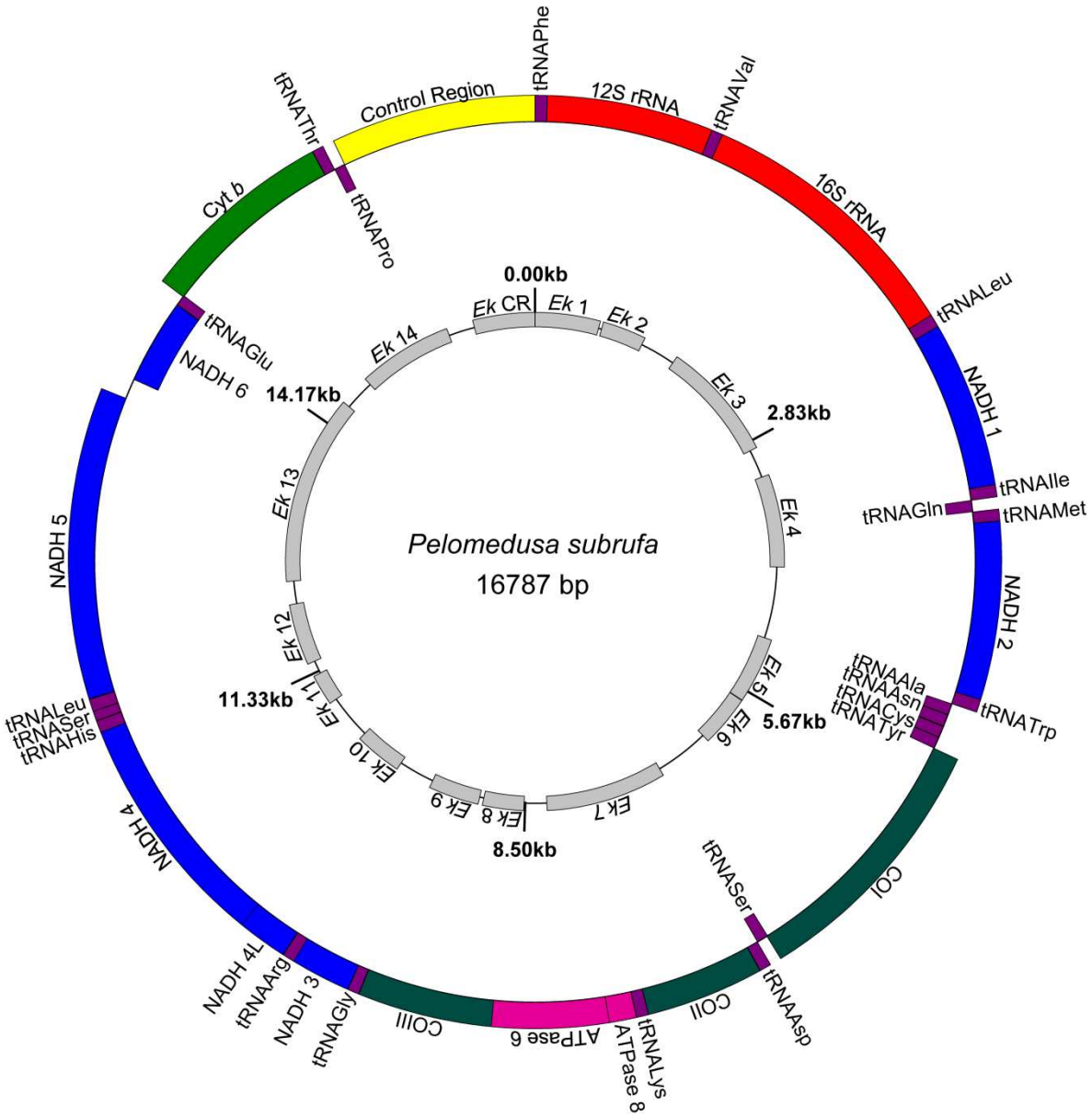

Fig. 2.3 Mitochondrial genome map of *Pelomedusa subrufa* (outer ring), indicating the position and coverage of mitochondrial sequence fragments identified within the *Emydura macquarii krefftii* 454-sequence set (inner ring). Location of genes, ribosomal RNAs (rRNA), and transfer RNAs (tRNA) are indicated for the *P. subrufa* genome according to GenBank record AF039066. Inner and outer annotations represent light and heavy strand orientation, respectively.

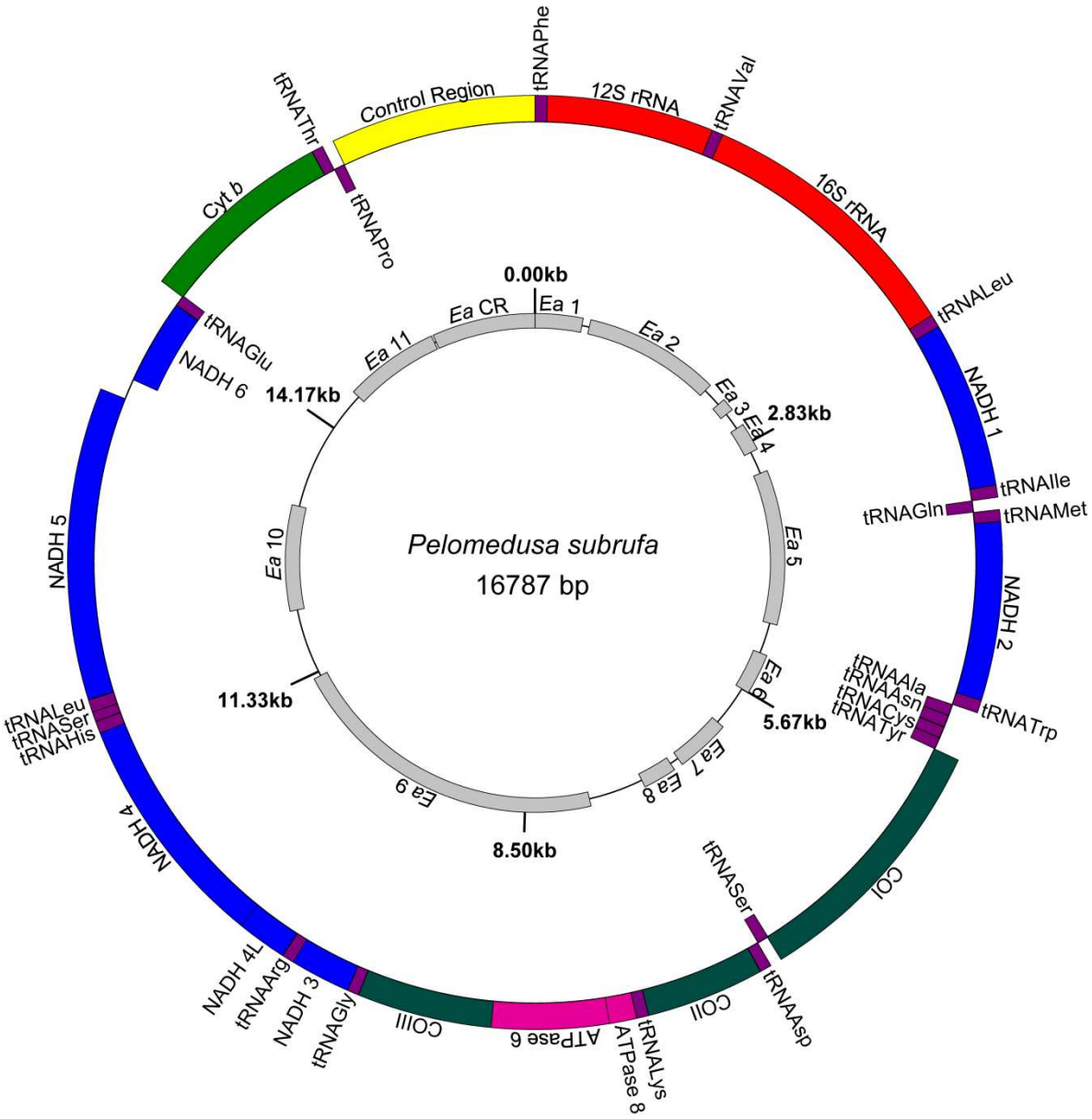

Fig. 2.4 Mitochondrial genome map of *Pelomedusa subrufa* (outer ring), indicating the position and coverage of mitochondrial sequence fragments identified within the *Elseya albagula* 454-sequence set (inner ring). Location of genes, ribosomal RNAs (rRNA), and transfer RNAs (tRNA) are indicated for the *P. subrufa* genome according to GenBank record AF039066. Inner and outer annotations represent light and heavy strand orientation, respectively.

Analysis of large contigs

Functional sequence homology searches (BLASTx) using large (> 500 bp) contigs returned 905 *Ek* and 721 *Ea* contigs with significant matches to the GenBank protein database. Of these, 342 (*Ea*) and 488 (*Ek*) could be assigned a GO annotation. However, across the three ontologies, only a small proportion of hits represented unique genes: 114 (13%) for *Ek* and 73 (10%) for *Ea* (Appendix 2C).

Querying large contigs against the GenBank non-redundant database (BLASTn) returned 301 hits for *Ea* and 393 hits for *Ek*. Most common hits were to various coding sequence and messenger RNA (mRNA) from a wide variety of mostly vertebrate species, retrotransposons including LINEs and SINEs identified in other turtle species, nuclear ribosomal sequence, and numerous hits to unannotated sequences within published BAC libraries for *Chrysemys picta* (north American painted turtle) and *Gallus gallus* (chicken). For example, there was complete coverage of a characterised CR1-like LINE from the South American chelid turtle *Acanthochelys spixii* (PsCR1, Kajikawa *et al.* 1997), in both species. However, only the *Ea* sequence had two functional open reading frames (Appendix 2D). There was also near-complete coverage of the nuclear ribosomal DNA complex in both species. Contigs with extensive coverage of this region are given in Appendix 2E. Table 2.8 summarises annotation results for the 10 largest contigs in each species.

Repetitive elements

Analyses in REPEATMASKER confirmed a high repetitive sequence content for both datasets (Table 2.2). For the *Ek* NR dataset, 10.2% of sequences (n = 12,361) returned one or more hits to a repetitive element (including tandem repeats, simple repeats, and low complexity sequence). For *Ea*, this figure was 9.3% (n = 9,094). LINEs were the most common repeat identified.

DISCUSSION

Chapter 2 demonstrates the utility of small-scale 454-sequencing as a technique for microsatellite discovery in two previously unstudied species of Australian freshwater turtle, *Emydura macquarii krefftii* and *Elseya albagula*. The method proved quick, simple, and economical, and was very efficient in terms of numbers of suitable loci isolated. Stringent search parameters identified thousands of microsatellite loci in the genome of each species, of which hundreds had suitable flanking sequence for the design of PCR primers (Appendix 2A). The subset of loci developed as genetic markers for high-resolution population genetic work in the study species represent the first published for any short-necked Australian turtle (Todd *et al.* 2011).

Table 2.8 Annotation results from BLASTn analysis of the 10 largest contigs assembled from 454-sequence data for *Emydura macquarii krefftii* and *Elseya albagula*.

Contig	Length in bp (no. of reads)	Best BLASTn result	E value
Emydura macquarii krefftii			
MID8_100bp_rep_c2	11,247 (232)	nuclear ribosomal DNA	0
MID8_100bp_rep_c210	3,175 (24)	*Chrysemys picta* BAC clone CHY3-26H12	0
MID8_100bp_rep_c1	3,167 (169)	*Mus musculus* BAC clone RP24-72L18	7.39E-10
MID8_100bp_rep_c9739	3,152 (26)	PsCR1 retrotransposon	0
MID8_100bp_rep_c212	2,562 (20)	PsCR1 retrotransposon	0
MID8_100bp_rep_c3	2,249 (71)	*Mus muscularis* BAC clone rp24-72I18	1.3E-11
MID8_100bp_rep_c254	2,249 (18)	no result	-
MID8_100bp_rep_c10210	2,221 (12)	PsCR1 retrotransposon	0
MID8_100bp_rep_c10283	2,021 (8)	PsCR1 retrotransposon	0
MID8_100bp_rep_c12	2,014 (24)	*Chrysemys picta* BAC clone CHY3-1H12	3.56E-157
Elseya albagula			
MID9_100bp_rep_c6	4,464 (48)	nuclear ribosomal DNA	0
MID9_100bp_rep_c4265	3,158 (65)	PsCR1 retrotransposon	0
MID9_100bp_rep_c7590	3,013 (12)	PsCR1 retrotransposon	0
MID9_100bp_rep_c11	2,750 (33)	*Chrysemys picta* BAC clone CHY3-26H12	0
MID9_100bp_rep_c7615	2,447 (14)	PsCR1 retrotransposon	0
MID9_100bp_rep_c40	2,335 (13)	Zebrafish clone CH211-160J9	3.42E-25
MID9_100bp_rep_c2	2,281 (33)	nuclear ribosomal DNA	0
MID9_100bp_rep_c28	2,169 (22)	PsCR1 retrotransposon	0
MID9_100bp_rep_c38	2,029 (16)	*Chrysemys picta* BAC clone CHY3-88H12	1.11E-11
MID9_100bp_rep_c15	2,002 (19)	*Chrysemys picta* BAC clone CHY3-26H12	0

Cross-species testing of these markers in 12 other Australian short-necked turtle taxa, representing all but one of six extant genera, showed them to be useful across a broad range of extant Australian turtle diversity. Further bioinformatic exploration of the sequence data also provided information about repetitive and functional content, and was useful in the development of additional genomic resources, including near-complete mitochondrial genomes. Molecular resources developed herein (microsatellite markers and mitochondrial genomes) provide the necessary toolkit for work described in Chapters 3, 4 and 5.

Microsatellite discovery by 454-sequencing

The two study species, sequenced together in a single run, yielded remarkably similar results in terms of numbers of loci isolated and proportion of loci for which primers could be designed. Although total sequence data was biased towards *Ek* (46.2 Mbp vs. 36.6 Mbp for *Ea*), which translated into more microsatellite loci with primers designed for this species (375 vs. 257), the total proportion of repeat-containing sequences was comparable (1.3% vs. 1.4%). Representation of the different repeat classes and motifs was also highly similar between species (Fig. 2.1 and 2.2). Direct comparison with other published studies reporting microsatellite discovery from 454-sequence data is difficult, due to variability in the amount of sequencing undertaken, equipment and chemistry used, and the specific search programs and parameters employed. However, results of the current study fall within the reported range for studies conducted in the same time period (years 2009-2010) (Table 2.1). Genome-wide frequency and composition of microsatellite repeats is known to vary between taxa (Primmer *et al.* 1997; Toth *et al.* 2000). Reptiles, for example, appear to have a genomic microsatellite frequency intermediate between mammals and birds (Shedlock *et al.* 2007). Although the dominant motif class recovered varied between studies, dinucleotide repeats dominated in six of the 11 species examined in Table 2.1. The proportion of isolated sequences suitable for PCR primer design also varied among studies, and is probably dependent on average sequence lengths and primer design stringency.

Microsatellite discovery via the 454 approach has multiple benefits over traditional protocols based on Sanger sequencing of enriched genomic libraries. Most notably, the genomic sequencing approach is quick, cheap, and efficient. Traditional enrichment protocols are laborious and expensive, taking weeks to months of lab work and costing ~$12,000US to obtain just 10's of suitable loci. The 454-sequencing approach used here can provide 100's-1000's of candidate markers in a matter of days or weeks, at a fraction of the cost and without a high level of local technical expertise. Sample preparation for shotgun sequencing only requires extracting genomic DNA, typically undertaken in 1-2 days. As sequencing itself is typically outsourced, turnaround

time is market-dependent, and was 6 weeks for the current analysis, but is often less. Sequencing costs depend on the volume of sequencing done. Pooling multiple taxa within a single 454 run is an effective cost-saving measure. In the present study, a half plate of sequencing with three pools cost $3,500US per taxa. Runs as small as 1/16 plate are now routinely used (Scheobel *et al*. 2013), costing as little as $1,000US (M. Gardiner, pers. comm.). Microsatellite identification and primer design from 454-sequence data was a quick and simple process, facilitated by user-friendly open-access software pipelines. Most are suitable for standard desktop computers and produce results in minutes, depending on the volume of sequence data. The software used here (QDD1, Meglécz *et al.* 2010) has in-built pipelines that also address several necessary bioinformatics/QC steps. These include pre-sorting of sequences by tag (for multiplexed samples), as well as removal of sequencing adapters, and redundant and repetitive reads.

By-passing the need for enrichment and cloning removes much of the time, cost, and error involved in microsatellite discovery. The random nature of the shotgun technique also eliminates selection bias associated with repeat enrichment for pre-selected motif size and composition. Isolated repeats are instead expected to reflect the natural distribution of repeat types within the target genome. A broad genomic distribution for microsatellites isolated from 454-sequence data has been demonstrated (Abdelkrim *et al.* 2009; Saarinen & Austin 2010). Shotgun sequencing of pre-enriched DNA libraries targeting specific microsatellite repeats can nevertheless dramatically increase the efficiency of microsatellite isolation (Santana *et al.* 2009). Santana *et al.* (2009) reported that between 25% and 97% of total sequences contained microsatellites using this method (compared to 1.4% in the current study). This approach certainly limits the amount of sequencing required, reducing cost, and would be useful in cases where certain motif classes and compositions are desired (e.g. microsatellite evolution studies).

Finally, the efficiency of the shotgun technique, in terms of numbers of amplifiable loci typically isolated, affords enormous flexibility in marker selection and array design. As a result, the often time-consuming downstream empirical testing of primers and optimisation of PCR conditions should become more streamlined. *In silico* selection of loci with the best chance of success should not only decrease time and cost wastage on poor or non-amplifying loci, but should also maximise efficiency and resolving power of the final marker set. In this chapter, purposefully stringent parameters for sequence selection and primer design ensured prioritisation of loci most likely to have suitable levels of polymorphism (i.e. longer repeats, Kelkar *et al.* 2008) with primers amenable to streamlined PCR multiplexing (i.e. similarly high annealing temperatures and low potential for non-target interactions, Henegariu *et al.* 1997). In the current study, a very high success rate was

observed during empirical primer testing of a subset of the identified loci. Of the 50 primer pairs tested herein, 43 (86%) amplified a single product of the expected size at the first attempt. Of these, 35 produced interpretable peak profiles and no further PCR optimisation was necessary. The 29 loci selected for development as markers (15 *Ek* and 14 *Ea* loci; Todd *et al.* 2011) compressed into seven multiplex PCR combinations without optimisation, using uniform PCR conditions (T_A 58 °C) and a commercial multiplex PCR system (Tables 2.6 and 2.7). Similarly high success rates have been reported by others applying markers isolated with this method (Table 2.1). Progressive improvements in 454-sequencing chemistry, now capable of producing reads up to 1,000 bp in length (http://454.com/products/gs-flx-system/index.asp), will only further improve efficiency and cost-effectiveness of the shotgun sequencing approach.

454 data exploration

Small-scale genome sequence surveys, as described in this chapter for microsatellite discovery, also generate a wealth of genome-wide sequence information potentially useful for further genomic resource development and for comparative genomic research (Bouck *et al.* 1998; Green 2007; Rasmussen & Noor 2009). Raw sequences were of a length (av. 340 bp) amenable to *de novo* assembly and BLAST searches. Even with < 2% genome coverage per turtle species and no reference genome, ~20% of reads per species were assembled into contiguous sequences up to 11,200 bp in length. Bioinformatic analyses of the NR datasets (contigs+singleton reads), via sequence similarity searches of public databases, revealed several features of these two previously unstudied genomes.

As no attempt was made to remove mtDNA from gDNA samples prior to sequencing, assembly of near-complete (> 70%) mitochondrial genomes was possible for each species. Completion of each genome has since been achieved using existing sequence flanking each gap for PCR primer design and traditional Sanger sequencing (E. V. Todd *et al.* unpubl. data). Complete mitochondrial genome sequences are invaluable resources for marker development for phylogenetics and phylogeography, including whole genome phylogenetics (Kumazawa & Nishida 1999; Miya *et al.* 2003) and genome evolution studies (Russell & Beckenbach 2008). Complete mitochondrial genomes for two separate genera of Australian Chelidae are an especially valuable resource and were used to design primers for mitochondrial loci used in Chapters 3 through 5. Published mitochondrial sequence for this group has previously been sparse, few species have been the focus of population-level studies, and relationships among living genera remain largely unresolved (Georges & Adams 1992).

Bioinformatics analyses also yielded information about functional and repetitive elements. Partial or full sequences of ~100 gene orthologues were predicted in each species (Appendix 2C). Although this value is low compared to the number of total hits, low numbers of unique genes reflects an overrepresentation of genes located within transposable elements (e.g. reverse transcriptase). Such high redundancy manifesting as an abundance of repetitive elements is expected from genomic sequence data, as opposed to transcriptome databases typically generated for gene research. This reflects the large repetitive component of the genomes of most organisms (Shedlock *et al.* 2007). Unsurprisingly, a reasonably large proportion of each dataset was characterised as repetitive sequence, including sequences with homology to known elements. However, the power of these analyses was limited by the availability of suitable reference data for turtles, and true functional and repetitive content will have been underestimated.

Assembly of a complete CR1-like LINE, homologous with one previously characterised from a South American chelid turtle (*A. spixii*, PsCR1, Kajikawa *et al.* 1997) (Appendix 2D), and of the nuclear ribosomal DNA repeat (Appendix 2E), was achieved in both species. As these are repetitive genomic regions, alignments possibly represent a composite of repeats from duplicates throughout the genome, which is presumably why they could be assembled. Such sequences are nevertheless potentially useful resources for comparative research into amniote retroelement evolution (e.g. Shedlock *et al.* 2007) and turtle phylogenetics (e.g. Kupriyanova *et al.* 2012), respectively. There were also several hits to uncharacterised sequences from an existing BAC library for the painted turtle, *C. picta* (Table 2.7). As genome-wide sequence data accumulate for an ever widening variety of species, the potential utility of the current datasets for comparative genomic studies will only expand.

CONCLUSIONS

Overall, Chapter 2 demonstrates the value of the genomic sequencing approach for developing novel genetic resources in non-model species. Together, the identified microsatellite loci and mitochondrial sequences represent a significant resource for genetic research in the poorly-studied Australian Chelidae. Specifically, these resources form a molecular toolkit for the phylogenetic and population genetic analyses presented in Chapters 3 through 5. Moreover, the combined 83 Mbp of genome-wide sequence data produced provides a wealth of sequence information for a thus-far poorly represented yet distinct vertebrate clade, useful for future comparative genomic research and marker development. Future utility of this data will only increase with the accumulation of genomic sequence information for turtles and related groups.

CHAPTER 3 – CONTEMPORARY GENETIC STRUCTURE REFLECTS HISTORICAL DRAINAGE ISOLATION IN AN AUSTRALIAN SNAPPING TURTLE, *ELSEYA ALBAGULA*

A version of this chapter has been published as a collaborative manuscript, with colleagues at the University of Canberra, as follows:

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Image: The study species– the white-throated snapping turtle, *Elseya albagula*. In this case, an adult female captured from the Connors River within the Fitzroy drainage basin. Photo by Erica Todd.

CHAPTER 3 – CONTEMPORARY GENETIC STRUCTURE REFLECTS HISTORICAL DRAINAGE ISOLATION IN AN AUSTRALIAN SNAPPING TURTLE, *ELSEYA ALBAGULA*

SUMMARY

Effective spatial classification of freshwater biodiversity remains a worldwide conservation challenge. Over evolutionary timescales, the isolating nature of drainage boundaries makes them potentially important in defining natural units for freshwater biodiversity management. This chapter sought to clarify biogeographic relationships among drainages within Australia's mid-eastern coastal region (Fitzroy, Burnett and Mary Catchments), where freshwater communities face considerable urban pressure. A locally endemic riverine specialist, the white-throated snapping turtle *Elseya albagula*, was examined as a model. Mitochondrial (1326 bp control region and ND4) and nuclear microsatellite datasets (12 polymporphic loci) were employed to investigate past and present influences on population connectivity and to identify units for management. Populations within drainage basins were largely well-connected. However, the Fitzroy drainage contained a distinct genetic lineage, deeply divergent from a second lineage present across the Burnett and Mary drainages. These two lineages can be considered evolutionarily significant units and apparently reflect historical isolation of the Fitzroy, and recent coalescence of the Burnett-Mary drainages during lowered Pleistocene sea levels. Congruence with geological evidence and genetic patterns reported for co-distributed fishes and macroinvertebrates supports the shared biogeographic history of a diverse regional biota. This work further highlights the need for better spatial classification of freshwater biodiversity at local as well as regional scales, including recognition of potentially cryptic diversity among individual drainage basins.

INTRODUCTION

The natural subdivision of freshwater environments by drainage boundaries imposes unique constraints on dispersal for freshwater-limited taxa, driving high levels of population divergence and cryptic diversity (Ward *et al*. 1994). As a result, local regions are assumed to contain characteristic biotic assemblages, determined by present drainage connections and the dispersal abilities of the component species. However, it is becoming apparent that changes to drainage architecture over geological timescales can be equally significant in shaping current spatial patterns of diversity (Waters *et al*. 2001). Understanding current community structures and recognising patterns of cryptic diversity are fundamental precursors for effective conservation management, yet

current frameworks regularly suffer from limited data on distinct freshwater communities thus limiting identification of potentially cryptic diversity within them (Cook *et al.* 2008).

Within the largely arid Australian continent, the eastern coastal margin supports a rich endemic freshwater fauna (Unmack 2001). This region is noteworthy for its complex biogeography, reflecting multifarious processes of landform development in an otherwise geologically quiescent continent. Volcanism, uplift, escarpment retreat and sea level fluctuations have produced complex drainage patterns that are not yet well understood (Jones 2006; Hodgkinson *et al*. 2007; Vasconcelos *et al*. 2008). In recent years, genetic studies have provided evidence for historical drainage rearrangements by revealing unexpected patterns in the distribution and relatedness of genetic lineages across now-isolated catchments (Hurwood & Hughes 1998; Hughes *et al.* 1999).

Especially high levels of genetic subdivision are reported among drainages in mid-eastern coastal Australia, which is dominated by the geographically extensive Fitzroy drainage (142,600 km^2) (Fig. 3.1) (Hughes *et al*. 1999; McGlashan & Hughes 2001b; Murphy & Austin 2004). This is somewhat unexpected, as the continental shelf is considerably wider here than further north or south, potentially providing greater opportunity for drainage coalescence during lowered sea levels (Thacker *et al.* 2007, 2008). High taxonomic diversity in the area coincides with its position as a transition zone between tropical and subtropical climates. High local endemism, including that found among many ancient relict lineages such as chelid turtles (Pleurodira), lungfish (*Neoceratodus forsteri*), and bonytongue fishes (Osteoglossiformes), also implies it may have been an important evolutionary refugium. A thorough understanding of biodiversity distribution in this area is critical, as growing urban, agricultural and mining sectors place increasing pressure on local freshwater habitats (Poff *et al.* 1997; Limpus *et al*. 2002; Stein *et al*. 2002).

Freshwater turtles were early models for understanding biogeographic patterns (Walker & Avise 1998) and often show high levels of cryptic lineage diversity (Roman *et al*. 1999; Spinks & Shaffer 2005; Pearse *et al*. 2006). Australian species, however, are poorly studied and often neglected in evolutionary studies and in management planning (Georges 1993; Fielder *et al*. 2012), yet they are a diverse and evolutionarily distinct group consisting almost exclusively of members of the family Chelidae. The mid-eastern coastal catchments of the Fitzroy, Burnett and Mary Rivers support the second highest turtle diversity (seven species) and the greatest concentration of local endemics (three species) in Australia. The area is a global conservation priority for chelonians (Buhlmann *et al.* 2009) and freshwater species generally (Abell *et al*. 2008; Vörösmarty *et al*. 2010).

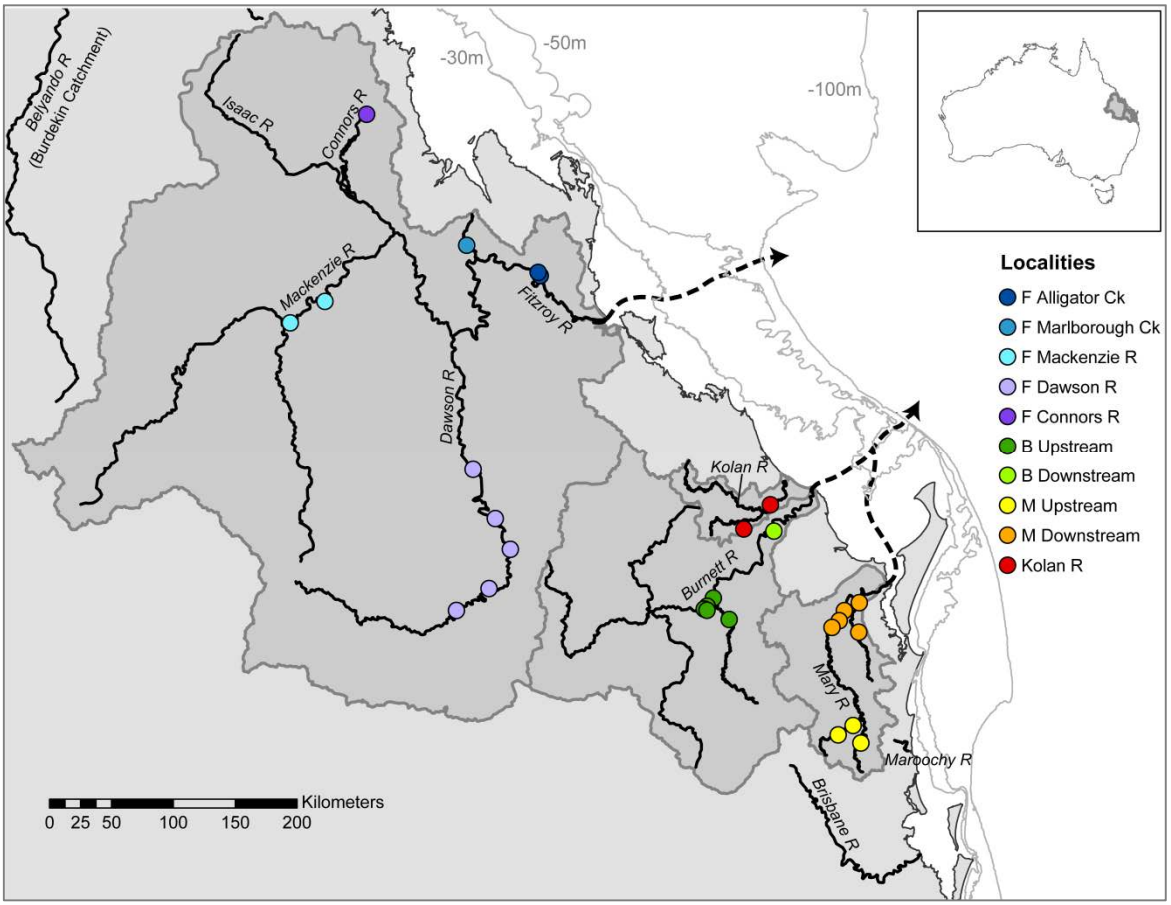

Fig. 3.1 Location map showing the geographical distribution (dark shading) of the white-throated snapping turtle, *Elseya albagula*, in mid-eastern coastal Australia and sampling locations (coloured circles) for the present study throughout four river drainages. Dashed arrows indicate likely paleochannels of the Fitzroy (F) (Ryan *et al*. 2007), Burnett (B) and Mary (M) Rivers during lowered sea levels, indicating potential confluence of the Burnett and Mary Rivers at the -50 m contour. Samples are grouped by colour; see Table 3.1 for sample sizes. Major rivers mentioned in text are in black, with drainage boundaries outlined in grey. Bathymetric contours at -30 m, -50 m and -100 m are shown (light grey) (Beaman 2010).

The white-throated snapping turtle, *Elseya albagula*, is a large-bodied riverine species locally endemic to the Fitzroy, Burnett and Mary catchments (Fig. 3.1). It is one of several recently described but distinct allopatric forms of Australian snapping turtle previously regarded as *E. dentata* (Georges & Adams 1996). *Elseya albagula* is considered a habitat specialist and co-exists with two other locally endemic riverine specialist turtles (*Rheodytes leukops* and *Elusor macrurus*). All three species share a requirement for well-oxygenated flowing waters, which seems closely associated with an interesting physiological adaptation allowing them to respire aquatically via highly vascularised cloacal bursae (Legler & Georges 1993; Mathie & Franklin 2006; Clark *et al*. 2009). Accordingly, these species are potentially highly sensitive to changes in habitat and flow regime associated with river regulation and climate change (Thomson *et al.* 2006; Hamann *et al.* 2007). Habitat specialisation is also expected to restrict dispersal ability and promote population sub-structure in *E. albagula*, especially between individual catchments, but potentially also within the stream hierarchy.

Chapter 3 examines patterns of population genetic structure and connectivity across the natural range of *E. albagula* to elucidate historical biogeography and address pressing conservation issues for this species and this region. Nuclear microsatellite and mitochondrial DNA sequence (mtDNA) markers were used to assess contemporary and historical influences on genetic structure and to identify cryptic diversity, including evolutionarily significant units (ESUs) and management units (MUs) (*sensu* Moritz 1994). ESUs and MUs are an established framework for recognising population units as evolutionarily or demographically distinct genetic entities, respectively, key for the development of effective management strategies. Specific aims of this chapter are to 1) describe patterns of connectivity within and across drainage divides, and 2) investigate regional phylogeography in order to understand historical relationships among catchments and potential connectivity during historical sea level fluctuations.

METHODS

Sample collection and laboratory procedures

Tissue samples were obtained from 199 individual *E. albagula* throughout this species' natural range, encompassing the Fitzroy, Burnett and Mary drainages in mid-eastern coastal Australia (Fig. 3.1; Appendix 3A). Within the geographically extensive Fitzroy drainage (142,600 km^2), sample sites represented all major sub-drainages: the Fitzroy, Dawson, Mackenzie, and Isaac-Connors Rivers. For the much smaller Burnett (39,500 km^2) and Mary (9,700 km^2) drainages, samples were divided into upstream and downstream catchment locations. Three additional samples were

obtained from the Kolan River, a short river adjacent the Burnett. Samples were sourced from existing collections, through collaborations with other researchers, and during targeted field work. During the latter, turtles were caught by seine net, baited trap, or by hand with the aid of mask and fins (Hamann *et al*. 2008). A small section of skin was removed from the forelimb of each turtle, preserved immediately in 95% ethanol and later stored at -20 °C. Genomic DNA was extracted from tissues using a modified salting-out protocol, as described in Chapter 2.

Twelve polymorphic microsatellite loci developed specifically for *E. albagula* (Chapter 2; Todd *et al.* 2011) were employed to assess contemporary genetic structure and gene flow (Ealb02, Ealb06, Ealb07, Ealb09, Ealb10, Ealb14, Ealb15, Ealb18, Ealb19, Ealb20, Ealb24, Ealb25). Loci were amplified via multiplex PCR as per Chapter 2. PCR product purification, fragment analysis and allele scoring were also performed as described in Chapter 2. Positive controls (included in each run) and allele binning were employed to ensure consistent scoring among samples and runs.

ARLEQUIN 3.5 (Excoffier & Lischer 2010) and GENALEX 6.4 (Peakall & Smouse 2006) were used to determine the number of alleles, observed and expected heterozygosities, and conformation to Hardy-Weinberg expectations for each microsatellite locus. Allelic richness was estimated in FSTAT 2.9 (Goudet 1995). Potential linkage disequilibrium between pairs of loci was tested using GENEPOP 4.0 (Rousset 2008) and the presence of null alleles and scoring error was evaluated using MICRO-CHECKER 2.2 (van Oosterhout *et al.* 2004). For multiple comparisons, significance levels were adjusted using a false discovery rate (FDR) correction (Benjamini & Hochberg 1995). No evidence for linkage disequilibrium or deviation from Hardy-Weinberg expectations was found. There was also no evidence for scoring error or large allele dropout, although null alleles were suggested at locus Ealb24 and Ealb07 in the upstream and downstream Mary River populations, respectively. Analyses performed with and without these markers did not alter the outcome and results are presented for all 12 loci.

To investigate historical population structure and phylogeography, for each specimen 1326 bp of mtDNA sequence was amplified, comprising 475 bp of control region (CR) (primers CREalb 5′-TGGTCTTGTAAACCAGAAACG-3′, present study; TCR500, Engstrom *et al*. 2002), 680 bp of the NADH dehydrogenase subunit 4 (ND4), plus 70 bp of tRNAHis, 61 bp of tRNASer and the first 46 bp of tRNALeu (primers ND4Ealb 5′-TGACTACCAAAAGC**A**CATGTA**C**AAGC-3′; LeuEalb 5′-CAT**G**ACTTTTACTTGGA**G**TTGCACCA-3′, modified after Arévalo *et al*. 1994). Primers ND4Ealb and LeuEalb were modified from published sources (substituted bases shown in bold) with reference to complete mitochondrial genomes for *E. albagula* and *Emydura macquarii krefftii* (E. V. Todd *et al.* unpubl. data; Chapter 2). PCR amplifications were performed in 40 µL reactions

containing 10-20 ng genomic DNA, 1x PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 1 U GoTaq® Flexi DNA polymerase (Promega), 1x BSA, and 0.2 µM each primer. Amplification was performed in a BioRad C1000 Thermo Cycler with the following cycling conditions: initial 3 min denaturation at 95 °C, followed by 35 cycles of 95 °C denaturation for 45 s, 55 °C annealing for 45 s, and 72 °C extension for 1 min, with a final extension of 5 min at 72 °C. PCR product purification and sequencing were performed commercially by Macrogen Inc. (Seoul, South Korea). Samples were sequenced in forward and reverse directions (CR) or twice in the forward direction (ND4, due to poor quality reverse sequence) to confirm sequence fidelity. Sequences were aligned and edited using GENEIOUS PRO 5.6 (Biomatters, available from http://www.geneious.com). ND4 sequences were translated and examined for premature stop codons that may indicate the presence of nuclear mitochondrial (numt) DNA paralogues. Sequence chromatograms were inspected visually for ambiguous nucleotide signals that may indicate co-amplification of mtDNA and numts. Finally, regions were aligned with published sequences from a range of turtle species to confirm amplification of the correct mitochondrial region. No evidence of numts was found.

Nucleotide diversity (π) and haplotype diversity (H_d) were estimated separately for CR and ND4 regions, and for the concatenated dataset, in ARLEQUIN and DNASP 5.10 (Librado & Rozas 2009). A maximum likelihood approach implemented in jMODELTEST (Guindon & Gascuel 2003; Posada 2008) was used to determine the most representative evolutionary model for each region separately and for the concatenated dataset, using the AIC criterion. Unique sequences for CR and ND4 were submitted to GenBank (Accession numbers: JX871334-JX871338, JX871340-JX871353).

Population structure and phylogeography

Fine-scale contemporary (microsatellites) and broad-scale historical (mtDNA) population structure were investigated within and across drainage divides using three complementary approaches: 1) calculation of pairwise population statistics and analysis of molecular variance (AMOVA) (microsatellites and mtDNA data), 2) haplotype networks (mtDNA data), and 3) Bayesian clustering analyses (microsatellite data). Pairwise comparisons (linearised F_{ST}) were made between sampling locations and catchments to test for significant genetic differentiation. Analyses were implemented in FSTAT for microsatellite data and ARLEQUIN for sequence data (pairwise difference, 10,000 permutations), with significance levels adjusted for multiple comparisons using FDR, as above. Hierarchical AMOVA were performed to investigate distribution of genetic variance within and among catchments. Calculations were performed in ARLEQUIN (10,000 permutations) using F_{ST} for microsatellite data and Φ_{ST} (haplotype frequencies only) for mtDNA. Mean genetic distances within and among catchments were calculated for the concatenated mtDNA dataset in MEGA 5

(Tamura *et al.* 2011) using the closest available model to that selected by jMODELTEST (TrN model), without gamma correction and with pairwise deletion of sites containing gaps. Relatedness and spatial distribution of mtDNA haplotypes were assessed with a minimum spanning network, computed in ARLEQUIN (pairwise distance model). The minimum spanning tree was drawn for clarity of presentation following visualisation in HAPSTAR 0.7 (Teacher & Griffiths 2011).

Bayesian clustering analyses were implemented in STRUCTURE 2.3 (Pritchard *et al*. 2000) to identify the number of distinct genetic clusters (*K*) best represented by the microsatellite data. The three individuals from the Kolan River were included in analyses. Ten independent simulations were run for *K* inferred clusters from one to 10, with an initial burn-in of 100,000 followed by 1 million iterations, found to be appropriate in pilot runs. An admixture model with correlated allele frequencies was assumed. Sampling locations were used as prior information (LOCPRIOR model), which improves clustering performance for datasets where the signal of genetic structure may be weak, without introducing bias (Hubisz *et al.* 2009). For each run, summary statistics (log likelihood and alpha) were monitored to verify convergence. The true value of *K* was determined following both posterior probability (Pritchard *et al*. 2000) and delta log likelihood methods (Evanno *et al*. 2005) implemented in STRUCTURE HARVESTER 0.6 (Earl & vonHoldt 2012). Each of the identified clusters was subsequently run independently to identify any further sub-structure within each major genetic unit.

RESULTS

Genetic diversity

Microsatellite polymorphism was greater within the large Fitzroy drainage relative to either of the smaller systems, both at the drainage level and within individual sampling locations (Table 3.1). Controlling for differences in sample size, allelic richness was still highest within the Fitzroy drainage and was 4.47 (±1.99 SD) compared to 3.27 (±2.67 SD) in the Burnett and 3.89 (±2.07 SD) in the Mary. The Fitzroy drainage also contained a considerable number of private alleles: of 60 alleles detected overall within this catchment, 25 (42%) were not found within the Burnett or Mary catchments. The Burnett sites had 8 private alleles (17%) and the Mary sites had five (11%). Only small numbers of private alleles (1-3) were detected within sample locations.

Table 3.1 Diversity statistics for 12 microsatellite loci analysed for *Elseya albagula* sampled across three river drainages. Statistics are presented for each drainage, individual sampling locations within drainages, and overall. Location abbreviations follow Fig. 3.1.

Drainage/ location	***N***	**Polymorphic loci**	P_A	N_A	A_R[1]	H_O	H_E
Fitzroy	**97**	**12**	**25**	**5.00(2.30)**	**4.47(1.99)**	**0.57(0.15)**	**0.59(0.14)**
F Alligator	33	12	1	4.00(1.65)	3.04(0.94)	0.57(0.17)	0.55(0.16)
F Marlborough	9	12	1	3.25(1.06)	2.87(0.85)	0.54(0.23)	0.51(0.14)
F Mackenzie	5	12	0	3.08(1.44)	3.08(1.44)	0.45(0.23)	0.51(0.18)
F Dawson	30	12	2	4.67(2.02)	3.29(1.04)	0.60(0.16)	0.59(0.15)
F Connors	20	12	0	4.00(1.86)	3.12(0.08)	0.56(0.13)	0.58(0.12)
Burnett	**68**	**10**	**8**	**3.92(3.09)**	**3.27(2.67)**	**0.48(0.27)**	**0.48(0.28)**
B Upstream	34	10	3	3.75(3.08)	2.78(1.51)	0.47(0.29)	0.46(0.30)
B Downstream	34	10	1	3.42(2.19)	2.63(1.15)	0.50(0.26)	0.48(0.26)
Mary	**34**	**11**	**5**	**3.92(2.11)**	**3.89(2.07)**	**0.48(0.28)**	**0.49(0.26)**
M Upstream	22	11	1	3.50(1.93)	2.63(1.27)	0.45(0.30)	0.45(0.29)
M Downstream	12	11	1	3.50(1.68)	2.93(1.16)	0.53(0.29)	0.54(0.22)
OVERALL	**199**	**12**	**-**	**4.28(2.51)**	**5.66(2.93)**	**0.51(0.23)**	**0.52(0.23)**

N, number of samples; P_A, private alleles. Statistics represent averages (SD) over all 12 loci: N_A, number of alleles; A_R, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity. No locus deviated significantly from Hardy-Weinberg expectations. [1]A_R values are based on a minimum sample size of n = 5 for sampling locations and n = 34 for drainage calculations and overall.

The mtDNA CR alignment contained 476 bp and 32 variable sites that defined 14 haplotypes. ND4 sequences were less variable, with six variable sites defining five haplotypes across 851 bp. Fifteen haplotypes were identified for the 1327 bp concatenated dataset. Haplotype frequencies by population are presented in Appendix 3.2. Genetic diversity was again considerably greater in the Fitzroy drainage compared to the Burnett and Mary drainages (Table 3.2). Results are hereafter reported for the concatenated dataset. Overall nucleotide diversity was low (0.008), and lowest for Burnett and Mary drainages (< 0.001) compared with the Fitzroy drainage (0.003). Haplotype diversity was high overall (0.74) and within the Fitzroy drainage (0.70) specifically, including at individual sample locations (range 0.50-0.70). Much lower haplotype diversity within the Burnett and Mary systems, at both individual sample locations (range 0-0.38) and drainage scales (0.23 and 0.30, respectively), signifies a much greater degree of haplotype sharing among individuals within these systems.

Population structure

Hierarchical AMOVA of both microsatellite data and mtDNA sequences revealed that a highly significant proportion of overall genetic variation was partitioned among drainages (15.84% and 41.43%, respectively) (Table 3.3). Little variation was partitioned among sample locations within drainages for microsatellites (< 2%), or mtDNA (< 12%), though the value was significant in both cases (P = 0.000). Overall fixation values were larger for mtDNA data (Φ_{ST} = 0.531) than for microsatellites (F_{ST} = 0.178) and were highly significant in both cases (P = 0.000, Table 3.3).

For pairwise comparisons between sample locations, the greatest differentiation (highest F_{ST} values) was seen among drainage basins (Table 3.4a). Within drainages, values were lower and often non-significant. Genetic differentiation between the upstream and downstream Burnett locations was significantly different from zero at both marker types. Interestingly, mtDNA data suggested the downstream Mary sample was differentiated from the upstream Mary sample (F_{ST} = 0.513) but not from either sample location within the Burnett (F_{ST} < 0.12). Otherwise, results from the mtDNA and microsatellite datasets were highly congruent. Comparing among drainages overall (Table 3.4b), the greatest differentiation occurred between the Fitzroy drainage and elsewhere. Burnett and Mary samples were less divergent, although the comparison was still significant.

Table 3.2 Diversity statistics for CR and ND4 mtDNA sequences from *Elseya albagula* sampled across four river drainages. Statistics are presented for CR/ND4/concatenated datasets for each drainage, individual sampling locations within drainages, and overall. Location abbreviations follow Fig. 3.1.

Catchment Location	***N***	H_h	***V***	H_d	**π**
Fitzroy-all	**94**	**9/3/10**	**13/2/15**	**0.65/0.44/0.70**	**0.007/0.001/0.003**
F Alligator	32	4/2/4	8/1/9	0.70/0.47/0.70	0.008/0.001/0.003
F Marlborough	9	2/1/2	1/0/1	0.50/0.00/0.50	0.001/0.000/<0.001
F Mackenzie	3	2/2/2	7/1/8	all 0.67	0.010/0.001/0.004
F Dawson	30	6/3/7	11/2/13	0.54/0.54/0.69	0.008/0.001/0.003
F Connors	20	5/2/5	9/1/10	0.57/0.39/0.57	0.007/<0.001/0.003
Burnett-all	**67**	**2/1/2**	**1/0/1**	**0.23/0.00/0.23**	**0.001/0.000/<0.001**
B Upstream	34	2/1/2	1/0/1	0.06/0.00/0.06	<0.001/0.000/<0.001
B Downstream	33	2/1/2	1/0/1	0.37/0.00/0.38	0.001/0.000/<0.001
Mary-all	**29**	**3/1/3**	**2/0/2**	**0.29/0.00/0.30**	**0.001/0.000/<0.001**
M Upstream	21	3/1/3	2/0/2	0.38/0.00/0.38	0.002/0.000/0.001
M Downstream	8	2/1/2	1/0/1	0.025/0.00/0.25	<0.001/0.000/0.001
Kolan	**3**	**2/2/2**	**6/1/7**	**all 0.67**	**0.008/0.001/0.004**
OVERALL	**193**	**14/5/15**	**32/6/35**	**0.73/0.62/0.74/**	**0.018/0.003/0.008**

N, number of samples; H_h, number of haplotypes; *V*, variable sites; H_d, haplotype diversity; π, nucleotide diversity.

Table 3.3 Hierarchical analysis of molecular variance (AMOVA) results for *Elseya albagula* microsatellite (F_{ST}) and mitochondrial sequence (Φ_{ST}) data.

Source of variation	**Variation (%)**	**Fixation index**	***P* value**
Microsatellite data			
Among drainages	15.84	F_{CT} = 0.158	0.002
Among sample locations within drainages	1.93	F_{SC} = 0.023	0.000
Within sample locations	82.24	F_{ST} = 0.178	0.000
MtDNA data			
Among drainages	41.43	Φ_{CT} = 0.414	0.006
Among sample locations within drainages	11.63	Φ_{SC} = 0.198	0.000
Within sample locations	46.95	Φ_{ST} = 0.531	0.000

Table 3.4 Pairwise F_{ST} comparisons for *Elseya albagula* populations based on microsatellite (below diagonal) and mtDNA data (above diagonal): a) among sampling locations within drainages, and b) among drainage basins. Bold values indicate significance at < 0.05 after correction for multiple comparisons. Negative values were converted to zero. Location abbreviations follow Fig. 3.1.

a)

	F Allig	F Marlb	F Mack	F Daw	F Con	B Up	B Down	M Up	M Down
F Alligator	-	**0.223**	0.000	0.012	0.000	**0.867**	**0.858**	**0.830**	**0.793**
F Marlborough	0.000	-	0.403	**0.180**	**0.211**	**0.992**	**0.977**	**0.960**	**0.978**
F Mackenzie	0.000	0.000	-	0.000	0.000	**0.974**	**0.955**	**0.922**	**0.904**
F Dawson	0.006	0.009	0.000	-	0.000	**0.886**	**0.877**	**0.850**	**0.818**
F Connors	**0.045**	0.053	0.012	**0.032**	-	**0.908**	**0.896**	**0.865**	**0.829**
B Upstream	**0.180**	**0.198**	**0.181**	**0.187**	**0.217**	-	**0.152**	**0.737**	0.092
B Downstream	**0.173**	**0.196**	**0.175**	**0.174**	**0.208**	**0.020**	-	**0.653**	0.116
M Upstream	**0.224**	**0.258**	**0.241**	**0.211**	**0.250**	**0.146**	**0.125**	-	**0.513**
M Downstream	**0.165**	**0.182**	**0.146**	**0.160**	**0.178**	**0.077**	**0.076**	0.040	-

b)

	Fitzroy	Burnett	Mary
Fitzroy	-	**0.858**	**0.818**
Burnett	**0.173**	-	**0.559**
Mary	**0.189**	**0.101**	-

Phylogeography

The mtDNA haplotype network (Fig. 3.2) further highlights the genetic distinctiveness of the Fitzroy drainage from the smaller drainages to its south. The two groups are reciprocally monophyletic as they share no haplotypes and are separated from one another by at least 16 mutational steps and 1.5% mean sequence divergence (Table 3.5). Within the Fitzroy, although not all haplotypes were found in every sample location, there was no geographical pattern to the observed diversity to suggest sub-structure within this system. The Burnett and Mary drainages together form a distinct group, characterised by a single common haplotype, with additional closely related haplotypes unique to either the Burnett (1 haplotype) or Mary (2 haplotypes) systems. A divergent haplotype (7 mutations) occurred in two of three samples collected from the Kolan River, with the third individual sharing the common Burnett/Mary haplotype. Mean sequence divergence (Table 3.5) between the Burnett and Mary drainages was only 0.04%, which is of the same scale as that observed within either catchment (0.02 and 0.05%, respectively), further highlighting their close genetic relationship.

Bayesian clustering analyses

Bayesian clustering analyses of the microsatellite data indicated the presence of two well-defined genetic clusters (highest posterior probability and delta K obtained for $K = 2$; Fig. 3.3a). The first included all individuals from the Fitzroy drainage, with individuals from the small southern catchments (Burnett/Mary/Kolan) forming a second well-defined group. When analysed separately, three discrete clusters are identified within the second group and correspond to the Burnett, Mary and Kolan Rivers (Fig. 3.3b). No sub-structure was detected within the Fitzroy basin.

DISCUSSION

Drainage boundaries typically present insurmountable barriers to dispersal by obligate freshwater species. Historically, these may be overcome only occasionally, when stochastic events (e.g. floods, sea level change and river capture) create rare opportunities for genetic exchange and range expansion. In this chapter, population connectivity in the riverine turtle *E. albagula* was found to be highly constrained across drainage boundaries, but not within. However, current drainage divides do not fully explain the observed patterns. The Fitzroy drainage contains a distinct genetic lineage, while the Burnett and Mary drainages display a close genetic affinity despite their current isolation. As *E. albagula* does not tolerate estuarine conditions and is not known to make overland journeys,

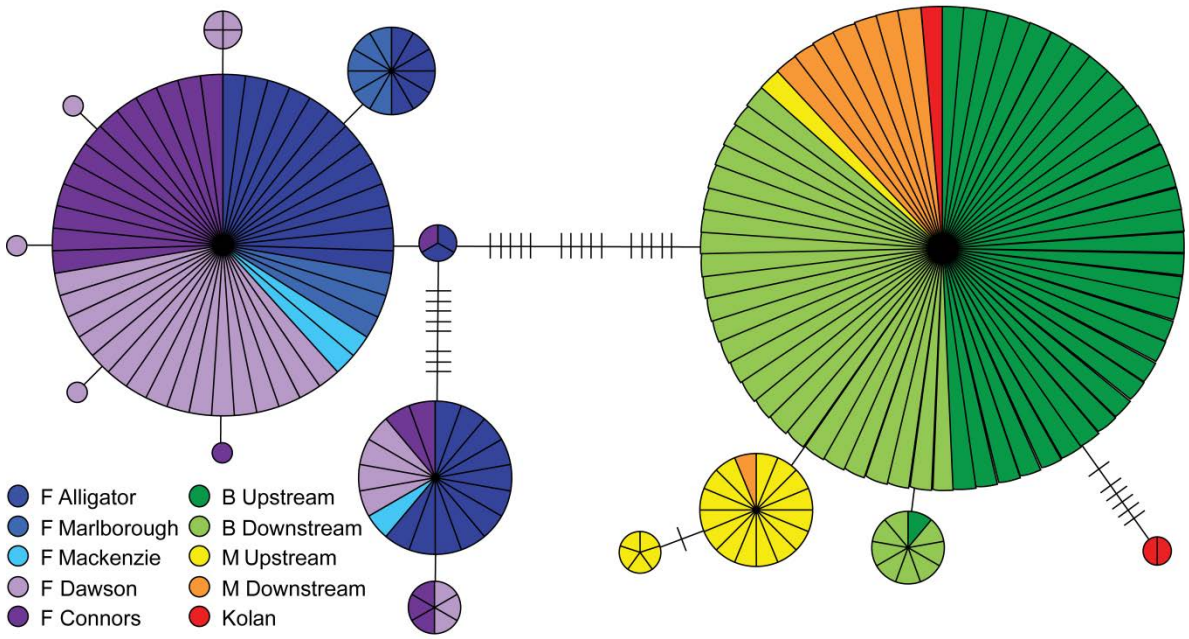

Fig. 3.2 Minimum spanning tree depicting haplotype relationships for concatenated CR/ND4 mtDNA sequences sampled from *Elseya albagula* across 10 locations. Circles represent unique haplotypes and are connected to one another by mutational changes (lines). Crossbars indicate additional mutational steps and circle size is proportional to haplotype frequency. Colours indicate geographic source of haplotypes. Location abbreviations and colours follow Fig. 3.1.

Table 3.5 TrN corrected mean genetic distances (%) for *Elseya albagula* populations sampled among drainages, based on a 1327 bp concatenated CR/ND4 mtDNA alignment. Below diagonal, percent divergence among drainages; above diagonal, SE values for percent divergence among catchments; italics diagonal, percent divergence within catchments.

	Fitzroy	Burnett	Mary	Kolan
Fitzroy	*0.30*	0.003	0.003	0.003
Burnett	1.48	*0.02*	0.000	0.001
Mary	1.47	0.04	*0.05*	0.001
Kolan	1.75	0.39	0.41	*0.38*

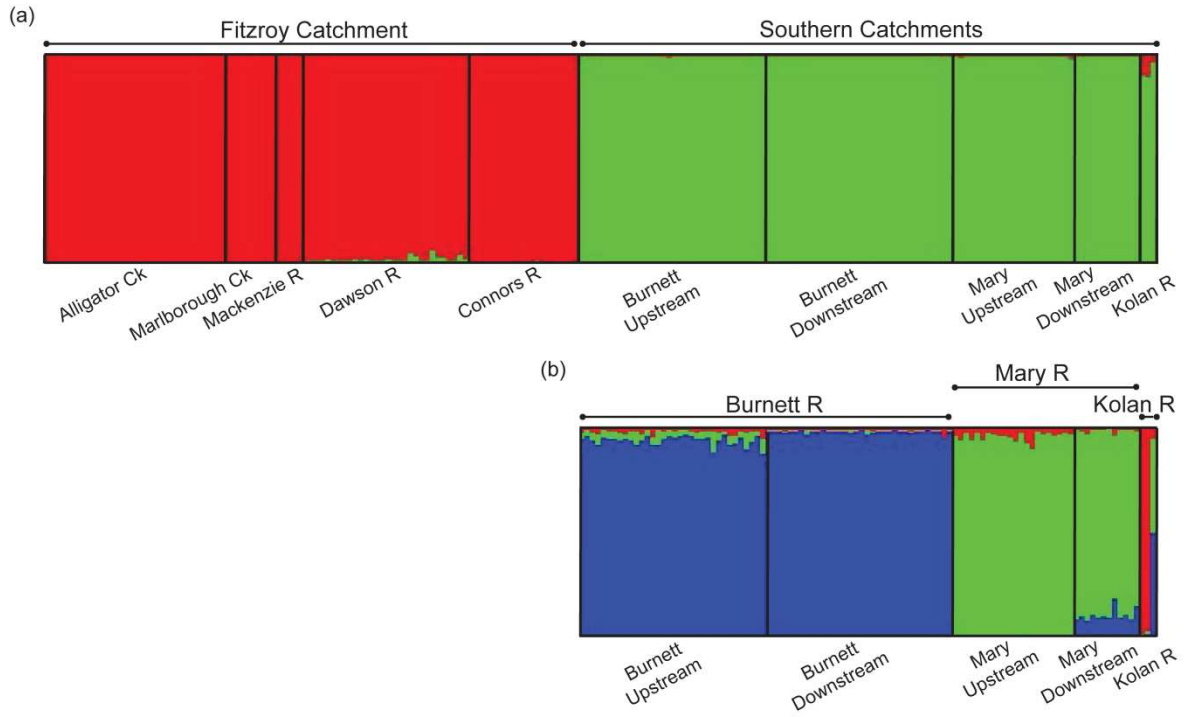

Fig. 3.3 Bayesian population assignments for *Elseya albagula* individuals based on analysis of 12 microsatellite loci in STRUCTURE, indicating structure by region and drainage. Individuals are represented by coloured vertical bars, which indicate percent genetic membership (y axis) within genetic units, as indicated above each diagram. Black lines separate sampling locations (x axis). a) Initial differentiation between two regional units (K = 2), and b) further substructure among drainages within the southern region (K = 3).

the observed patterns likely reflect historical drainage architecture. Strong agreement between mtDNA and microsatellite datasets indicates that current restrictions on gene flow across the Fitzroy drainage boundary also have a deep historical aspect, while subtle frequency differences between the Burnett and Mary systems suggest a shared history despite a lack of current gene flow.

Molecular studies continue to reveal deep genetic partitioning and cryptic diversity in freshwater communities. Patterns often reflect historical drainage structure and therefore the isolating nature of watershed boundaries over evolutionary timescales (Berendzen *et al*. 2008; Cook *et al*. 2008; Smith 2009). However, for habitat specialists and species with limited dispersal capacity, fine-scale genetic structure within riverine networks has been reported (Hughes *et al.* 1999, 2012). This was also anticipated for *E. albagula*, given its apparent narrow niche requirements (Thomson *et al*. 2006), sedentary nature (Hamann *et al*. 2007), and the tendency for female turtles to show strong natal philopatry to nesting areas (Sheridan *et al.* 2010). More extensive tagging-recapture studies are required to define the extent of breeding movements made by *E. albagula* within drainages. In the Burnett River, Limpus *et al*. (2011) recorded movements of adult male and female *E. albagula* in the order of low tens of kilometres to aggregated nesting areas, and the upstream movement of approximately 60 km of an adult male at courtship time. Higher F_{ST} values for mtDNA may indicate some degree of natal philopatry or male biased dispersal within drainages (see Karl *et al*. 2012). Overall, *E. albagula* appears to be an effective disperser within drainage boundaries, even among sub-catchments of the geographically extensive Fitzroy drainage. The same is reported for freshwater turtle populations elsewhere (Roman *et al*. 1999; Pearse *et al*. 2006). The exceptionally long life spans of these animals (Finch 1990) probably provide substantial opportunities for life-time dispersal and gene flow within hydrologically connected systems (i.e. river basins). Perceived habitat specialisation clearly does not necessarily predict a greater degree of population genetic structure for long-lived species.

Phylogeographic structure and drainage evolution

Several lines of evidence suggest a historical barrier to freshwater dispersal between the Fitzroy drainage and systems to its north and south. Of 34 freshwater fish species recorded across the Fitzroy, Burnett, Mary and Brisbane drainages, 16 are not shared between the Fitzroy River and the more southerly catchments (Unmack 2001). The Fitzroy and Burnett-Mary regions each have their own endemic species, including several ancient relict lineages of great interest. In the Fitzroy these include bonytongues and the Fitzroy River turtle (*R. leukops*). Australia's only lungfish species is endemic to the Burnett and Mary Rivers (despite anthropogenic introductions into the Brisbane River, Frentiu *et al*. 2001), and a further two species (Mary River turtle, *E. macrurus*, and Mary

River cod, *Maccullochella mariensis*) are endemic to the Mary River. An even stronger pattern of lineage disjunction occurs between the Fitzroy and the almost equally extensive Burdekin drainage directly to its north (Unmack 2001). Another inferred barrier occurs south of the Mary River, based on genetic data from various species including several fishes (Hughes *et al.* 1999; Wong *et al*. 2004; Thacker *et al*. 2007) and macroinvertebrates (Murphy & Austin 2004; Sharma & Hughes 2009; Bentley *et al*. 2010). These barriers appear to define the natural distributional limits of *E. albagula* within the Eastern Province despite its capacity to survive in the Brisbane and Maroochy drainages following apparent human introductions (see published version of this chapter, Todd *et al*. 2013a). Even in studies of widespread species that span these natural barriers, populations from the Fitzroy, Burnett and Mary Rivers often form a distinct clade, though with considerable internal substructure among individual river basins (McGlashan & Hughes 2001b; Wong *et al*. 2004; Thacker *et al*. 2007). This region may therefore form a natural bioregion of considerable conservation value.

The inference of shared history despite a lack of current gene flow for the Burnett and Mary drainages demands closer attention. Drainage re-arrangement due to Pleistocene volcanism presents an explanation for complex genetic patterns observed for freshwater species in far north Queensland (Hurwood & Hughes 1998) and drainage coalescence during lowered sea levels has been invoked to explain relatedness among catchments elsewhere (Bermingham & Avise 1986). Data presented here for a freshwater turtle is consistent with a recent connection between the Burnett and Mary Rivers. Although precise paleodrainage patterns are difficult to reconstruct, bathymetric data suggest a possible confluence of the Burnett and Mary rivers at the -50 m contour (Fig. 3.1). The Late Pleistocene eustatic sea-level curve (Chappell *et al*. 1996) indicates that within the last 120,000 years, sea levels were at or below 50 m approximately 55% of the time (Harris *et al*. 2005), suggesting a timeframe for admixture to occur. Such a connection would have been severed approximately 12,000 ya, as sea levels rose rapidly following the Last Glacial Maximum (LGM) (Yokoyama *et al*. 2006). The very low level of divergence between, and presence of shared haplotypes across, these systems is consistent with this timeframe. Genetic similarity between Burnett and Mary populations is reported for several widespread fish species, including hardyhead (*Craterocephalus stercusmuscarum*) (McGlashan & Hughes 2001b), gudgeons (Eleotridae) (Thacker *et al*. 2007, 2008), and catfish (*Tandanus tandanus*) (Jerry 2008), as well as the locally endemic lungfish (Frentiu *et al*. 2001). Many of the above studies also report genetic distinctiveness of Fitzroy lineages.

The level of genetic divergence observed across the Fitzroy drainage boundary suggests it has been comparatively stable, and over a much longer timeframe. This is in line with strong geological

evidence supporting the antiquity of the Fitzroy River system, which is thought to have expanded during the Paleogene (65-35 Mya) as the eastern escarpment retreated inland (Jones 2006). Bathymetric expression of the Fitzroy River paleochannel is remarkably clear and indicates that during the LGM lowstand (30,000 to 18,000 ya) it very likely extended to the continental shelf edge more than 100 km east of the current shoreline (Fig. 3.1; Ryan *et al.* 2007). Its path is such that confluence with neighbouring catchments is unlikely at this time, or indeed any time since the last marine regression began 120,000 ya. Such a conclusion is supported by the presented molecular data.

It is difficult to distinguish between possible patterns of colonisation for *E. albagula* between the Fitzroy, Burnett and Mary Rivers given the considerable timescale involved. Several scenarios could explain the observed patterns of diversity. Although the Fitzroy apparently did not connect to other catchments during lowered sea levels, cyclic wetting and drying of the Australian continent since the Mid-to-Late-Miocene no doubt dramatically influenced the connectivity of freshwater environments. Conceivably, this allowed interchange or vicariance among freshwater populations in separate drainages. Low genetic diversity in the Burnett-Mary drainages relative to the Fitzroy may reflect a founder event from ancestral populations within the Fitzroy system. Low diversity may also reflect genetic erosion due to bottleneck or much smaller effective population size in the Burnett-Mary following vicariance from the Fitzroy, which would have maintained larger population sizes and thus greater genetic diversity over time. In support of the latter case, neutrality tests for historical changes in population size were non-significant (data not shown).

Conservation management

Effective management of freshwater biodiversity requires an understanding of its spatial distribution across the landscape, reflecting geographic isolation at evolutionarily and ecologically relevant time scales. At the species level, this is recognised within the framework of ESUs and MUs, applicable to long and short-term population management goals, respectively (Ryder 1986; Moritz 1994). For *E. albagula*, genetic data suggest the Fitzroy and Burnett-Mary lineages be recognised as separate ESUs, given reciprocal monophyly of mtDNA haplotypes and strong allelic differences at nuclear markers (*sensu* Moritz 1994). MUs can be further defined at the drainage level, recognising subtle yet significant allele frequency differences between the Burnett and Mary populations that imply current demographic independence. However, molecular data presented here does not measure ecological exchangeability in terms of niche differences and adaptive differentiation, which are also important considerations in classifying population distinctiveness and determining whether genetically distinct units warrant separate management, as recognised by

Crandall *et al*. (2000). Further sampling of *E. albagula* from the Kolan River (and other peripheral streams) is warranted to resolve whether it contains a distinct genetic lineage and to clarify its historical relationship with the larger catchments. The Kolan River is a small coastal stream typically considered part of the Burnett system by water supply managers (Van Manen 1999). However, genetic distinctiveness between these rivers is also described for carp gudgeons (*Hypseleotris* sp. 5 Midgley's) (Thacker *et al*. 2007), Pacific blue-eye (*Pseudomugil signifier*) (Wong *et al*. 2004), and *Caridina* shrimp (Cook *et al*. 2008).

Population fragmentation by serial water storage impoundments is a major global freshwater management issue (Liermann *et al*. 2012), and one concerning the long-term viability of *E. albagula* throughout its range (Hamann *et al.* 2007; Limpus *et al*. 2011). Impoundment infrastructures have the potential to significantly impede in-stream movement of turtles, except for downstream dispersal during over-topping events associated with floods. Gene flow is critically important for maintaining genetic diversity at a metapopulation level. Small, isolated populations become vulnerable to genetic losses associated with genetic drift and inbreeding, less adaptable to further environmental change, and more vulnerable to local extinction (Reed & Frankham 2003; Hughes 2007). Negative genetic impacts on populations fragmented by dams have been demonstrated in simulation studies (Jager *et al.* 2001), and recorded for freshwater-limited (Macquarie perch *Macquaria australasica*, Faulks *et al*. 2011) and anadromous fishes (brown trout *Salmo trutta*, Horreo *et al.* 2011). The present study offers a unique window into patterns of genetic structure and connectivity pre-human influence on the environment, as long generation times mean that even the oldest dams in drainages inhabited by *E. albagula* (< 100 years) will not yet have had a detectable influence on genetic structure. Gene flow is important for maintaining genetic diversity within drainages for this species and serial modern impoundments may have serious negative impacts for long-term population persistence. Maintenance of natural levels of connectivity across artificial in-stream barriers as well as the continuum of permanent flowing habitat via environmental flows should be management priorities.

CONCLUSIONS

Chapter 3 highlights the shared evolutionary history of a diverse regional biota. Biological and geological evidence together support the stability of the Fitzroy River system over considerable evolutionary time, and by contrast, a recent historical confluence of the Burnett and Mary Rivers. Stability of some and alteration of other drainage boundaries in the study area possibly contributed to its particularly high levels of taxonomic diversity, endemism, and genetic population structure.

These systems may also represent important historical freshwater refugia, making them significant targets for conservation in the face of predicted global climate change.

Work presented in Chapter 3 also provides important information for the spatial classification of freshwater biodiversity within eastern Australia relevant to conservation management. As an indicator species, conservation efforts targeted towards *E. albagula* are likely to have positive flow-on effects for other sensitive aquatic taxa in this region (e.g. other riverine turtles, lungfish and others). More generally, targeted conservation efforts for freshwaters should recognise the spatial distribution of aquatic biodiversity at regional and local scales, including important subdivision among individual river drainages. Better characterisation of such patterns should be a global conservation priority as increasing pressure is put on riverine ecosystems.

CHAPTER 4 – A BIOGEOGRAPHIC HISTORY AND TIMELINE FOR THE EVOLUTION OF AUSTRALIAN SNAPPING TURTLES (*ELSEYA*: CHELIDAE) IN AUSTRALIA AND NEW GUINEA

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Image: An Australian snapping turtle. In this case, an adult female *Elseya albagula*. Photo by Blanche D'Anastasi.

CHAPTER 4 – A BIOGEOGRAPHIC HISTORY AND TIMELINE FOR THE EVOLUTION OF AUSTRALIAN SNAPPING TURTLES (*ELSEYA*: CHELIDAE) IN AUSTRALIA AND NEW GUINEA

SUMMARY

Australian snapping turtles (*Elseya* spp.) were used to examine biogeographic hypotheses associated with changes to riverine connectivity through eustatic sea level change, landform development and aridity across their natural range in Australia and New Guinea. Phylogenetic relationships were reconstructed for all extant species of *Elseya* plus two putative species not yet described, using a multi-locus molecular approach employing mitochondrial (control region, ND4 and 16S) and nuclear (R35 intron) loci. A calibrated relaxed molecular clock was used to estimate divergence times, using calibration information from the fossil and geological records. Intra-specific lineage structure and diversity was investigated using control region sequences analysed via haplotype networks and AMOVA. Four divergent clades within the genus *Elseya* corresponded geographically to all of New Guinea, Southern New Guinea plus northern Australia, north-eastern Australia, and south-eastern Australia. These are estimated to have arisen in the Late-Miocene (between ~5.82-9.70 Ma), diversifying further in the early Pleistocene (between ~2.20-2.43 Ma), coincident with major phases of aridity and climatic upheaval. The current study provides fresh insights into historical freshwater faunal relationships between Australia and New Guinea, with convincing evidence for separate vicariant events establishing the two endemic New Guinean *Elseya*– one recent (Pleistocene, *E. branderhorsti*) and another considerably older (Late-Miocene, *E. novaeguineae*). Genetic data reveal a long vicariant history for the genus *Elseya* in Australia and New Guinea, which appears closely tied to the disconnection of fluvial habitat through landform evolution, sea level rise, and ongoing aridification since the Late-Miocene. Data presented in Chapter 5 paints a more complete picture of freshwater biogeography in Australia and congruence with patterns well described for terrestrial groups implies a collective response of the Australian fauna to aridification.

INTRODUCTION

Australia's rich and highly endemic biota has evolved largely in isolation and against a unique backdrop of remarkable geological stability but dramatic climatic change. Since the Mid-to-Late-Miocene, Australia has undergone a progressive transition from its temperate Gondwanan origins to increasingly arid and more capricious climatic conditions, culminating in extreme aridity during

periodic Pleistocene glacial maxima (Crisp *et al*. 2004; Martin 2006; Byrne *et al*. 2008). These events generated striking patterns of regional endemism across the continent for terrestrial vertebrates (Cracraft 1991) and freshwater fishes (Unmack 2001), and are hypothesised to reflect long-term patterns of habitat availability. Much recent phylogeographic research has confirmed this for terrestrial taxa, whereby deepest divergences between genera and infrageneric groups coincide with the onset of desiccation in the Mid-to-Late-Miocene, while Pleistocene oscillations drove mostly species and population-level diversification (reviewed in Byrne *et al*. 2008, 2011). Conversely, evolution of Australia's freshwater biodiversity in this context remains largely speculative, as detailed molecular phylogenies of widespread freshwater-dependent groups are rare. Aquatic taxa are expected to be particularly susceptible to aridity and have unique dispersal constraints, making them especially sensitive biogeographic models. Therefore, understanding how freshwater communities have responded to paleoclimatic change is integral to our appreciation of Australia's unique biogeographic history.

Australia's extant freshwater biodiversity is concentrated along its northern, eastern, and south-western coastlines, within remaining mesic habitats divided by a vast arid interior. Studies to date suggest the response of some freshwater groups to advancing aridity parallels that of their terrestrial mesic-adapted counterparts, with vicariance by habitat disjunction being a major driver of divergence. For example, Late-Miocene regional vicariant speciation is described for *Cherax* crayfish (Munasinghe *et al*. 2004), and simultaneous vicariance among isolated mountain refugia drove Pliocene speciation within cold-adapted rainforest specialist crayfish, *Euastacus* spp. (Ponniah & Hughes 2004). A detailed biogeographic assessment of Australian freshwater fishes (Unmack 2001) revealed gradation of species ranges across northern and eastern coastlines that implies either a lack of distinct biogeographic barriers or largely idiosyncratic dispersal. Molecular studies of a few ichthyofaunal groups nevertheless reveal complex biogeographic patterns that do not necessarily reflect accepted zoogeographic provinces (McGuigan *et al*. 2000; Unmack & Dowling 2010). Moreover, while some species-level studies reveal deep (pre-Pleistocene) phylogenetic divisions implying long-standing barriers to dispersal (Wong *et al*. 2004; Jerry 2008), others report limited genetic structure across extensive areas (Bostock *et al*. 2006). There is clearly a biogeographic complexity to Australian freshwaters that is not yet fully appreciated.

Freshwater turtles have proven sensitive biogeographic models in northern latitudes (Walker & Avise 1998), but remain largely overlooked in biogeographic studies on the southern continents despite unique family-level diversity. Freshwater turtles (Chelidae, Pleurodira) are among a handful of freshwater vertebrate taxa of Gondwanan origin still taxonomically and ecologically diverse and

geographically widespread in Australia. Their long evolutionary history *in situ*, extant diversity, and high local endemism make them good models for assessing competing theories regarding freshwater biodiversity evolution in Australia.

Australian snapping turtles (genus *Elseya*) are remarkable for their large size (up to 9 kg) and specialised ecophysiology, whereby they possess highly vascularised cloacal bursae facilitating aquatic respiration (Mathie & Franklin 2006). Being largely restricted to perennial riverine habitat, snapping turtles as a group are expected to be particularly sensitive to changes in surface flow and drainage architecture, both historically and contemporaneously (i.e. through river regulation and climate change). The genus occupies an extensive yet patchy distribution throughout northern and eastern Australia and New Guinea (Fig. 4.1), implying a long vicariant history. Fossils found outside the current range of *Elseya* indicate this genus once occupied now-arid drainages of central Australia (Thomson 2000), though they lack accurate age estimates. Allozyme electrophoresis has revealed that the genus constitutes a series of divergent allopatric taxa, once thought to be a single widespread species (Georges & Adams 1996). Though relationships within the genus remain unclear, a high reliance on riverine habitat means they may be expected to reflect historical fluvial connections across the landscape rather than long distance dispersal. Phylogeographic relationships within the group should thus provide important insights into multiple aspects of freshwater biogeography, including historical connectivity across major biogeographic regions, the location of potential long-term freshwater refugia, and the significance of specific biogeographic barriers relevant over more recent timescales.

Distribution of *Elseya* species covers two major areas of recent interest in relation to Australian freshwater biogeography: the first is the periodic formation of Lake Carpentaria and the second is a major arid corridor associated with the Burdekin Gap. Lake Carpentaria (Fig. 4.1) formed periodically on the Sahul shelf off northern Australia during low sea levels (Torgersen *et al*. 1983; Chivas *et al*. 2001; Reeves *et al*. 2008). The vast paleolake system potentially facilitated historical though intermittent freshwater connectivity between Australia and New Guinea. Plio-Pleistocene connectivity throughout the Gulf and between northern Australia and southern New Guinea is demonstrated for several freshwater groups (freshwater shrimp, de Bruyn *et al*. 2004; snake-necked turtles, Alacs 2008; and several fishes, Cook & Hughes 2010; Huey *et al*. 2010; Cook *et al*. 2012). However, all of these species tolerate swampy or brackish conditions and riverine specialist taxa have not yet been investigated. Direct riverine connectivity between northern Australia and New Guinea may also have occurred west of Lake Carpentaria, via coastal rivers draining west off the Arafura Sill (see Voris 2000).

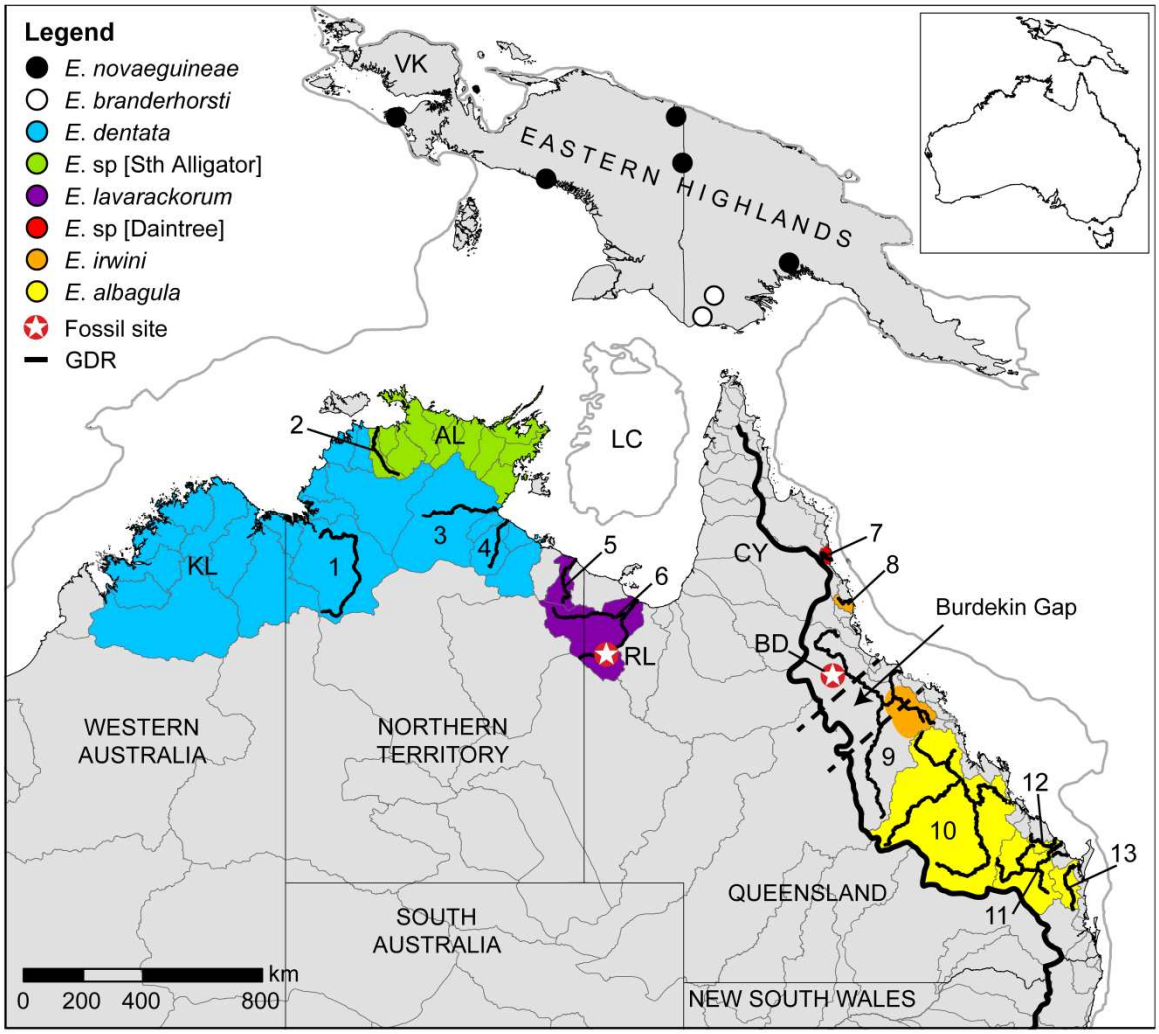

Fig. 4.1 Map showing distributions of *Elseya* species across Australia and New Guinea (Georges & Thomson 2010; present study), with features mentioned in-text. Lake Carpentaria (LC) and the Australian continental shelf (-200m contour) are outlined in light grey. On the Australian mainland, grey lines delineate drainage boundaries and black lines depict river networks where Australian *Elseya* spp. specimens were collected, as follows (left to right). Northern Territory: 1, Victoria R; 2, Mary R; 3, Roper R; 4, Limmen-Bight R; 5, Calvert R. Queensland: 6, Gregory-Nicholson R; 7, Daintree R; 8, Johnstone R; 9, Burdekin R; 10, Fitzroy R; 11, Burnett R; 12, Kolan R; 13, Mary R. Black dots (*E. novaeguineae*) and white dots (*E. branderhorsti*) represent sample locations for New Guinean taxa (Georges *et al*. 2013). Fossil sites are indicated at Riversleigh (RL) and Bluff Downs (BD), as are regions of the Kimberley (KL), Arnhem Land (AL), Cape York (CY), and Vogelkop Peninsula (VK). The Great Dividing Range (GDR) is indicated by a thick black line, running the length of the east coast. Position of the Burdekin Gap is indicated by dashed lines.

In the case of the Burdekin Gap on the east coast of Queensland (Fig. 4.1), the Burdekin drainage currently experiences the most severe aridity of any east coastal river system. Present and historical aridity in the Burdekin lowlands is a well-known habitat barrier defining north-eastern and south-eastern terrestrial lineages (Keast 1961; Simpson 1961; Cracraft 1986). Recent genetic studies of widespread freshwater fauna, including fishes (*Pseudomogil signifier*, Wong *et al*. 2004; *Tandanus* catfish, Jerry 2008) and platypus (*Ornithorhynchus anatinus*, Gongora *et al*. 2012), also implicate a major biogeographic barrier disrupting freshwater connectivity in this region. However, the depauperate fish fauna of the Burdekin has prevented appropriate sampling to discriminate between other possible drivers of divergence, for example, a major drainage divide between the extensive Burdekin and Fitzroy basins (Fig. 4.1).

The genus *Elseya* presents an ideal model to examine the response of a widespread freshwater group to ongoing aridification of the ancient Australian landmass, and as its intermittent connection with the much younger island of New Guinea. Accordingly, the current study examines the vicariant history of this group across its range using a molecular phylogenetic approach to 1) investigate patterns of lineage divergence at continental, regional and local scales, 2) propose a timeline for the evolutionary diversification of the genus to establish important drivers of diversification, and 3) examine alternative hypotheses relating to important biogeographic features. Specifically, the following scenarios are investigated: a) that Lake Carpentaria acted as a habitat barrier to riverine *Elseya* spp., isolating populations to the west and east of what is now the Gulf of Carpentaria in northern Australia; b) whether riverine connectivity between northern Australia and New Guinea occurred to the west of Lake Carpentaria; and c) whether genetic relationships among east coast taxa are consistent with a barrier posed by climatic aridity (i.e. divergence of populations across the Burdekin Gap) or a major drainage divide (i.e. divergence of populations across the Burdekin-Fitzroy boundary).

METHODS

Taxa

Distributions of *Elseya* species are summarised in Fig. 4.1. In the south-east, *E. albagula* occupies the Fitzroy, Burnett, Mary and Kolan drainages. *Elseya irwini* occurs in adjacent headwaters of the Burdekin drainage (Bowen and Broken Rivers) as well as disjunct populations in the Johnstone River and Hartley Creek, further north. The Hartley Creek population may be introduced. An undescribed form of uncertain affinity was recently discovered in the nearby Daintree River (J. Schaffer, E. V. Todd and C. J. Hoskin, unpubl. data). *Elseya lavarackorum* is known from spring-

fed streams of the Gregory-Nicholson and Calvert drainages in the southern Gulf of Carpentaria. Populations currently assigned to *E. dentata* occupy a wide distribution from the MacArthur River in the western Gulf to the Fitzroy River in Western Australia, though these populations may represent a species complex (Georges & Thomson 2010). A distinct undescribed species inhabits rivers flowing north from the Arnhem Land plateau (*Elseya* sp. [Sth Alligator] of Georges & Adams 1992). Two further species are known from New Guinea, but have overlapping ranges that differ vastly in extent. *Elseya branderhorsti* is restricted to southern New Guinea. *Elseya novaeguineae* is the only chelid widespread throughout New Guinea and associated islands. Throughout, taxonomy follows that of Georges and Thomson (2010) with the exception of *Elseya novaeguineae*, which is retained in the genus *Elseya* (van Dijk *et al*. 2012; Le *et al*. 2013; Georges *et al*. 2013).

Tissue samples were obtained from 325 individuals across 13 Australian drainages representing all six described *Elseya* species plus two putative species not yet described (Table 4.1; Fig. 4.1; Appendix 4A). For Australian taxa, samples were collected from multiple drainages per species to investigate intra-specific lineage diversity and among-drainage divergence. Tissues were sourced from existing collections, through collaborations with other researchers, or during targeted field work. Field capture of turtles and tissue sampling was performed as described in Chapter 3. Phylogeography of the New Guinean endemics *Elseya branderhorsti* and *E. novaeguineae* is to be published elsewhere (Georges *et al*. 2013). Here, *E. novaeguineae* is represented by two haplotypes from each of three major clades (northern, southern, and western New Guinea), and *E. branderhorsti* by two haplotypes from its limited southern New Guinean distribution, drawn from the above-mentioned study of Georges *et al*. Three other Australian chelid turtles, *Emydura macquarii krefftii*, *Myuchelys latisternum* and *M. georgesi*, were used as outgroups.

Genes

Sequence data from three mitochondrial DNA (mtDNA) fragments: control region (CR, ~500 bp), NADH dehydrogenase subunit 4 with 3′ transfer RNA (ND4, ~800 bp), and 16S ribosomal RNA (16S, ~300 bp), plus one nuclear intron: RNA fingerprint protein 35 (R35, ~500 bp), were used. Loci were selected to provide markers evolving at relatively fast, medium, and slow rates so to be phylogenetically informative at various levels of divergence. CR and ND4 were sequenced for all samples. Up to five specimens from individual drainage basins were sequenced at 16S. R35 was sequenced for single individuals from a subset of five species representing major lineages identified in preliminary analyses (*E. albagula*, *E. irwini*, *E. dentata*, *E. branderhorsti*, *E. novaeguineae*).

Table 4.1 Sample numbers and sampling locations of *Elseya* spp. collected for phylogeographic analysis. Sequences from New Guinean samples are derived from Georges *et al*. (2013). See also Fig. 4.1.

Drainage (Region)	**Species**	***N***
New Guinea		
Aer Besar R (Western NG)	*E. novaeguineae*	1
Missool R (Western NG)	*E. novaeguineae*	1
Sepik R (Northern NG)	*E. novaeguineae*	1
Tami R (Northern NG)	*E. novaeguineae*	1
Aika R (Southern NG)	*E. novaeguineae*	1
Kikori R (Southern NG)	*E. novaeguineae*	1
Merauke R (Southern NG)	*E. branderhorsti*	1
Fly R (Southern NG)	*E. branderhorsti*	1
Australia		
Victoria R (Northern Territory)	*E. dentata*	5
Roper R (Northern Territory)	*E. dentata*	2
Limmen-Bight R (Northern Territory)	*E. dentata*	3
Mary R (Arnhem Land)	*Elseya* sp. [Sth Alligator]	1
Gregory-Nicholson R (Gulf)	*E. lavarackorum*	28
Calvert R (Gulf)	*E. lavarackorum*	10
Daintree R (Qld Wet Tropics)	*Elseya* sp. [Daintree R]	5
Johnstone R (Qld Wet Tropics)	*E. irwini*	47
Burdekin R (Qld Wet Tropics)	*E. irwini*	31
Fitzroy R (SE Qld)	*E. albagula*	94
Burnett R (SE Qld)	*E. albagula*	67
Mary R (SE Qld)	*E. albagula*	29
Kolan R (SE Qld)	*E. albagula*	3

Laboratory procedures and sequencing

Genomic DNA extractions were performed as described in Chapter 2. Target mtDNA loci were PCR-amplified under identical reaction and cycling conditions, and using primers for CR and ND4, described in Chapter 3, except in the following circumstances. For some samples, CR required amplification using a 'touchdown' protocol to inhibit non-specific product. Annealing temperature was held initially at 55 °C for 10 cycles, decreased stepwise by 0.5 °C per cycle for 10 cycles, and then held at 50 °C for a final 25 cycles. CR amplification in *E. lavarackorum* required alternative primers (13a_F, 5'-GACCTACTAATCCTAACATGAATCG-3′; 13a_R, 5′-TGCCATGCTTTGGTATAAGC-3′) initially designed to amplify the entire CR between 3′ Cytochrome *b* and 5′ tRNA-Phe during whole mitochondrial genome reconstruction in *E. albagula* (E. V. Todd *et al*. unpubl. data), and a touchdown protocol as above with 2.0 mM $MgCl_2$. 16S sequences were amplified using primers published previously (M89(L), 5′-AGGAGTGATGCCTGCCCAGTGAC-3′; M90(H), 5′-CCTTAATAGCGGCTGCACCATTAGGA-3′, Georges *et al*. 1998). PCR products were purified and sequenced commercially by Macrogen Inc. (Seoul, South Korea) in forward and reverse directions (CR, 16S, R35) or forward in duplicate (ND4, due to poor quality reverse sequence) to confirm sequence fidelity. CR and ND4 sequences for *E. albagula* and *E. m. krefftii* were available from Chapter 3 and 5, respectively. MtDNA sequences for *M. latisternum* and *M. georgesi* were obtained from complete mitochondrial genomes (A. Georges *et al*. unpubl. data). R35 sequences were provided by Arthur Georges at the University of Canberra, and were generated using primers and methodology described in Alacs (2008).

Sequences were aligned and edited in GENEIOUS PRO 5.6 (Biomatters, available from http://www.geneious.com/) and checked for ambiguous bases and numts as described in Chapter 3. Sequences were then collapsed to haplotypes for phylogenetic analysis, with final alignments generated using CLUSTALX in MEGA 5 (Tamura *et al*. 2011). Unique sequences generated during the current study were submitted to GenBank (accession numbers KF255934- KF255982).

Phylogenetic analysis

Phylogenetic relationships within the genus *Elseya* were reconstructed using maximum likelihood (ML), Bayesian inference (BI), and maximum parsimony (MP) approaches implemented in GARLI 2.0 (Zwickl 2006), MRBAYES 3.2 (Ronquist *et al*. 2012), and MEGA 5 (Tamura *et al*. 2011), respectively. Because single-locus analyses produced largely congruent, though often poorly-supported trees (data not shown), analyses were performed on a concatenated alignment of CR,

ND4, 16S and R35 sequences using data partitioning (described below). The alignment represented 339 individuals from 11 species and included three outgroups. Data were missing for 16S at six of 11 *E. albagula* multi-locus haplotypes, and for R35 in *Elseya* sp. [Sth Alligator], *E. lavarackorum*, *Elseya* sp. [Daintree], *Emydura m. krefftii* and *Myuchelys georgesi*. Hypervariability of the CR makes it potentially unsuitable for inferring deeper evolutionary relationships and analyses were also performed without this locus. Alignment gaps were treated as missing data in all analyses.

Prior to ML and BI analyses, optimal partitioning strategies for the concatenated datasets, including best-fit models of nucleotide substitution for each sequence subset, were selected from among all possible strategies in PARTITIONFINDER 1.0 (Lanfear *et al*. 2012) using the Bayesian Information Criterion (BIC) (Table 4.2).

ML analyses were conducted from a random starting tree in each of 10 independent runs. BI analyses used default settings as priors, random starting trees, and four Markov chains (three hot, one cold), sampled every 100 generations over 5 million MCMC generations in each of two independent analyses. Branch lengths and rates were unlinked and model parameters estimated independently across partitions. Convergence was assessed by ensuring the average standard deviation of split frequencies (SDSF) of independent runs fell below 0.01 and that effective sample sizes (ESS) of posterior estimates were suitably high (> 200). Convergence of model and tree parameter estimates were assessed by plotting outputs in TRACER 1.5 (available from http://beast.bio.ed.ac.uk/Tracer) and AWTY (Nylander *et al*. 2008), respectively. MP analyses were performed using heuristic closest-neighbour-interchange searches with 1000 initial random starting trees and a search level of three. Statistical support for nodes was determined using non-parametric bootstrap values (1000 ML/MP pseudoreplicates) and Bayesian posterior probabilities (pp) (first 25% of sampled trees discarded as ‘burn-in’). Bootstrap values of at least 70% and pp values of at least 95% were considered adequate support for a given node.

ND4 evolutionary distances (mean uncorrected *p*-distances) within and among taxa were calculated in MEGA as a common measure of genetic divergence comparable across studies of other reptiles and fishes (see Vargas-Ramírez *et al*. 2010; Fritz *et al*. 2012).

Table 4.2 Details of data partitioning strategies[1] employed for different DNA alignments used in phylogenetic analyses of *Elseya* spp. from Australia and New Guinea.

Alignment	No. of Subsets	Subset partitions	Best model	No. of parameters	Log-likelihood	BIC Score
Without CR	3	ND4_1, tRNA, 16S	TVM+G	78	-4995.6	10577.1
		ND4_2, R35	HKY+I			
		ND4_3	TrN+I			
With CR	4	CR	HKY+G	114	-7837.9	16558.9
		ND4_1, tRNA, 16S	TVM+G			
		ND4_2, R35	HKY+I			
		ND4_3	TrN+I			

[1]Partitioning scheme and models selected by PARTITIONFINDER using the BIC criterion.

Divergence date estimation

Divergence dates for major *Elseya* lineages were estimated using a Bayesian relaxed molecular clock approach (Drummond *et al*. 2006) performed in BEAST 1.7.4 (Drummond *et al*. 2012). Analyses were performed without the CR and additional outgroup taxa were used (*Emydura victoriae*, *E. subglobosa worrelli* and *Rheodytes leukops*, and the more distantly related snake-necked species *Chelodina rugosa* and *C. longicollis*) (see Appendix 4A for sample details). A relaxed uncorrelated lognormal molecular clock with a Birth-Death speciation prior (Gernhard 2008) was applied, enforcing monophyly of the ingroup and partitioning the dataset into mitochondrial (ND4, 16S) and nuclear (R35) data with a HKY+G and HKY+I substitution model, respectively.

Fossils and geological events were used as time calibrations to constrain the tree. Few reliable fossil calibrations are available for Australian chelid turtles, owing to a sparse fossil record and a lack of consistent osteological characters for diagnosing fossil taxa to genus (Thomson 2000). *Elseya nadibadjagu*, a well-described fossil from early Pliocene deposits in Bluff Downs, Queensland (Thomson & Mackness 1999), was applied to constrain the age of the 'Queensland radiation' (i.e. *E. albagula*, *E. irwini*, *Elseya* sp. [Daintree] and *E. lavarackorum*), because it is a likely ancestor of this group (Thomson *et al*. 1997; S. Thomson pers. comm.). Radiometric dating of the lava flow overlying the fossil formation provides a minimum date of 3.6 ±0.5 Ma (Mackness *et al*. 2000) to which a lognormal prior distribution (offset 3.1, median 5.1, 95% CI 3.6-11.0 Ma) was applied. A second calibration point, based on geological knowledge of the uplift of New Guinea's Central Highlands (Pigram & Davies 1987), was used to constrain divergence of northern and southern *E. novaeguineae* lineages (normal distribution, median 5.0, 95% CI 3.0-7.0 Ma). A date of 72.2 Ma (95% CI 59.8-116.8 Ma) was used to constrain the root of the tree, following the estimated divergence between *Elseya* and *Chelodina* lineages by Heath (2012).

Two independent MCMC simulations were run in BEAST over 50 million generations, sampling every 5,000 generations. Runs were combined using LOGCOMBINER 1.7.2 (10% 'burn-in' each) and trace files and summary statistics were monitored for convergence using TRACER and AWTY. The final calibrated chronogram with node estimates and confidence intervals was produced in TREEANNOTATOR 1.7.2 and edited using FIGTREE 1.3.1 (available from http://tree.bio.ed.ac.uk/software/figtree/).

Population genetics

Intra-specific genetic diversity and population structure (i.e. within and among drainage basins) were investigated using CR sequences, which are hypervariable and therefore suitable for population-level comparisons. Diversity statistics including number of haplotypes (N_h), number of variable sites (V), nucleotide diversity (π) and haplotype diversity (H_d) were calculated in DNASP 5.10 (Librado & Rozas 2009) and ARLEQUIN 3.5 (Excoffier & Lischer 2010). For species sampled across multiple catchments, population genetic structure and the geographical distribution of haplotype diversity were visualised with minimum spanning networks estimated in ARLEQUIN using the pairwise distance method. Global Φ-statistics were also calculated by partitioning total genetic variance among catchments using AMOVA in ARLEQUIN (pairwise distance, 10,000 permutations).

RESULTS

Molecular diversity

The final combined alignment including the CR (of 339 individuals from 11 species) contained 2314 nucleotides, including 415 (17.9%) parsimony informative (PI) sites, which described 45 unique multilocus haplotypes. The dataset excluding CR contained 1872 nucleotides, including 249 (13.3%) PI sites describing 30 unique multilocus haplotypes. Further details are presented in Table 4.3.

Phylogeny

Phylogenetic analyses performed with or without the CR produced congruent topologies strongly supporting monophyly of the genus *Elseya* and revealing four divergent lineages: 1. *E. novaeguineae* clade comprising the six haplotypes from New Guinea; 2. *E. dentata* clade, comprising *E. dentata* and *Elseya* sp. [Sth Alligator] from the Northern Territory, and *E. branderhorsti* from southern New Guinea; 3. *E. lavarackorum* clade comprising *E. lavarackorum*, *E. irwini* and *Elseya* sp. [Daintree] from north-eastern Queensland; and 4. *E. albagula* clade in south-eastern Queensland. The Bayesian phylogenetic tree for the partitioned analysis of the dataset including the CR, with branch support values for ML, BI, and MP analyses, is presented in Fig. 4.2.

Table 4.3 Details of aligned DNA regions used for phylogenetic analysis of *Elseya* spp. from Australia and New Guinea, including total alignment length (in bp), number of conserved (C) and variable (V) sites, number of parsimony informative (PI) sites and G/C content (GC%).

Alignment	**Total bp**	**C**	**V**	**PI**	**GC%**
Individual loci					
CR	487	273	212	170	35.6
ND4	870	657	213	180	38.3
16S	455	380	73	55	42.8
R35	502	482	20	12	36.0
Concatenated					
Without CR	1872	1519	306	249	39.0
With CR	2314	1793	517	415	41.2

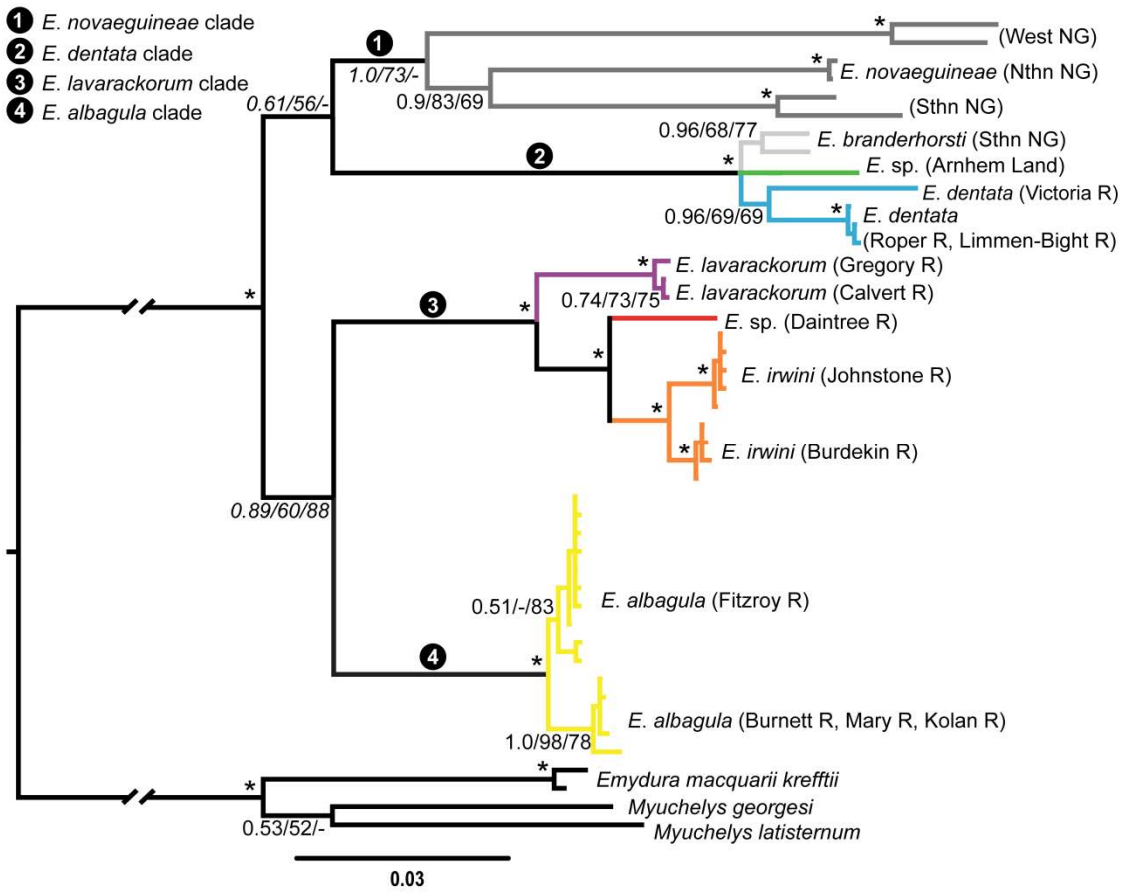

Fig. 4.2 Bayesian majority rule consensus tree from a partitioned phylogenetic analysis of snapping turtles (genus *Elseya*) from Australia and New Guinea (2314 bp: CR, ND4, 16S, R35). This tree is congruent with those generated by maximum likelihood and parsimony analyses. Stars indicate nodes that received strong statistical support (≥ 70% bootstrap support for likelihood or parsimony; ≥ 0.95 posterior probability for Bayesian) at all three analyses (Bayesian, likelihood and parsimony). Deep node support values (italics) reflect analyses performed without the CR data (see text for details). Terminal branches are coloured by species, consistent with Fig. 4.1.

Each of the four major clades, and most major divisions within them, received strong statistical support. However, relationships among the major clades were poorly supported, especially the node uniting the *E. novaeguineae* and *E. dentata* clades. In analyses excluding the CR, support values for deeper nodes were on average 14.6% (range 2.1%-25.4%) higher than in analyses including this locus. The former values are presented for deep nodes in Fig. 2. Support values at shallower nodes were similar despite inclusion or exclusion of the CR, suggesting this locus is saturated at deeper nodes but phylogenetically informative for more recent relationships in this group.

Within each clade, regional patterns were also evident and are further supported by mean inter-specific *p*-distances for ND4 haplotypes (Table 4.4). In agreement with the analysis of Georges *et al*. (2013), the three sub-clades within *E. novaeguineae*, corresponding to western, northern, and southern New Guinea, are considerably divergent (intra-specific ND4 divergence 5.7%), with haplotypes from the Vogelkop Peninsula basal to a clade comprising northern and southern haplotypes (Fig. 4.2). *Elseya branderhorsti*, from southern New Guinea, is most closely related to species from the Northern Territory. An undescribed species from Arnhem Land, previously identified from allozyme data (Georges & Adams 1992), is distinct from *E. dentata* and *E. branderhorsti*. However, relationships among these three taxa were unresolved, with the Arnhem Land form being variably placed as either basal, sister to *E. branderhorsti*, or in an unresolved trichotomy. ND4 sequence divergences suggest it is genetically more similar to *E. branderhorsti* (1.4% divergence) than *E. dentata* (2.3% divergence). There is differentiation within *E. dentata* among westerly (Victoria River) and easterly (Roper and Limmen-Bight Rivers) flowing drainages. Intra-specific ND4 divergence within *E. dentata* was 1.08%, but was ≤ 0.5% within other taxa. Relationships within the *E. lavarackorum* clade were especially well-supported. *Elseya lavarackorum* from the southern Gulf is most closely related to species on the north-east coast (100% support) despite its close geographic proximity to Northern Territory taxa. The undescribed Daintree River form is distinct and most closely related to *E. irwini* (1.5% ND4 divergence). Disjunct populations of *E. irwini* inhabiting the Johnstone and Burdekin drainages are differentiated but closely related. Within the final clade, which consists solely of *E. albagula* from south-eastern coastal Australia, differentiation is evident between the Fitzroy and Burnett/Mary/Kolan drainages.

Table 4.4 Percent evolutionary divergences (S.E.) among species in the genus *Elseya*, calculated for an 870 bp fragment of the mitochondrial ND4 locus (uncorrected *p*-distances). Values in bold highlight groups with high genetic similarity. Values in italics represent intra-specific divergences.

	1	2	3	4	5	6	7	8	9
1. *E. novaeguineae*	*5.73(0.59)*								
2. *E. branderhorsti*	6.9(0.7)	*0.51(0.26)*							
3. *Elseya* sp. [Sth Alligator]	6.9(0.8)	**1.4(0.4)**	-						
4. *E. dentata*	7.0(0.7)	**1.2(0.3)**	**2.3(0.4)**	*1.08(0.25)*					
5. *E. lavarackorum*	7.3(0.8)	7.6(0.9)	8.0(1.0)	7.3(0.8)	*0.12(0.11)*				
6. *Elseya* sp. [Daintree]	7.6(0.7)	7.8(0.9)	7.9(0.9)	7.3(0.9)	**2.0(0.5)**	*0.00(0.00)*			
7. *E. irwini*	7.7(0.8)	8.3(0.9)	8.4(0.9)	7.9(0.9)	**2.1(0.5)**	**1.5(0.4)**	*0.21(0.11)*		
8. *E. albagula*	7.2(0.8)	7.0(0.9)	7.2(1.0)	6.7(0.9)	**4.9(0.7)**	**4.8(0.7)**	**5.4(0.7)**	*0.38(0.15)*	
9. *Emydura m. krefftii*	11.4(1.0)	10.7(1.1)	11.0(1.1)	10.1(1.0)	11.2(1.0)	10.5(1.0)	11.3(1.1)	9.6(1.0)	*0.46(0.22)*

Divergence date estimation

Inclusion of additional outgroup taxa resulted in a final alignment of 1835 nucleotides, with 469 variable and 348 (74.2%) PI characters. The BEAST phylogeny produced an alternative topology that placed *E. novaeguineae* reciprocally monophyletic to the Australian clades (Fig. 4.3). This arrangement received full statistical support (Table 4.5). Examination of summary statistics found the coefficient of variation to be close to zero (mtDNA mean 0.23, 95% HPD $2.20E^{-4}$-0.45; R35 mean 1.11, 95% HPD $3.68E^{-1}$-2.49), meaning the data cannot reject a strict molecular clock and that there is not a high degree of rate variation among branches. However, the covariance parameters spanned zero (mtDNA mean -0.03, 95% HPD -0.45-0.21; R35 mean 0.0, 95% HPD -0.20-0.22), giving no strong evidence for autocorrelation and indicating the uncorrelated relaxed molecular clock model was appropriate in this case.

Divergence date estimates from BEAST suggested the four major lineages within *Elseya* diversified in the Late-Miocene. *Elseya novaeguineae* is estimated to have diverged from mainland lineages 9.7 Ma (95% HPD 6.96-12.54), while Northern Territory/southern New Guinea and Queensland lineages diverged 8.67 Ma (95% HPD 6.05-11.27). North-eastern and south-eastern Queensland lineages diverged in the very Late-Miocene, ~5.82 Ma (95% HPD 4.02-7.86). The three major clades within *E. novaeguineae* shared a common ancestor in the Late-Miocene (7.91 Ma, 95% HPD 5.47-10.47). Most recent common ancestors of the *E. dentata* and *E. lavarackorum* clades are estimated around the Plio-Pleistocene boundary: *E. lavarackorum* in the Australian Gulf diverged from relatives on the north-east coast coincidentally with initial divergence among Northern Territory/southern New Guinea species, 2.43 and 2.20 Ma (95% HPD 1.41-3.51 and 1.23-3.16), respectively. Further diversification within these clades: between *Elseya* sp. [Sth Alligator] and *E. branderhorsti* (southern New Guinea), *Elseya* sp. [Daintree] and *E. irwini*, and western and eastern *E. dentata* (Northern Territory) occurred in the late Pleistocene, 1.36-1.66 Ma.

Phylogeography and population genetics

Population genetic analyses of Australian *Elseya* spp. using CR data revealed generally low levels of intra-specific diversity despite extensive sampling in many areas. Values for H_d, π, and V within drainage basins were considerably lower for each species overall when sampled across multiple drainages (Table 4.6). Global Φ_{ST} values were large and highly significant in all cases, with between 82% and 99% of total variance distributed among drainages (Fig. 4.4). Variability within species can thus be attributed mostly to divergence among drainage basins. This result was reflected

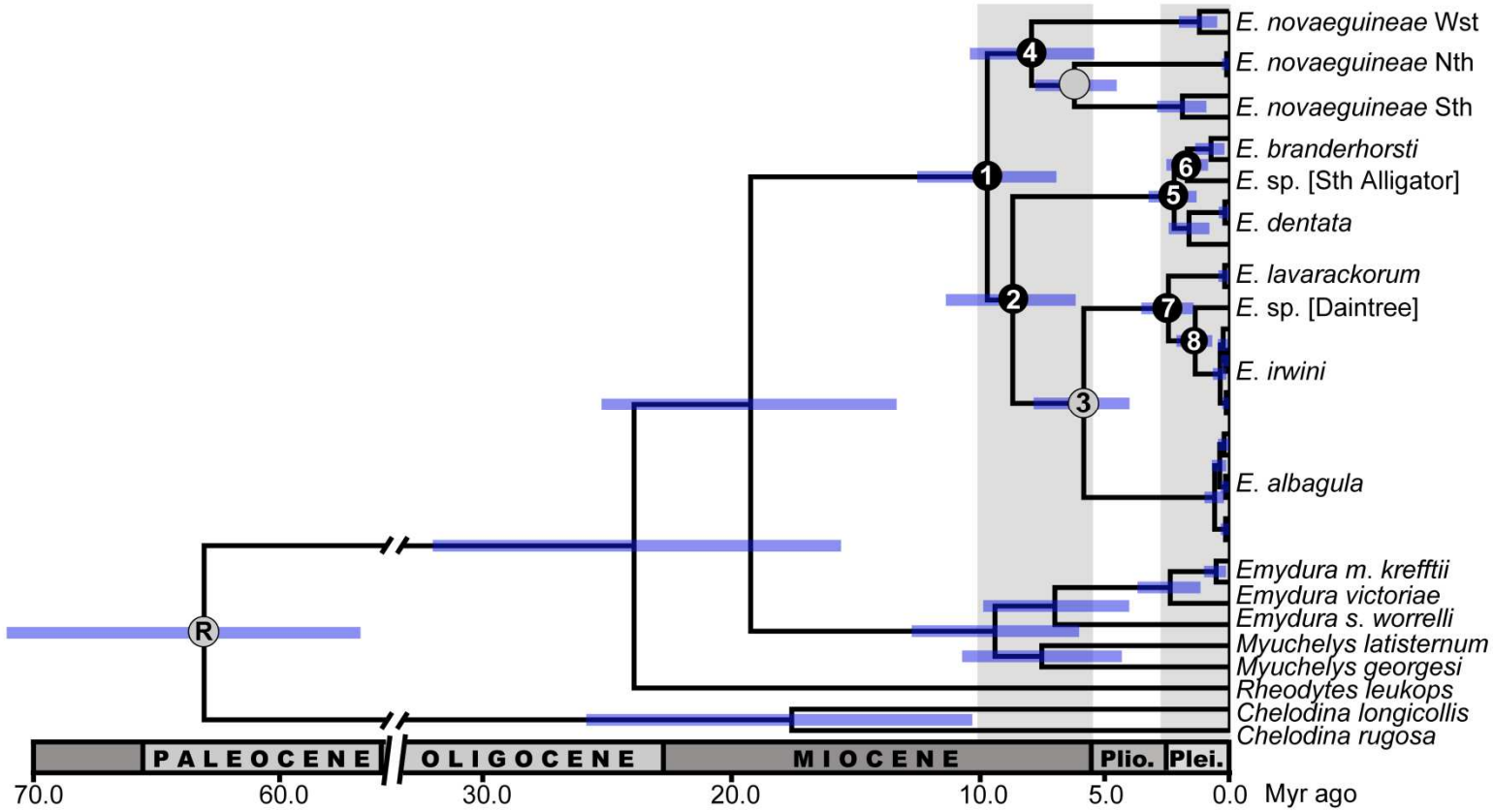

Fig. 4.3 Chronogram and topology of estimated divergence events for snapping turtles (*Elseya* spp.) from Australia and New Guinea, resulting from a partitioned (mitochondrial:nuclear) BEAST analysis of a 2287 bp dataset (ND4, 16S, R35; Birth-Death speciation tree prior; 2x50 million generations). A relaxed uncorrelated lognormal molecular clock was applied using three calibration points (grey nodes, see main text for details). Horizontal bars represent 95% highest posterior density estimates for node ages. Age estimates and branch support values are presented for numbered nodes within the *Elseya* in Table 4.5. Vertical grey bars highlight periods of major aridity in Australia coinciding with episodes of divergence for *Elseya* spp.

Table 4.5 Divergence estimates and support values for major nodes within the genus *Elseya* based on analyses in BEAST. Numbers are consistent with nodes in Fig. 4.3.

Node	**Branch support**	**Age estimate (Ma)**	**95% HPD (Ma)**
1. *Elseya*	1.0	9.70	6.96-12.54
2. Mainland	0.98	8.67	6.05-11.27
3. North-eastern+south-eastern	1.0	5.82	4.02-7.86
4. *E. novaeguineae*	1.0	7.91	5.47-10.47
5. Northern Territory+*E. branderhorsti*	1.0	2.20	1.23-3.16
6. *E. branderhorsti*+*Elseya* sp. [Sth Alligator]	0.68	1.66	0.87-2.53
7. North-eastern	1.0	2.43	1.41-3.51
8. *E. irwini*+*Elseya* sp. [Daintree]	1.0	1.36	0.64-2.09

Table 4.6 Diversity statistics for *Elseya* spp. sampled across northern and eastern Australia, based on a 487 bp fragment of the mitochondrial control region.

Species	***N***	N_h	***V***	H_d	π
Catchment					
E. dentata	**9**	**3**	**33**	**0.75**	**0.041**
Victoria R	4	1	0	-	-
Roper R	2	2	1	1.00	0.002
Limmen-Bight R	3	1	0	-	-
E. lavarackorum	**38**	**3**	**5**	**0.42**	**0.004**
Calvert R	10	2	1	0.82	0.002
Gregory R	28	1	0	-	-
***E.* sp. [Daintree]**	**5**	**1**	**0**	**-**	**-**
E. irwini	**86**	**6**	**22**	**0.53**	**0.020**
Johnstone R	55	3	2	0.07	<0.001
Broken R	31	3	3	0.24	0.001
E. albagula[1]	**193**	**14**	**32**	**0.73**	**0.018**
Fitzroy R	94	9	12	0.65	0.007
Burnett R	67	2	1	0.23	0.001
Mary R	29	3	2	0.29	0.001
Kolan R	3	2	6	0.67	0.008

N, sample size; N_h, number of haplotypes; *V*, variable sites; H_d, haplotype diversity; π, nucleotide diversity. [1]Values for *E. albagula* derived from Todd *et al*. (2013a).

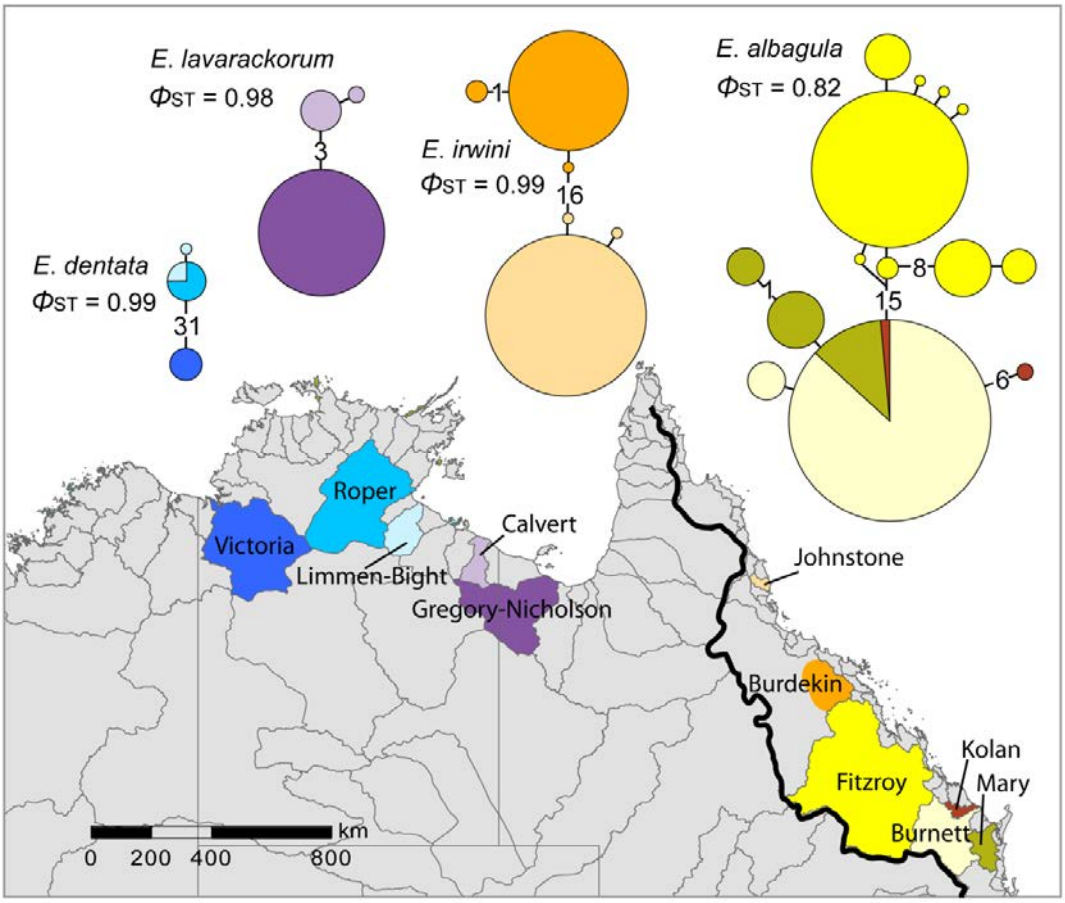

Fig. 4.4 Haplotype networks for four Australian species of *Elseya* based on control region mtDNA sequences, indicating divergence among individual drainage basins (global Φ_{ST} values). Individual circles represent unique haplotypes. Colours indicate drainage basins in which haplotypes were sampled and circle size represents sampling frequency. Connections represent one base pair differences between related haplotypes and numbers indicate additional changes.

in the species haplotype networks (Fig. 4.4), which demonstrate drainage basins are typically dominated by a single common haplotype and are differentiated from others by multiple mutational steps (from four in *E. lavarackorum* to 32 in *E. dentata*). Haplotype sharing across catchment boundaries was observed on only two occasions: between the Roper and Limmen-Bight Rivers (*E. dentata*), and the Burnett, Mary, and Kolan Rivers (*E. albagula*) (Fig. 4.4).

DISCUSSION

A phylogeny for the genus *Elseya*

A comprehensive hypothesis of phylogenetic relationships among Australian snapping turtles (genus *Elseya*) is presented, along with a timeline for their evolution throughout Australia and New Guinea. Molecular phylogenetic data highlight the diversity and endemism of this group across its range and resolves four divergent, geographically correlated lineages. Species from the Northern Territory and southern New Guinea form a distinct clade (*E. dentata* clade), as do species from eastern Australia, which exhibit further north-south divergence (*E. lavarackorum* and *E. albagula* clades). The widespread New Guinean taxon *E. novaeguineae* is either sister to the *E. dentata* group (Fig. 4.2) or basal within the genus (Fig. 4.3). Though the latter relationship received stronger statistical support (Table 4.5), the former reflects earlier analyses of allozyme data (Georges & Adams 1992) and the morphological assessment of Thomson *et al*. (1997), which discern two subgroups within the genus *Elseya* that distinguish Northern Territory and New Guinean species ('*E. dentata* subgroup', including *E. novaeguineae*) from Queensland forms ('Queensland subgroup', including *E. lavarackorum*).

The above phylogenetic hypotheses conflict with the recent molecular phylogeny of Le *et al*. (2013) (ND4, cytochrome *b*, R35 genes), which places *E. albagula* basal within the genus, *E. novaeguineae* as sister to *E. branderhorsti*, and *E. dentata* as sister to *E. irwini* within the *E. lavarackorum* clade. However, sequences for *E. dentata* referred to by Le *et al*. (2013) have since been identified as *E. irwini*, partially accounting for the conflicting topology and leaving Northern Territory species unsampled in their analysis. Reanalysis of the Le *et al*. data by Georges *et al*. (2013) corrects the miss-identification and results in a phylogeny consistent with that of the current study, excepting that *E. albagula* remains basal instead of being sister to other Queensland forms as was found herein (Fig. 4.2 and 4.3).

Affinities of extant clades may lie with extinct lineages, making it difficult to reconstruct deeper relationships. Primitive undated fossil *Elseya* uncovered from paleo-lakes in South Australia may represent an extinct radiation from Australia's now-arid interior (Thomson 2000). The basal uncertainty in the current tree may also reflect close successive divergence of major lineages, as implied by the molecular dating analyses (Fig. 4.3). Similarly low support for basal

nodes is reported in studies of other Australian freshwater taxa despite multi-gene phylogenies, especially with regards to divergent New Guinean clades (e.g. *Cherax* crayfish, Munasinghe *et al*. 2004; Melanotaeniid rainbowfish, McGuigan *et al*. 2000; hardyhead *Craterocephalus* spp., Unmack & Dowling 2010). Rapid diversification or simultaneous vicariance can make it difficult to resolve phylogenies even with increasing amounts of genetic data (Hoelzer & Melnick 1994). Additional morphological, paleontological and (potentially) nuclear genetic data will be important in unravelling the deepest relationships within the genus *Elseya*.

Continental biogeography and drivers of diversification

The genus *Elseya* exhibits a striking degree of local endemism, which together with their serially allopatric distribution spanning northern and eastern Australia and New Guinea, makes them exemplary models for uncovering biogeographical processes underlying the evolutionary development of Australia's extant freshwater biodiversity. Genetic relationships within the group reflect a hierarchical pattern of divergence at continental (among regions), regional (within regions among species), and local (within species among drainages) scales. Vicariant speciation within the group apparently reflects progressive regional isolation of once much more widespread lineages, consistent with the known climatic and geological history of Australia.

Divergence date estimates (Fig. 4.3) suggest the four major *Elseya* lineages arose in the Late-Miocene, between ~5.82-9.70 Ma. This was a time of global drying and cooling (Zachos *et al*. 2001; Flower & Kennett 1994), which in Australia marked the end of previously stable, warm-wet environments (Byrne *et al*. 2008). Australia's rainforest-dominated vegetation gave way to sclerophyllous communities (Crisp *et al*. 2004; Martin 2006) and the collapse of major depositional systems implies reduced rainfall and the demise of once extensive interior drainages (Quilty 1994; Jones 2006). The onset of major aridity in Australia conceivably drove contraction and fragmentation of previously more continuous fluvial habitats that provided greater dispersal opportunities for freshwater taxa. Regional endemic speciation associated with Late-Miocene aridity is also suggested for *Cherax* crayfish (Munasinghe *et al*. 2004), another Gondwanan relic. Phylogenetic analyses presented herein provide further evidence that the onset of aridity in Australia drove regional vicariant divergence among freshwater lineages.

Younger divergence estimates correlate with more recent episodes of climatic instability. Regional diversification within major snapping turtle clades coincides with the Plio-Pleistocene transition (~2.7 Ma). Following a brief return of more mesic conditions in the Early-Pliocene, the Plio-Pleistocene transition marks the onset of global glacial-interglacial climatic oscillations (Kennett & Hodell 1993). Though Australia remained essentially unglaciated, glacial maxima were characterised by low sea levels, low temperatures, and extreme aridity (Williams 1984).

Further speciation events (at 2.20-2.43 Ma and 1.36-1.66 Ma) are coincident with documented transitions in global climate dynamics at ~2.7 Ma and ~1.4 Ma (Lisiecki & Raymo 2007). The youngest *Elseya* fossils recovered west of the Great Dividing Range are from Plio-Pleistocene deposits in south-eastern Australia (*E. uberima*, Thomson 2000). Therefore, *Elseya* spp. apparently persisted in inland Australia, where extant populations no longer occur, until its full aridification 1 to 4 Ma (Fujioka & Chappell 2010), concurrent with the demise of lungfish, flamingos and crocodiles from the Murray Basin (Hope 1982).

Australia-New Guinea relationships

The current study provides fresh insights into historical freshwater faunal relationships between Australia and New Guinea, with evidence for separate vicariant events establishing the two endemic New Guinean *Elseya*– one recent (Pleistocene, *E. branderhorsti*) and another considerably older (Late-Miocene, *E. novaeguineae*). New Guinea's complex biogeography reflects its development as a collage of landmasses at the collision boundary between the Australian and Pacific plates (Pigram & Davies 1987). Northern and western regions of New Guinea consist of aggregated island arcs that docked from the Late-Oligocene to the Pliocene (Pigram & Davies 1987). Southern New Guinea is part of the Australian craton and shares a close biogeographic relationship with northern Australia, considered to reflect extensive faunal exchange during low Pleistocene sea levels that exposed the Sahul shelf (Heinsohn & Hope 2006). New Guinea's freshwater biodiversity is concentrated in the southern lowlands, consistent with most dispersal to and from Australia occurring since uplift of the Central Highlands ~5 Ma. *Elseya branderhorsti* apparently also shares this biogeographic history. Divergence between it and Northern Territory *Elseya* (1.2-1.4%, Table 4.4.) was lowest of any species pair in the current analysis, indicating gene flow between Australia and New Guinea before sea levels rose.

Elseya novaeguineae is the only chelid found north of the New Guinean Highlands. Its distinctiveness, widespread distribution, and deep lineage structure among western, northern, and southern populations implies a long evolutionary history *in situ* (discussed in Georges *et al*. 2013). The current phylogenetic analysis (Fig. 4.3) indicates an ancestral *E. novaeguineae* initially came to occupy New Guinea in the Late-Miocene, potentially facilitated by low sea levels ~10 Ma (Haq *et al*. 1987). Given the basal position of *E. novaeguineae* in the dated phylogeny (Fig. 4.3), a New Guinean origin for the genus *Elseya* may be possible. However, I consider this less likely and suggest *E. novaeguineae* simply reached early reproductive isolation, as the result of rising sea levels, from a geographically structured, but widespread ancestor present on mainland Australia. Miocene dispersal between Australia and New Guinea is also suggested for *Cherax* crayfish (Baker *et al*. 2008), rainbowfish (McGuigan *et al*. 2000)

and terrestrial elapid snakes (Wüster *et al*. 2005), all of which show signals of further Pleistocene dispersal. Little is known regarding these much earlier colonisation events, yet repeated terrestrial and freshwater connections throughout the Late-Cenozoic clearly generated considerable lineage diversity across this region.

Australian relationships

Deepest divergence among Australian snapping turtle clades occurs among rivers of the western and southern Gulf, supporting the hypothesis that Plio-Pleistocene Lake Carpentaria was a habitat barrier for these riverine specialists. However, at 8.67 Ma (95% HPD 6.06-11.27) their divergence vastly pre-dates Plio-Pleistocene connectivity estimated across this region for other freshwater groups (de Bruyn *et al*. 2004; Alacs 2008; Cook & Hughes 2010; Huey *et al*. 2010; Cook *et al*. 2012). Although first occurrence of Lake Carpentaria has not been documented, it may have occurred as early as the Late-Miocene low-stand (McConneckie 1996 cited in Georges & Thomson 2006). The close relationship between *E. branderhorsti* from New Guinea and *Elseya* species from the Northern Territory and Arnhem Land supports riverine connectivity with New Guinea to the west of Lake Carpentaria, along the Arafura Sill. Viewing Lake Carpentaria as an ecological filter can explain both deep genetic disjunctions and recent widespread connectivity for different freshwater taxa across northern Australia. Disjunction between riverine and lacustrine habitat may account for the high levels of freshwater endemism in the Kimberly and western Arnhem Land for turtles (six species, Georges & Thomson 2006) and freshwater fishes (Unmack 2001). Studies of more westerly turtle populations, including further sampling of *E. dentata*, should be a focus of further research. This region's biogeography is especially poorly explored but seems particularly complex (Bowman *et al*. 2010; Eldridge *et al*. 2011; Potter *et al*. 2012).

By contrast, Plio-Pleistocene divergence between *E. lavarackorum* (southern Gulf) and north-east coast taxa (*E. irwini*, *Elseya* sp. [Daintree]), at 2.43 Ma (95% HPD 1.41-3.51), implies recent connectivity across low-lying southern Cape York. Freshwater connectivity is possible during floods (Unmack 2013) or river capture events. The latter are documented geologically or inferred from biological data between the southern Gulf and north-east coast (Pusey *et al*. 1998; Unmack 2001), southern Gulf and interior (Twidale 1966; Coventry *et al*. 1985), and interior and east coast across the Great Dividing Range (Unmack & Dowling 2010; McGuigan *et al*. 2000). Chelids have a long fossil history in the southern Gulf, with several genera present in Early-to-Mid-Miocene deposits at Riversleigh (Gaffney *et al*. 1989), including *Elseya* spp. (S. Thomson, pers. comm.). *Elseya lavarackorum* was originally described from Pleistocene Riversleigh fossils (White & Archer 1994; Thomson *et al*. 1997) (Fig. 4.1), and persists in the same location today within spring-fed drainages in an otherwise semi-arid landscape.

Divergence between the north-eastern and south-eastern clades (at 5.82 Ma, 95% HPD 4.02-7.86), notalbly the geographically abutting species *E. irwini* and *E. albagula*, is inconsistent with the Burdekin Gap. Were the Burdekin Gap involved in a genetic divide for these turtles on the east coast, deeper divergence would be expected between disjunct populations of *E. irwini* from the Burdekin and Johnstone Rivers (Fig. 4.1). Divergence between *E. irwini* and *E. albagula* invokes an ancient drainage divide between the extensive Burdekin (130,400 km^2) and Fitzroy (141,100 km^2) basins. Uniquely on Australia's eastern seaboard, these systems expanded during the Paleogene, capturing westward-flowing drainage through scarp retreat (Jones 2006). This potentially provided a direct colonisation route between ancient inland and modern coastal drainages. Bathymetric data also imply independent paleochannel trajectories for these systems during glacial low-stands, when coastlines extended >100 km offshore (Fielding *et al*. 2003; Ryan *et al*. 2007). Surprisingly, this major drainage boundary has not previously been considered an important biogeographic feature. It would nevertheless explain genetic breaks reported for several widespread freshwater species (Wong *et al*. 2004; Jerry 2008; Gongora *et al*. 2012). Although the Burdekin basin's northern boundary currently defines northern and eastern freshwater provinces (Unmack 2001), reflecting a dramatic change in species distributions, this pattern may be largely driven by the basin's oddly depauperate fish fauna (Pusey *et al*. 1998) and Plio-Pleistocene events including extreme local aridity and volcanism (Griffin & McDougall 1975; Sutherland *et al*. 1977).

Le *et al*. (2013) estimated older divergences within the genus *Elseya*, suggesting an origin in the Early-Miocene then diversification in response to aridity events in the Mid-to-Late-Miocene and Pliocene. Differences in gene sampling, in estimated tree topologies, and in the use of fossil calibrations could cause major differences in age estimates between studies. If our fossil calibration (*E. nadibadjagu*) is not a true ancestor of the 'Queensland radiation' as currently supposed, but a younger branch within it, our timeline will underestimate ages of divergence events within the genus *Elseya*. Well-dated chelid fossil material with accurate taxonomy will be especially important for future work into the timescale of Australian freshwater turtle evolution.

Population genetics

Population genetic analyses of Australian *Elseya* spp. and the deep intra-specific divergences observed across adjacent drainages together imply a highly limited dispersal potential across watersheds. Two instances of haplotype sharing among drainages (*E. albagula*– Burnett and Mary Rivers; *E. dentata* – Roper and Limmen-Bight Rivers; Fig. 4.4) are more consistent with low sea level drainage coalescence during the most recent glacial cycle (last 20,000 years) (see Chapter 3 for discussion concerning *E. albagula*), than recent cross-drainage dispersal. In both

cases, adjacent river mouths extend over shallow continental shelves and shared haplotypes are internal (ancestral), indicating retention of ancestral polymorphism. Drainage basins represent natural units for conservation of freshwater taxa and typically contain distinct genetic diversity (Cook *et al*. 2008). Observed among-drainage divergence in snapping turtles is more typical of evolutionarily significant units (ESUs) (*sensu* Moritz 1994; Chapter 3). Under expectations of increased climatic drying, greater hydrological extremes, and continued sea level rise in Australia associated with projected climate change (Hughes 2003; Kershaw *et al*. 2003), sensitive freshwater groups like *Elseya* spp. can be expected to experience increased habitat fragmentation and erosion of genetic diversity. Work presented in Chapter 4 establishes a critical phylogenetic framework for the genus *Elseya* necessary for future taxonomic and conservation work.

Freshwater refugia

Reliance of *Elseya* spp. on permanent riverine habitat and genetic divergence among locally endemic units also imply isolation in persisting aquatic habitats. It follows that their extant distribution may indicate important freshwater evolutionary refugia. This possibility remains largely overlooked despite considerable research focus on terrestrial refugia in Australia (Byrne 2008) and globally (Hewitt 1996). Regions including the Fitzroy-Burnett-Mary Rivers region (*E. albagula*), which collectively supports especially high richness and endemism for freshwater Gondwanan relics, Queensland's Wet Tropics (*E. irwini*, *Elseya* sp. [Daintree]), Arnhem Land (*Elseya* sp. [Sth Alligator]), and the Gregory-Nicholson drainage (*E. lavarackorum*), all contain high freshwater biodiversity and endemism relative to neighbouring regions, and may therefore represent evolutionary refugia.

CONCLUSIONS

Phylogenetic analysis of a freshwater Gondwanan relic paints a more complete picture of the evolution of Australia's unique freshwater biodiversity as aridity progressed since the Mid-Miocene. The genus *Elseya* clearly has a long vicariant history, closely associated with the disconnection of fluvial habitats by long-term drainage boundaries, sea level rise, and ongoing aridification of the Australian landmass. Moreover, congruence with patterns already well described for terrestrial groups implies a collective response of the Australian fauna to progressive aridification over the last 20 My. As one of the first inclusive studies of any freshwater turtle group in the Southern Hemisphere, this work provides important insights into processes shaping freshwater biogeography in this understudied region. Further studies of freshwater turtle diversity across Australia and New Guinea are warranted, and will likely reveal much about historical climatic and landscape influences on current freshwater biodiversity.

CHAPTER 5 – INFLUENCE OF A MAJOR DRAINAGE DIVIDE VERSUS A HISTORICAL ARID CORRIDOR ON GENETIC STRUCTURE AND DEMOGRAPHY IN THE WIDESPREAD FRESHWATER TURTLE, *EMYDURA MACQUARII KREFFTII*

This chapter has been submitted for publication, as follows:

Todd, E.V., Blair, D. & Jerry, D.R. Influence of drainage divides versus arid corridors on genetic structure and demography of the widespread freshwater turtle, *Emydura macquarii krefftii*, from Australia. *Ecology and Evolution*, under review.

Image: The study species– Krefft's river turtle, *Emydura macquarii krefftii*. In this case, an adult individual captured from the Pioneer River in central Queensland. Photo by Erica Todd.

CHAPTER 5 – INFLUENCE OF A MAJOR DRAINAGE DIVIDE VERSUS A HISTORICAL ARID CORRIDOR ON GENETIC STRUCTURE AND DEMOGRAPHY IN THE WIDESPREAD FRESHWATER TURTLE, *EMYDURA MACQUARII KREFFTII*

SUMMARY

Krefft's river turtle, *Emydura macquarii krefftii*, are common throughout eastern coastal Australia and their extensive longitudinal distribution spans landscape and climatic barriers recently proposed as important in structuring freshwater biodiversity. Their evolutionary history in response to climatic oscillations and putative biogeographic barriers was examined using range-wide sampling (649 individuals representing 18 locations across 11 drainages) and analysis of mitochondrial sequence (~1.3 kb control region and ND4) and nuclear microsatellite (12 polymorphic loci) data. Phylogeographic (haplotype networks), demographic (neutrality tests, mismatch distributions) and population genetic analyses (pairwise F_{ST}, AMOVA, Bayesian clustering analysis) were implemented to investigate patterns of genetic diversity and population structure, and to examine competing demographic (local persistence vs. range expansion) and biogeographic (arid corridor vs. drainage divide) hypotheses. Genetic data revealed a complex evolutionary history for *E. m. krefftii*, with significant genetic structure across their range consistent with isolation across drainage divides. Mitochondrial and microsatellite markers revealed considerable north-south regional divergence consistent with long-term isolation across the Burdekin-Fitzroy drainage boundary, not the adjacent Burdekin Gap dry corridor. The level of divergence implies Krefft's turtles persisted within the arid Burdekin region through multiple Pleistocene episodes of aridity, though with very low contemporary genetic diversity indicating population bottlenecks. There was also evidence of rare contemporary overland dispersal across the Burdekin-Fitzroy watershed and for hybridisation with *Emydura tanybaraga* at the northern range limit.

INTRODUCTION

Climatic cycles over the past several million years have driven important evolutionary changes in the world's biota, though research has focused largely on the Northern Hemisphere where land-based glaciation was extensive. Widespread glaciations never occurred in Australia, which instead experienced cycles of severe aridity that also profoundly influenced current patterns of biodiversity (Byrne *et al*. 2008, 2011). Extreme hydrological variability also accompanied these cycles (Kershaw *et al*. 2003), though evolutionary responses of freshwater species to such cyclic aridity are not yet well-understood. Understanding how evolutionary histories of freshwater species are

tied to the underlying biotic and geological evolution of a region is significant, because their dispersal depends upon direct hydrological connectivity within and across drainage divides and because the history of drainage interconnectivity reflects historical influences of landscape, sea level, and climate (Bermingham & Avise 1986; Bernatchez & Wilson 1998; Waters *et al*. 2001).

Within an otherwise largely arid and geologically quiescent continent, Australia's narrow eastern margin still supports widespread permanent drainages and a diverse endemic freshwater fauna. Volcanism, erosion, scarp retreat and sea level fluctuations have shaped complex drainage patterns (Jones 2006; Hodgkinson *et al*. 2007; Vasconcelos *et al*. 2008) among a series of coastal-flowing systems formed by Cenozoic uplift of the Great Dividing Range (GDR). The GDR parallels the entire length of Australia's east coast, separates coastal and interior drainages, and shelters the eastern margin from widespread aridity. In recent years, phylogeographic studies of fishes and macroinvertebrates have highlighted the biogeographic complexity of the eastern margin, revealing cryptic diversity among individual drainage basins and deep lineage disjunctions at higher geographic scales (Wong *et al*. 2004; Jerry 2008; Page *et al*. 2012).

Lowlands within the geographically extensive Burdekin drainage (130,400 km^2) (Fig. 5.1) currently experience the most severe seasonal aridity of anywhere along Australia's east coast. The degree of aridity experienced here was probably more extreme during glacial maxima (Williams 1984). The 'Burdekin Gap' arid corridor is a well-recognised vicariant barrier enforcing north-south divergence in many terrestrial animal lineages (Cracraft 1991; Chapple *et al*. 2011), dating to the Mid-to-Late-Miocene or Pliocene (James & Moritz 2000; Moussalli *et al*. 2005). Recent molecular studies of several wide-ranging freshwater taxa also report deep north-south divergence congruent with the Gap (freshwater fishes, Wong *et al*. 2004; Jerry 2008; Unmack & Dowling 2010; platypus, Gongora *et al*. 2012). However, the Burdekin hosts substantially lower species diversity of freshwater fishes compared to adjacent drainages, and inadequate sampling has so far precluded accurate assessment of possible alternative biogeographic barriers. Genetic breaks observed in previous studies are also consistent with a major drainage divide between the Burdekin and equally extensive Fitzroy basin (141,100 km^2), immediately to its south. These basins may be particularly ancient (Jones 2006), and bathymetric data suggest they maintained independent paleochannels during glacial low-stands despite a broad continental shelf (~160 km wide) (Fielding *et al*. 2003; Ryan *et al*. 2007). It remains unclear whether north-south genetic disjunction in Australia's eastern freshwater fauna reflects climatic aridity, as for terrestrial taxa, or a physical drainage divide. *Emydura m. krefftii* presents an excellent opportunity to examine the relative importance of climate versus drainage topography in influencing patterns of freshwater biodiversity.

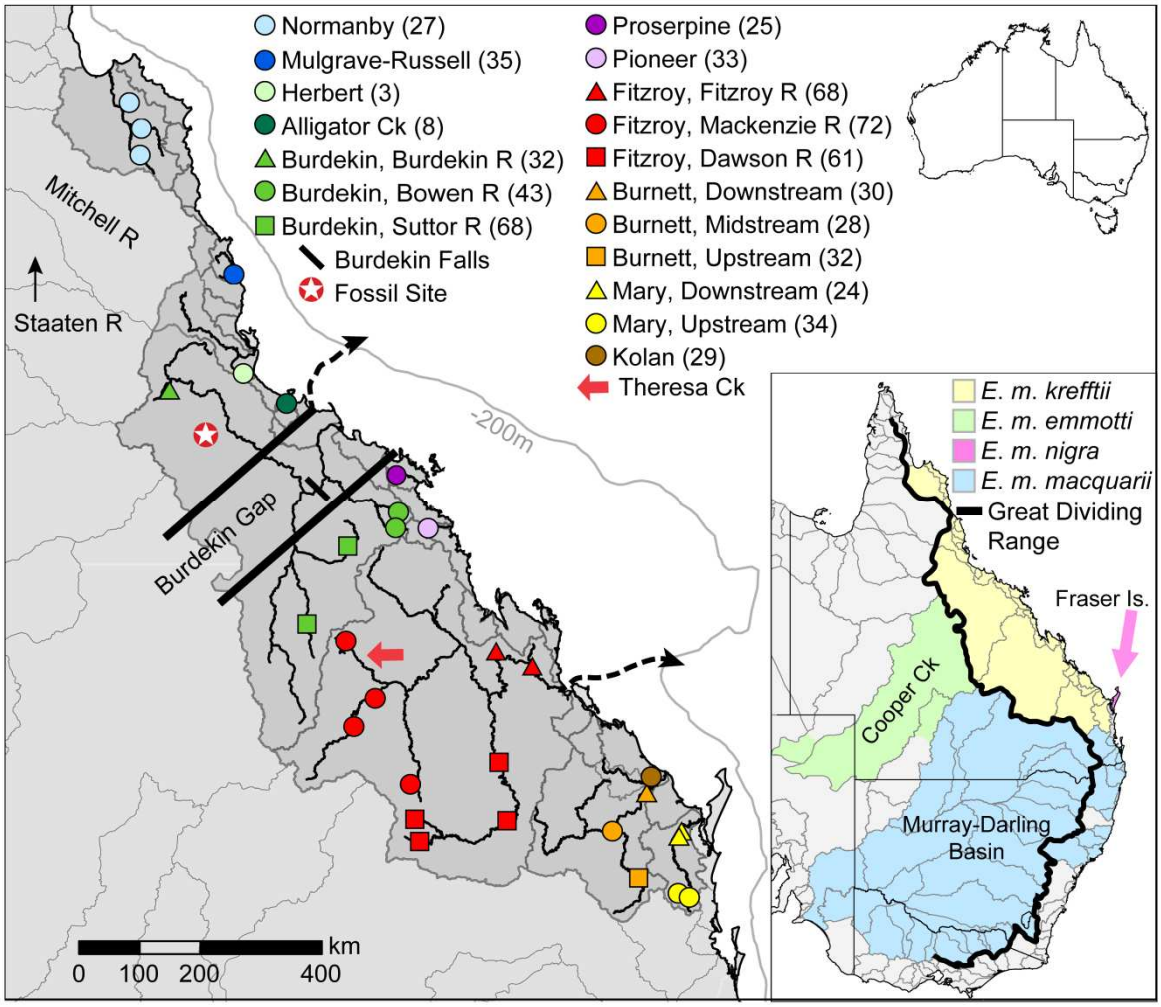

Fig. 5.1 Map showing the distribution of *Emydura macquarii* subspecies (inset) and sampling locations (left) for *E. m. krefftii* across 11 drainages in eastern Australia. Samples are grouped by drainage (colour), then sub-region (shape, where appropriate). Samples sizes are given in brackets. Drainage boundaries are outlined in dark grey. The distribution of *E. m. krefftii* is in darker grey, and river networks are in black for drainages where samples were collected. Dashed arrows indicate likely paleochannels of the Fitzroy (Ryan *et al*. 2007) and Burdekin (Fielding *et al*. 2003) Rivers, indicating independent trajectories to the continental shelf, indicated in light grey at the -200 m contour. Important features mentioned in text are indicated, including the Burdekin Gap, Burdekin Falls, Bluff Downs fossil site, and Theresa Ck.

Freshwater and terrestrial turtles have proven sensitive phylogeographic models for inferring how historical evolutionary forces shape observable patterns of intra-specific molecular diversity (Walker & Avise 1998; Weisrock & Janzen 2000), but remain poorly studied outside the Northern Hemisphere. Australian freshwater turtles (Chelidae: Pleurodira) are part of the continent's original Gondwanan fauna. They represent underutilised model taxa for uncovering regional biogeographic patterns and for inferring underlying evolutionary forces driving Australia's unique freshwater biodiversity. Species of the genus *Emydura* are especially common throughout eastern Australia. They have broad opportunistic diets and generalist habitat requirements, and although they are primarily riverine, occupy a variety of permanent freshwater habitats from rivers to billabongs (Georges & Thomson 2006). Though southern and northern species groups are recognised, electrophoretic surveys have revealed *Emydura* species to be very closely related genetically and presumably represent a recent radiation (Georges & Adams 1996). Their apparently shallow evolutionary history may imply an ecological capacity to survive climatic perturbation and to exploit opportunities during favourable conditions.

Krefft's river turtle, *E. macquarii krefftii*, is one of four subspecies within the southern *Emydura macquarii* complex, currently defined largely by non-overlapping geographic ranges (Fig. 5.1) and minor morphological differences in facial coloration and body shape/size (Georges & Thomson 2010). Krefft's turtle is ideally suited to investigating patterns of genetic differentiation, as it occupies an extensive longitudinal distribution east of the GDR that includes major regions of coastal aridity, as well as wetter regions to the north and south. It is one of the few freshwater species, and the only turtle species, found throughout the arid Burdekin drainage. Though its response to historical aridity in this region is unknown, fossil evidence places it within the Burdekin drainage in the early Pliocene (Thomson & Mackness 1999), prior to initiation of Pleistocene aridity. However, whether Krefft's turtles survived glacial aridity in this region within isolated local refugia– experiencing bottlenecks and latter demographic expansions, or whether there was northwards range expansion from a southern stronghold– e.g. since the most recent aridification, is yet to be determined.

Chapter 5 describes a range-wide examination of genetic structure in Krefft's river turtle, to investigate the relative importance of a major drainage divide versus a historical arid corridor as barriers to movement of freshwater animals in eastern Australia. A combination of mitochondrial sequences and sensitive nuclear microsatellite loci were used to 1) describe population genetic structure in *E. m. krefftii* at multiple spatial scales, including within and among individual drainage basins and regionally across its whole distribution, and 2) differentiate between competing

biogeographic (arid corridor vs. drainage divide) and demographic (local persistence vs. range expansion) hypotheses. If the Burdekin Gap has been an important long-term biogeographic barrier, greatest genetic divergence is expected among populations either side of the Burdekin lowlands. Alternatively, if the Burdekin-Fitzroy drainage divide has had the greatest influence on regional genetic structure, greatest divergence is expected across the Burdekin's southern boundary. Similarly, if *E. m. krefftii* survived climatic perturbation in local northern refugia, a) northern populations are expected to be strongly differentiated genetically from southern populations, and b) show signals of historical bottlenecks and, potentially, recent demographic expansion. By contrast, recent range expansions northwards from southern refugia would be evidenced by a) a steady northwards decrease in genetic diversity and b) less pronounced genetic structure. In either case, southern populations are expected to have been demographically more stable. Potential genealogical concordance with phylogeographic signatures reported previously for other east-Australian freshwater taxa are discussed in order to identify major biogeographic patterns influencing freshwater biodiversity evolution in this understudied region.

METHODS

Sample collection and DNA extraction

Tissue samples were obtained from 656 *E. m. krefftii* across 18 locations in 11 drainage basins, representing the native distribution of this subspecies in eastern coastal Australia (Fig. 5.1; Appendix 5A). A hierarchical sampling design enabled examination of genetic structure at three spatial scales: local river (among locations within drainages), drainage basin (among drainages within regions) and regional (whole distribution). To investigate the role of specific biogeographic features in structuring turtle populations, samples were especially sourced from across the Burdekin Gap dry corridor within the Burdekin drainage, and the Burdekin-Fitzroy drainage divide, including geographically intermediate, peripheral coastal streams (Proserpine and Pioneer Rivers, Fig. 5.1). Samples were also obtained from five representatives of the other three *Emydura macquarii* subspecies (*E. m. macquarii*, Murray-Darling drainage; *E. m. emmotti*, Cooper Ck drainage; *E. m. nigra*, Lake Birrabeen, Fraser Is; Appendix 5A) for comparison. Tissues were obtained from existing collections, through collaborations with other researchers, or during targeted field work. In the latter case, turtle capture and tissue sample collection were performed as described in Chapter 3. Genomic DNA was extracted from tissues using a modified salting-out protocol described in Chapter 2.

Phylogeographic analysis of mtDNA

Historical population structure was investigated, and major genetic lineages within *E. m. krefftii* identified, by sequencing ~1.3 kb of the mitochondrial genome. Turtle-specific primers were used to amplify sections of the hypervariable control region (CR) (439 bp) and NADH dehydrogenase subunit 4 (ND4) (670 bp ND4 plus 70 bp of tRNAHis, 63 bp of tRNASer and the first 34 bp of tRNALeu) using primers and PCR and sequencing protocols described in Chapter 3. Sequence alignment, editing and examination for the presence of nuclear mitochondrial (numt) DNA paralogues was performed as described in Chapter 3.

Relatedness and spatial distribution of mtDNA haplotypes (*E. m. krefftii* and related subspecies) were assessed with a minimum spanning network, computed in ARLEQUIN 3.5 (pairwise distance model) (Excoffier & Lischer 2010). The minimum spanning tree was drawn for clarity of presentation following visualisation in HAPSTAR 0.7 (Teacher & Griffiths 2011). Evolutionary distances within and among major genetic lineages of *E. m. krefftii* and related subspecies were estimated for ND4 sequences using mean uncorrected *p*-distances in MEGA 5 (Tamura *et al*. 2011), with pairwise deletion of sites containing gaps and using 1000 bootstrap replicates for variance estimation. Calculations were based on ND4 only as a common measure of genetic divergence that will be comparable across studies of other reptiles and fishes (see reviews in Vargas-Ramírez *et al*. 2010; Fritz *et al*. 2012).

Genetic diversity and historical demography of major mtDNA lineages

Measures of mtDNA diversity within major *E. m. krefftii* lineages and overall were calculated in ARLEQUIN and DNASP 5.10 (Librado and Rozas 2009), including number of haplotypes (N_h), number of variable sites (V), haplotype diversity (H_d), nucleotide diversity (π), and average number of nucleotide differences (k).

Neutrality statistics and mismatch distributions were used to test for signatures of population expansion within major *E. m. krefftii* clades. Fu's F_S (Fu 1997) is one of the most powerful neutrality tests for detecting population expansion and was calculated along with Tajima's D (Tajima 1989) in ARLEQUIN, with 10,000 coalescent simulations to assess significance. Neutrality statistics are sensitive to historical demography, selection and/or population structure. Significant negative values indicate population expansion or purifying selection, while significant positive values indicate population bottleneck, balancing selection, or admixture. The hypothesis of recent population expansion was evaluated by calculating the mismatch distribution of pairwise

differences between haplotypes (Rogers & Harpending 1992) against a model of sudden population expansion in ARLEQUIN. A smooth or unimodal distribution signifies recent population expansion, whereas a ragged or multimodal distribution represents demographic equilibrium or decline (Rogers & Harpending 1992; Excoffier 2004). A bimodal distribution may reflect admixture of two previously separate lineages. To test whether the data matched a sudden expansion model, sum of squared deviations (SSD) and raggedness indices (r) were calculated, using 2000 bootstrap replicates to assess significance. Significant SSD or r values indicate that an expansion model is rejected by the data.

Microsatellite amplification and screening

To assess contemporary genetic structure and gene flow, individuals were genotyped at 12 polymorphic microsatellite loci designed specifically for *E. m. krefftii* (Chapter 2; Todd *et al*. 2011; Ekref04, Ekref06-Ekref10, Ekref12-Ekref15, Ekref18, Ekref20). Multiplex PCR conditions and fragment analysis details are presented in Chapter 2. Tests for conformation to Hardy-Weinberg expectations (HWE) were performed for each locus in GENALEX 6.5 (Peakall & Smouse 2006, 2010). Potential linkage disequilibrium between pairs of loci was examined in GENEPOP 4.0 (10,000 permutations) (Rousset 2008) and the presence of null alleles was evaluated using MICRO-CHECKER 2.2.3 (van Oosterhout *et al*. 2004). Significance levels for multiple comparisons were adjusted using a false discovery rate (FDR) correction (Benjamini & Hochberg 1995).

For microsatellite markers, genetic diversity was assessed by calculating number of alleles (N_A), and observed (H_O) and expected (H_E) heterozygosities for each sample location, drainage and region in ARLEQUIN. Private alleles were identified using GENALEX. Because of sample size variation, allelic richness was also calculated for each population, region, and overall using FSTAT 2.9 (Goudet 1995). A hierarchical analysis of allelic richness (Kalinowski 2004) was also performed in FSTAT (1000 permutations) to test for differences in allelic richness among regions.

Population structure and differentiation calculated for microsatellite data

To quantify population genetic structure within and across drainage boundaries, pairwise linearised F_{ST} values were calculated among sampling locations and drainages. For the large Burdekin and Fitzroy locations, samples were pooled by sub-catchment. Analyses were implemented in FSTAT, with significance levels adjusted for multiple comparisons. Hierarchical distribution of genetic variance across the range of *E. m. krefftii* at a drainage and regional scale was estimated using AMOVA, performed in ARLEQUIN (10,000 permutations).

Bayesian clustering analysis

An individual-based Bayesian clustering approach, performed in the program STRUCTURE 2.3 (Pritchard *et al*. 2000), was used to identify the most probable number of genetically homogeneous groups (*K*) represented within the microsatellite data. All samples were utilised, including those with small numbers from the Herbert (n = 2) and Alligator Ck (n = 8) drainages. Ten independent simulations were run for each value of *K* from one to 10, using 5 million MCMC replications with an initial burn-in of 1 million, found to be appropriate in pilot runs. An admixture model with correlated allele frequencies was assumed and sampling locations were used as prior information (LOCPRIOR model). This improves clustering performance for datasets where the signal of genetic structure may be weak, without biasing the outcome (Hubisz *et al*. 2009). Summary statistics (log likelihood and alpha) were monitored for each run to verify convergence. The true value of *K* was determined following the posterior probability (Pritchard *et al*. 2000) and delta log likelihood methods (Evanno *et al*. 2005), implemented in STRUCTURE HARVESTER 0.6 (Earl & vonHoldt 2012). To investigate the potential for further sub-structure within major genetic units and reconstruct potential hierarchical relationships among clusters, each of the identified clusters was subsequently run independently. For the chosen *K* value in each analysis, average pairwise similarity (*H′*) of STRUCTURE runs was assessed in the program CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007), using the Greedy algorithm with 10,000 random additions on the 10 independent runs. Results were imported into DISTRUCT 1.1 (Rosenberg 2004) for graphical representation.

RESULTS

MtDNA phylogeographic structure and historical demography

Among 646 individuals sequenced at both CR and ND4, a total of 72 unique haplotypes were identified, with 121 variable sites across a 1276 bp concatenated alignment. Three individuals failed to amplify at both loci and were excluded from the concatenated dataset. For CR only, 56 haplotypes were identified among 649 individuals (62 variable sites across 439 bp) [GenBank: KF181795-KF181853]. Twenty-nine ND4 haplotypes were identified among 647 individuals (59 variable sites across 837 bp) [GenBank: KF181854- KF181885]. The haplotypic network, based on concatenated CR+ND4 data, distinguished three divergent clades within *E. m. krefftii* (Fig. 5.2). Two main clades, separated by 37 mutational changes, had a clear geographical break corresponding to the Burdekin-Fitzroy drainage divide. Turtles from the Pioneer and Proserpine Rivers, geographically intermediate between the Burdekin and Fitzroy drainages, belong within the

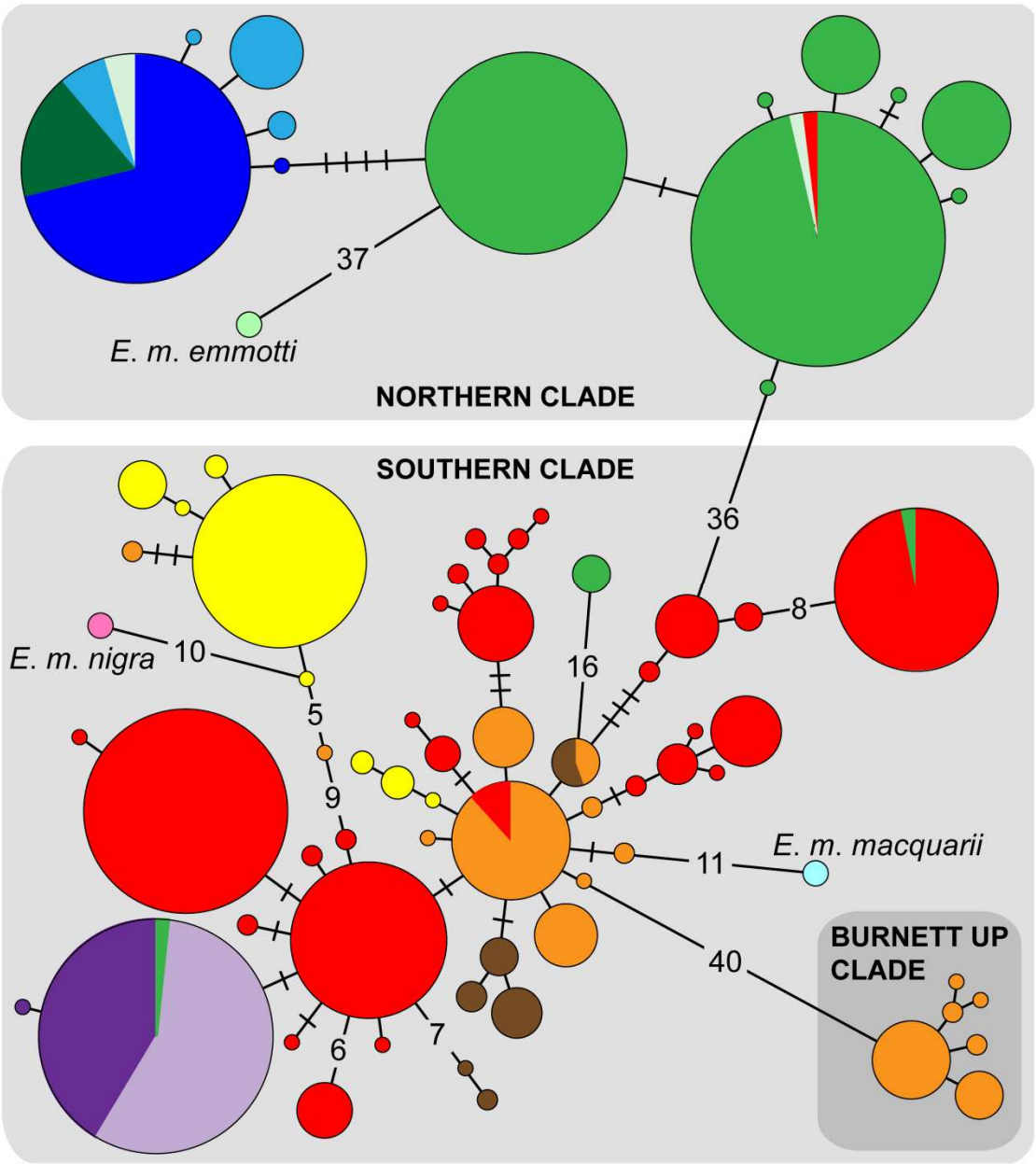

Fig. 5.2 Minimum spanning tree depicting relationships among mtDNA haplotypes for concatenated control region+ND4 sequences from *E. m. krefftii* across 11 river drainages. Haplotypes sampled from related subspecies *E. m. macquarii* (pale blue), *E. m. nigra* (pale pink) and *E. m. emmotti* (pale green) are also included. Circles represent unique haplotypes and are connected to one another by mutational changes (lines). Cross bars indicate additional mutational changes between haplotypes, which are represented numerically when there are many. Circle size is proportional to haplotype frequency and colours represent sampling location (drainages) following Fig. 5.1.

southern lineage. There were three instances of haplotype sharing across the Burdekin-Fitzroy boundary. Three individuals sampled close to the drainage divide possessed a haplotype from the opposite lineage, suggesting recent dispersal across the watershed in both directions (Fig. 5.2). A single haplotype, sampled from six individuals from the upstream Suttor R (Burdekin drainage), was also nested within the southern lineage, but distinguished by 17 mutations. A single common haplotype occurred across the four most northerly drainages, and another was common to the Pioneer and Proserpine Rivers. Otherwise, few haplotypes were shared among drainages. However, in the southern clade, haplotypes unique to particular drainages did not necessarily cluster together. A central (potentially ancestral) haplotype occurs in the Burnett and Fitzroy Rivers. A cluster of haplotypes from the Mary River is distinct by several mutations. Within the northern clade, subdivision occurs between the large Burdekin drainage and elsewhere. A third divergent clade within *E. m. krefftii* consists of six closely related haplotypes sampled exclusively from a location in the upstream Burnett River, separated by 41 mutations from other Burnett River haplotypes.

For the related subspecies, only a single haplotype per subspecies was identified among the five sampled individuals. Their relationships with *E. m. krefftii* haplotypes are also shown in Fig. 5.2. Haplotypes from the southernmost subspecies, *E. m. macquarii* (Murray-Darling Basin) and *E. m. nigra* (Fraser Is), were nested within the southern *E. m. krefftii* clade and were closely related to haplotypes from the Burnett River and Mary River, respectively. *Emydura m. emmotti* (Cooper Ck) was distinct, separated by 38 mutational changes from the northern *E. m. krefftii* clade. ND4 evolutionary distances are presented in Table 5.1 and show divergence among *E. m. krefftii* clades (2.23-3.03%) to be considerably greater than that between the southern *E. m. krefftii* clade and *E. m. nigra* (0.83%) or *E. m. macquarii* (0.64%). The upstream Burnett clade was as divergent from the southern clade (2.31%) as from the other subspecies (range 2.35-2.83%), giving no indication of a potential historical relationship. Greatest within-clade divergence was seen within the southern clade (0.53%), compared with the northern (0.19%) or upstream Burnett (0.16%) clades.

Measures of mtDNA diversity, estimates of demographic parameters and neutrality tests for major Krefft's turtle clades are presented in Table 5.2. The northern clade was divided into Burdekin and Far North subclades as per the haplotype network. In order to obtain an estimate of evolutionary divergence among lineages, three individuals geographically located within one lineage but carrying a haplotype from another (i.e. suggestive of recent dispersal) were excluded. Greatest genetic diversity was found within the southern clade (68 haplotypes, $H_d = 0.940$, $\pi = 0.662$). The northern clade shared just three common haplotypes across 200 individuals ($H_d = 0.812$, $\pi = 0.325$).

Table 5.1 Mean evolutionary distances within and among major *Emydura macquarii krefftii* clades and *E. macquarii* subspecies based on uncorrected ND4 *p*-distances (% divergence). Lower matrix, percent divergence among lineages/subspecies; upper matrix, SE values for percent divergence among lineages/subspecies; italics diagonal, percent divergence within lineages/subspecies. *Emk*, *E. m. krefftii*; *Emm*, *E. m. macquarii*; *Emn*, *E. m. nigra*; *Eme*, *E. m. emmotti*.

	Emk "N"	Emk "S"	Emk "BU"	*Emm*	*Emn*	*Eme*
Emk "Northern"	*0.19*	0.46	0.56	0.48	0.49	0.44
Emk "Southern"	2.23	*0.53*	0.46	0.20	0.25	0.45
Emk "Burnett Up"	3.03	2.31	*0.16*	0.49	0.50	0.55
Emm	2.06	0.64	2.35	-	0.28	0.49
Emn	2.23	0.83	2.51	0.72	-	0.50
Eme	1.63	2.01	2.83	1.91	2.15	-

Table 5.2 Measures of mitochondrial DNA diversity (1276 bp, concatenated control region and ND4), neutrality tests and demographic parameters within three major clades (bold) and two subclades of Krefft's river turtle *Emydura macquarii krefftii*. Results of the observed mismatch distribution against a sudden expansion model include the raggedness index (*r*) and the sum of squared deviations (SSD).

	Northern	**Northern:** Far North	**Northern:** Burdekin	**Southern**	**Burnett Up**	**OVERALL**
N	200	65	135	412	30	642
V	17	4	9	68	5	121
$\boldsymbol{N_h}$	13	5	8	53	6	72
$\boldsymbol{H_d}$ **(SD)**	0.812(0.012)	0.477(0.062)	0.706(0.022)	0.940(0.005)	0.685(0.067)	0.957(0.002)
$\boldsymbol{\pi}$ **(SD)**	0.325(0.180)	0.041(0.039)	0.108(0.074)	0.662(0.339)	0.076(0.059)	1.971(0.958)
k **(SD)**	4.140(2.069)	0.552(0.444)	1.37(0.85)	8.42(3.91)	0.96(0.67)	25.15(11.05)
D	1.440	-1.399*	-0.167	-0.862	-0.726	na
$\boldsymbol{F_S}$	2.066	-1.662	-0.551	-8.710	-1.761	na
r	0.039	0.161	0.059	0.018	0.118	na
SSD	0.029	0.013	0.016	0.011	0.010	na

N, sample size; *V*, number of variable sites; N_h, number of haplotypes; H_d, haplotype diversity; π, nucleotide diversity (expressed as percentages, i.e. 0.001 = 0.1%); *k*, average number of nucleotide differences; SD, standard deviation; F_S, Fu's statistic; *D*, Tajima's *D*-test. Significant tests are indicated with asterisks as follows: $*P < 0.05$, $**P < 0.01$. na, not applicable.

There was weak support for a model of demographic expansion in the northern *E. m. krefftii* clade. *D* and F_S values were negative for most lineages, suggesting population expansion, but only the *D* statistic in the Far North clade was significant. However, significant *r* and SSD values indicated that a sudden expansion model could not be conclusively rejected in any case. Smooth mismatch distributions characterised the upstream Burnett clade and the Burdekin and Far North subclades, also indicating demographic expansion (Fig. 5.3). However, in each of these cases, the number of haplotypes was very small, potentially limiting power of the test. A more ragged distribution was observed for the southern clade, indicating stable demography. Analysis of the northern clade produced a bi-modal distribution, indicating the presence of at least two distinct lineages (i.e. Burdekin and Far North subclades) (Fig. 5.3).

Microsatellite diversity and contemporary population structure

A total of 128 alleles were identified across the 12 microsatellite loci within 644 Krefft's river turtles with < 5% missing data (5-17 alleles per locus). Deviations from HWE were detected at six loci (Ekref04, Pioneer R, Burdekin Suttor R; Ekref18, Burdekin Suttor R; Ekref20, Normanby R; Ekref07, Mary downstream; Ekref12, Mary upstream; Ekref09, Fitzroy Dawson R, Fitzroy Mackenzie R). There was no evidence of scoring error due to stuttering or large allele dropout at any locus, though tests for homozygote excess in MICRO-CHECKER suggested null alleles at locus Ekref15 and Ekref12 in the Fitzroy (Fitzroy R sub-catchment) and Normanby R populations, respectively. There was no evidence for linkage disequilibrium. As there was no consistent evidence for null alleles or deviation from HWE for any particular locus or sampling location, and as analyses with and without these data did not alter the interpretation, the entire dataset was retained and examined.

As for mtDNA, stark differences were found in measures of genetic diversity among regions and some sampling locations at microsatellites (Table 5.3). Values of genetic diversity were considerably and consistently lower within the northern clade compared with the southern clade, both overall and when averaged across sampling locations (Table 5.3). The only exception was the Pioneer and Proserpine Rivers within the southern clade, which showed similarly low levels of genetic diversity as recorded for northern locations. Differences in A_R, H_O, and gene diversity among regions were all statistically significant ($P < 0.05$). For the southern locations collectively, 48 (40%) private alleles were detected, compared to eight (10%) in the northern locations. Most individual drainages also contained private alleles (range: 0 in Proserpine R, Mulgrave-Russell R and Normanby R to 8 in Fitzroy R and Burdekin R).

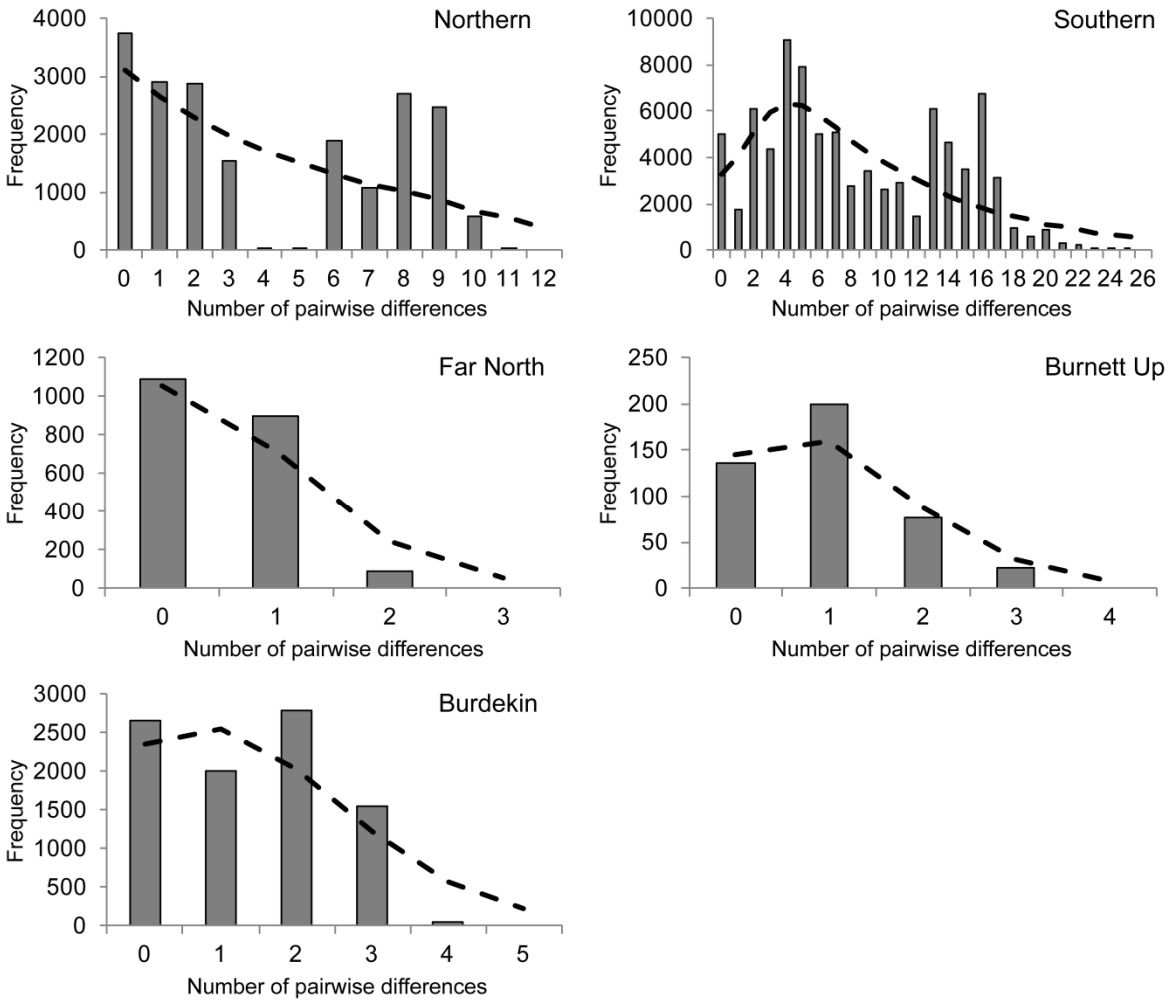

Fig. 5.3 Mismatch distribution among mitochondrial DNA haplotypes for major *E. m. krefftii* clades in Northern, Southern and Burnett Upstream locations. Northern lineage is further subdivided into Far North and Burdekin lineages. Grey bars show the observed values and black dashed lines indicate the expected distribution based on a model of sudden demographic expansion.

Table 5.3 Microsatellite diversity (12 loci) across sampling locations (≥ 10 sample size) for Krefft's river turtle (*Emydura macquarii krefftii*). Values are given for each catchment, region, and overall, with averages across multiple sampling locations given in brackets where appropriate.

Drainage (No. sample locations)	**Sample size (±SD)**	**Mean No. alleles (±SD)**	**Private alleles**	H_E **(±SD)**	H_O **(±SD)**	A_R[1] **(±SD)**
Mary	58	7.33(3.08)	4	0.66(0.20)	0.66(0.20)	6.39(2.00)
Average(2)	*29(7.1)*	*6.63(0.53)*		*0.66(0.01)*	*0.66(0.01)*	*6.31(0.22)*
Burnett	90	7.25(2.73)	2	0.69(0.14)	0.68(0.13)	6.06(2.05)
Average(3)	*30(2.0)*	*6.14(0.24)*		*0.69(0.02)*	*0.68(0.04)*	*5.85(0.15)*
Kolan	29	5.08(2.06)	1	0.65(0.16)	0.68(0.19)	4.99(1.95)
Fitzroy	201	8.42(3.50)	8	0.70(0.21)	0.69(0.22)	6.40(2.44)
Average(3)	*67.0(5.6)*	*7.50(0.14)*		*0.70(0.01)*	*0.69(0.01)*	*6.32(0.08)*
Pioneer	33	4.75(2.22)	2	0.49(0.24)	0.50(0.25)	4.35(1.99)
Proserpine	25	3.25(1.54)	0	0.44(0.26)	0.48(0.26)	3.19(1.48)
South	**436**	**10.00(3.67)**	**48**	**0.71(0.18)**	**0.65(0.17)**	**9.48(3.50)**
***Average*(11)**	***39.6(18.0)***	***6.11(1.34)***		***0.64(0.09)***	***0.64(0.08)***	***5.61(1.03)***
Burdekin	143	6.17(3.54)	8	0.53(0.27)	0.51(0.27)	4.49(2.57)
Average(3)	*47.7(18.4)*	*4.83(0.47)*		*0.53(0.03)*	*0.53(0.05)*	*4.26(0.19)*
M-Russell	33	3.50(2.19)	0	0.46(0.28)	0.57(0.21)	3.25(1.90)
Normanby	22	2.58(1.83)	0	0.30(0.23)	0.30(0.23)	2.58(1.83)
North[2]	**208**	**6.67(4.16)**	**8**	**0.53(0.26)**	**0.48(0.25)**	**6.67(4.16)**
***Average*(5)**	***34.3(20.3)***	***4.06(0.98)***		***0.47(0.09)***	***0.50(0.11)***	***3.72(0.78)***
OVERALL[2]	**644**	**10.67(4.01)**		**0.70(0.14)**	**0.60(0.15)**	**9.69(3.56)**

H_E, expected heterozygosity; H_O, observed heterozygosity; A_R, allelic richness. [1]A_R standardised to min. sample size of 22 for location and catchment, 208 for region and overall. [2]Includes samples from Alligator Ck (n = 8) and Herbert R (n = 2).

Microsatellites revealed a considerable level of genetic differentiation across the range of *E. m. krefftii*. Pairwise F_{ST} values among drainages were all highly significant after correction for multiple comparisons (Table 5.4). However, within drainages, F_{ST} values among sampling locations were low (< 0.016) (mean F_{ST} 0.008, SD 0.005). Only three comparisons were significant after correction: Burnett downstream vs. upstream (F_{ST} 0.008), Burdekin Bowen R vs. Suttor R (F_{ST} 0.016) and Burdekin Bowen R vs. Burdekin R (F_{ST} 0.015), suggesting drainage basins are the most local unit of population structure. Distinctiveness of the upstream Burnett R population, as supported by mtDNA (Fig. 5.2), was not strongly supported by nuclear data. Therefore, this location was included in all analyses of microsatellite data. Pairwise comparisons among drainages were highest across regions (mean F_{ST} 0.274, SD 0.090), compared to within regions (mean F_{ST} 0.144, SD 0.074). AMOVA analyses also revealed significant hierarchical population structure (Table 5.5). At a regional scale, 10.4% of genetic variation was attributed to variation among regions ("north" and "south") (P = 0.012), and 9.9% to variation among drainages within regions (P = 0.000). A drainage-level analysis attributed 15.9% of genetic variation to among drainages (P = 0.000), with a small but significant 0.7% (P = 0.000) was attributed to among locations within drainages.

Analyses in STRUCTURE further highlighted hierarchical population subdivision in *E. m. krefftii*. A primary division between two genetic clusters (highest delta K for K = 2) (Fig. 5.4a) was revealed that corresponded to northern and southern mtDNA clades and the Burdekin-Fitzroy drainage divide. However, distinct regions of admixture were evident in the Pioneer and Proserpine Rivers and a group of six individuals sampled from the upstream Fitzroy drainage (Theresa Ck, Mackenzie R sub-catchment). These three locations coincide with instances of haplotype sharing across the Burdekin-Fitzroy boundary, and have adjacent headwaters with the Burdekin, implying contemporary dispersal across a long-term barrier. Independent analysis of the southern cluster revealed further genetic subdivision into three distinct clusters of neighbouring drainages (highest delta K for K = 3, Fig. 5.4b), corresponding to the Mary-Burnett-Kolan drainages, the Fitzroy drainage, and the Pioneer-Proserpine drainages. When investigating individual assignments within the southern cluster beyond the best K, individual drainages became increasingly differentiated at higher values of K, up to K = 6 (Fig. 5.4b) when all drainages were differentiated and beyond which no further substructure was evident. There was no evidence for a divergent third lineage within the upstream Burnett R as for mtDNA. Three distinct clusters also best described subdivision within the northern cluster (highest delta K for K = 3, Fig. 5.4c), differentiating between the Burdekin-Herbert-Alligator Ck drainages, the Mulgrave-Russell drainage, and the Normanby drainage. Higher values of K further differentiated individual drainages, up to K = 4 (Fig. 5.4c), when all

Table 5.4 Pairwise F_{ST} comparisons between drainage basins for Krefft's river turtle, *Emydura macquarii krefftii*, based on 12 polymorphic microsatellite markers. Bold values are comparisons across "northern" and "southern" geographic regions. All values are significant at 0.001 after correction for multiple comparisons.

Drainage	Mary	Burn	Kolan	Fitzr	Pion	Pros	Burd	MR	Norm
Mary	-								
Burnett	0.038	-							
Kolan	0.060	0.044	-						
Fitzroy	0.076	0.059	0.100	-					
Pioneer	0.187	0.150	0.215	0.087	-				
Proserpine	0.226	0.204	0.237	0.148	0.186	-			
Burdekin	**0.161**	**0.171**	**0.212**	**0.173**	**0.240**	**0.294**	-		
M-Russell	**0.216**	**0.223**	**0.269**	**0.199**	**0.270**	**0.330**	0.114	-	
Normanby	**0.307**	**0.304**	**0.334**	**0.291**	**0.457**	**0.484**	0.198	0.272	-

Table 5.5 Hierarchical analysis of molecular variance (AMOVA) results for *Emydura macquarii krefftii* microsatellite data analysed at two spatial scales.

Source of variation	**Variation (%)**	**Fixation index**	***P* value**
Regional scale			
Among regions	10.42	F_{CT} = 0.104	0.012
Among drainages within regions	9.93	F_{SC} = 0.111	0.000
Within drainages	79.64	F_{ST} = 0.204	0.000
Drainage scale			
Among drainages	15.87	F_{CT} = 0.159	0.000
Among locations within drainages	0.73	F_{SC} = 0.009	0.000
Within locations	83.39	F_{ST} = 0.167	0.000

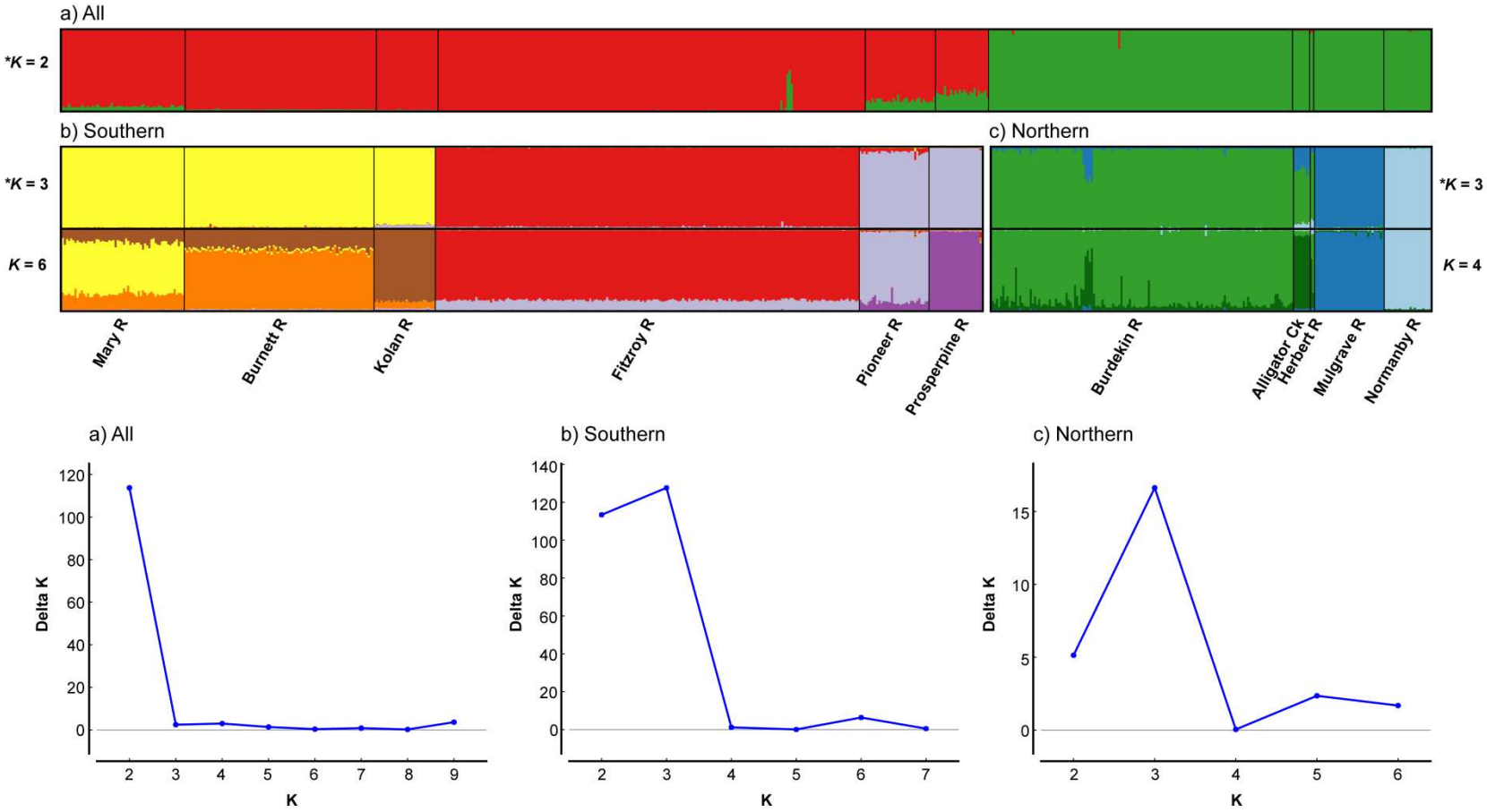

Fig. 5.4 Bayesian population assignment plots (top) for *E. m. krefftii* individuals sampled from 11 drainages, based on STRUCTURE analyses of 12 microsatellite loci. Individuals are represented by coloured vertical bars indicating their percent genetic membership (y axis) within *N* genetic units (*K*). Black lines separate individuals sampled from different drainages (x axis). *Indicates the most likely number of clusters (as per Evanno's delta log method, bottom): a) for the full dataset, indicating primary division between southern and northern genetic clusters; and for the southern b) and northern c) clusters analysed individually, indicating further substructure within regions. Highest level of substructure observed within southern and northern datasets are also indicated, showing differentiation among individual drainage basins.

drainages were differentiated except two Herbert R individuals, which showed mixed ancestry between the nearby Burdekin and Alligator Ck drainages. No further substructure was evident.

Hybridisation

Seven *E. m. krefftii* individuals, collected from the Normanby R (n = 5) and Mulgrave-Russell R (n = 2) in the north of the subspecies distribution, were found to have highly divergent mtDNA sequences (ND4 uncorrected *p*-distance from northern clade 8.19%, SE 0.99) and were excluded from all above analyses. Comparison with mtDNA sequences of other *Emydura* spp. (E. V. Todd unpubl. data) revealed 100% sequence identity (CR and ND4) with samples from the northern yellow-faced turtle, *E. tanybaraga* (n = 5, collected from Mitchell R and Staaten R, Fig. 5.1; see Appendix 5A for collection details). *Emydura tanybaraga* is poorly-known and closely resembles *E. m. krefftii* morphologically, but is currently thought to occupy rivers of northern Australia, west of the GDR. To investigate the possibility of potential hybridisation with *E. tanybaraga*, or misidentification of true *E. tanybaraga* occurring in a previously unrecognised sympatry with *E. m. krefftii* (i.e. cryptic species), multi-locus microsatellite genotypes from the seven "*E. m. krefftii*" samples in question were compared with those of the same five *E. tanybaraga* individuals sequenced for mtDNA (microsatellite data from Chapter 2; Todd *et al*. 2011). Though the five *E. tanybaraga* shared many microsatellite alleles found in the wider *E. m. krefftii* sample, eight alleles across six loci were unique to this species. Six of these unique alleles appeared within the seven "*E. m. krefftii*" with *E. tanybaraga* mtDNA signatures (Table 5.6). In all cases, these seven individuals were heterozygous and exhibited one allele unique to *E. tanybaraga* (in two individuals at one locus, an allele was shared between the two species), and another otherwise exclusive to the wider *E. m. krefftii* sample. This suggests an *E. tanybaraga* ♀ x *E. m. krefftii* ♂ hybrid origin for the seven individuals in question. No other individuals in the wider dataset exhibited alleles otherwise unique to *E. tanybaraga*, indicating no *E. m. krefftii* maternal hybrids or full *E. tanybaraga* individuals were sampled.

DISCUSSION

The present study examined range-wide genetic diversity and population structure for Krefft's river turtle, *E. m. krefftii*, throughout eastern coastal Australia. Fine-scale sampling and comparison of this subspecies' phylogeographic history (as measured from ~1.3 kb of mitochondrial sequence) with its more contemporary genetic structure (assessed by 12 nuclear microsatellite loci) permitted assessment of factors influencing genetic structure and diversity over its recent evolutionary history. This subspecies' range in eastern coastal Australia covers a region of particular biogeographic interest, spanning landscape and climatic barriers recently proposed as important evolutionary drivers of current freshwater biodiversity. Three divergent mtDNA clades were identified within *E. m. krefftii* and a strongly hierarchical population

Table 5.6 Multilocus microsatellite genotypes for seven potential hybrid individuals, between the study species (*Emydura macquarii krefftii*) and *E. tanybaraga*, for four diagnostic loci. Alleles unique to *E. tanybaraga* are in bold. A single allele common to both species is in italics.

Individual	**Drainage**	**Multi-locus genotype**[1]			
		Ekref06	*Ekref10*	*Ekref13*	*Ekref21*
EK504	Mulgrave-Russell	**201**/104	*104*/112	101/**104**	102/**112**
EK522	Mulgrave-Russell	**201**/104	*104*/112	101/**104**	102/**112**
EK633	Normanby	**201**/104	**102**/108	101/**104**	**200**/108
EK639	Normanby	**201**/104	**102**/109	101/**104**	**201**/108
EK642	Normanby	**201**/104	**102**/108	101/**104**	**201**/108
EK643	Normanby	**201**/104	**102**/108	101/**104**	**200**/108
EK650	Normanby	**201**/104	**102**/108	101/**104**	**201**/108

[1]Alleles are identified in relative repeat units.

structure was evident across its extensive distribution. A deep north-south genetic disjunction was consistent with an historical barrier in the form of the Burdekin-Fitzroy drainage divide, not the Burdekin Gap dry corridor (located entirely within the Burdekin basin). The degree of divergence across the drainage divide is inconsistent with a hypothesis of recent range expansion from southern refugia, impliying *E. m. krefftii* persisted during historical aridity in the Burdekin region. Nevertheless, the dramatically lower genetic diversity of northern populations suggests they suffered population bottlenecks.

Hierarchical population structure

Despite generalist habitat requirements and an ability to traverse land, *E. m. krefftii* is highly genetically structured across its extensive longitudinal distribution. Regional divergence in *E. m. krefftii* reflects isolation across a major drainage divide – the Burdekin-Fitzroy drainage boundary – not the Burdekin Gap dry corridor (Fig. 5.2, 5.4). As for other drainages, turtles sampled among locations within the Burdekin drainage were very closely related at mtDNA and microsatellite data, despite separation by the Burdekin Gap. Low but significant F_{ST} values among Burdekin locations for microsatellites (range 0.011-0.016) suggest only weak restrictions on dispersal, consistent with distance, a large waterfall (the Burdekin Falls, Fig. 5.1), and seasonal habitat isolation due to aridity. The Burdekin (130 400 km^2) and Fitzroy (141 100 km^2) basins are unique on Australia's eastern seaboard for their large size and geological antiquity, having expanded westwards during the Paleogene (Jones 2006). Bathymetric data also imply independent paleochannel trajectories for these systems during glacial low stands, when coastlines extended ~160 km offshore relative to today (Fielding *et al*. 2003; Ryan *et al*. 2007). North-south lineage divergence reported in this region for other riverine species (see Introduction), though consistent with the Burdekin Gap, can also be explained by this major drainage boundary. However, inadequate sampling within the Burdekin itself has previously precluded differentiating between these barriers as alternative biogeographic hypotheses. In Chapter 4, greatest divergence among east-coastal riverine specialist turtles in the genus *Elseya* also occured across this boundary (estimated at 5.82 Ma, 95% HPD 4.02-7.86), while disjunct populations inhabiting the southern Burdekin drainage and the Johnstone River further north (i.e. either side of the Burdekin Gap) were distinct only at the level of populations of a single species, *E. irwini*. The ancient Burdekin-Fitzroy drainage divide may represent a previously unrecognised, long-standing biogeographic barrier dividing northern and southern riverine taxa along Australia's eastern coastline.

The most local unit of population structure in this subspecies is represented by individual drainage basins, which contained unique genetic diversity at mitochondrial and nuclear markers. Most drainages contained unique haplotypes and pair-wise comparisons among drainages at

microsatellite data were all highly significant (mean F_{ST} 0.209, SD 0.104), with weak or no differentiation among locations within drainages (mean F_{ST} 0.008, SD 0.005). Therefore, though dispersal within drainages appears largely unconstrained, watershed boundaries act as long-term dispersal barriers. Clusters of related drainages were also evident, particularly in STRUCTURE analyses (Fig. 5.4). In the south, populations from the Burnett, Mary and Kolan Rivers are closely related, as are those within the Pioneer and Proserpine Rivers. Turtles from the Fitzroy drainage are distinct within the southern clade. Similar inter-drainage relationships are reported for co-distributed freshwater fish, in particular, genetic similarity of Burnett and Mary populations (Frentiu *et al*. 2001; McGlashan & Hughes 2001b; Thacker *et al*. 2007, 2008; Jerry 2008). For the locally endemic freshwater turtle *Elseya albagula* (Burnett, Mary and Fitzroy Rivers), examined in Chapter 3, the close Burnett-Mary relationship was found to be consistent with geological evidence of a recent confluence between these two river systems, severed by rising sea levels only ~12,000 years ago. Such a confluence is also supported by the *E. m. krefftii* data. In the north, the Burdekin drainage population is closely related to those in the neighbouring Alligator Ck and Herbert drainages, while the Mulgrave-Russell and Normanby populations are distinct.

There was, however, evidence for contemporary and historical dispersal across drainage divides. Contemporary (presumably overland) dispersal between divergent northern and southern clades was inferred in both directions across the Burdekin-Fitzroy drainage boundary. Instances of haplotype sharing coincide geographically with three regions of admixture uncovered in STRUCTURE analyses of microsatellite data, and indicate dispersal across adjacent headwaters via the geographically intermediate Pioneer and Proserpine Rivers and an upstream Fitzroy location (Theresa Ck). Closer relatedness of one Burdekin haplotype to Fitzroy haplotypes may also indicate historical crossing of the drainage boundary. Complex relationships among southern haplotypes, despite low haplotype sharing among drainages, may reflect periodic cross-drainage dispersal followed by isolation and divergence. Isolation by drainage divides coupled with occasional cross-drainage dispersal may be important in generating considerable genetic diversity observed across the whole distribution of *E. m. krefftii*.

Historical demography

Stark differences in levels of genetic diversity observed either side of the Burdekin-Fitzroy boundary may reflect differences in historical demography and climatic regime. Regions south of the Burdekin did not experience as extreme aridity in the recent past. The southern lineage contained high levels of genetic diversity at mtDNA and nuclear markers, and demographic analyses suggest a stable population history (Fig. 5.3). The region of the Fitzroy, Burnett and Mary Rivers supports high freshwater species diversity, especially for freshwater turtles (seven

species, three local endemics) and other old relict Gondwanan lineages (e.g. lungfish and saratoga), implying enduring aquatic habitat (discussed in detail in Chapters 3 and 4). By contrast, genetic diversity estimates for northern *E. m. krefftii* were strikingly low. However, the considerable divergence observed among northern and southern *E. m. krefftii* clades does not imply recent northwards expansion from a southern stronghold, but long-term isolation and persistence of northern populations, presumably throughout multiple arid cycles. In support, fossil *Emydura* unearthed at Bluff Downs in the north-west Burdekin (Fig. 5.1) are indistinguishable from living *E. m. krefftii* occupying the same locality today (Thomson & Mackness 1999). Radiometric dating of the overlying volcanic basalt provides a minimum age of 3.62 ±0.05 Ma (Mackness *et al*. 2000). The fossil deposits also contain water birds and other turtle species, including riverine specialist *Elseya* sp. and swamp-adapted *Chelodina* sp., indicating diverse aquatic habitat including permanent rivers and streams at the time of preservation (Thomson & Mackness 1999). Therefore, Krefft's turtles already occupied the region in the early Pliocene, which is well-known to have been globally warmer and wetter than the present day, with evidence for more mesic conditions in eastern coastal Australia before the establishment of major cyclic aridity (Martin 2006).

Northern populations may be expected to have undergone recurrent bottlenecks during glacial aridity, known to be especially severe in the Burdekin, followed by demographic expansions as favourable conditions returned. However, there was only weak support for a demographic expansion model. Many Northern Hemisphere species, including freshwater fishes and turtles, show clear signatures of severe bottlenecks and range reductions into isolated glacial refugia, followed by dramatic demographic and range expansion since the end of the last ice age (e.g. Lenk *et al*. 1999; Starkey *et al*. 2003; Seifertova *et al*. 2012). Cyclic Pleistocene aridity in Australia appears not to have produced molecular signatures of wide-scale contraction to major refugia and subsequent macrogeographical range expansion as seen for temperate Northern Hemisphere biotas (reviewed in Hewitt 1996, 2000). Recent work in Australia's arid zone suggests a less overwhelming and more idiosyncratic response of taxa, whereby divergence builds up over repeated cycles of localised contraction and expansion from patchwork refugia throughout a species' range (Byrne *et al*. 2008; Lanier *et al*. 2013). In the case of *E. m. krefftii*, bottlenecks may have been less severe and geographically widespread, and therefore expansions less dramatic. Their generalist ecology likely facilitated survival in multiple peripheral habitats (e.g. isolated waterholes and spring-fed streams), and swift re-colonisation once freshwater connections re-established. Divergence between turtle populations from the Burdekin drainage and further north may indicate multiple refugia, where northern drainages were colonised from an isolated population that has since expanded farther northwards. An ability to move overland, abandoning drying habitat in search of permanent waterbodies, probably also allowed *E. m.*

krefftii to survive periods of climatic harshness in this region when many other freshwater taxa were extirpated, as currently reflected in the Burdekin's dramatically low species diversity.

Coalescent-based analyses were not performed in the current study, but would facilitate more robust statistical testing of competing hypotheses of local persistent versus historical range expansion, evaluation of historical dispersal routes, and population size fluctuations. Such analyses would strengthen biogeographical conclusions of the current work and are currently being explored.

A 3rd divergent matrilineage in *E. m. krefftii*

A third divergent matrilineage in *E. m. krefftii*, found exclusively in an upstream Burnett River location, is difficult to explain as nuclear microsatellite data indicates essentially free gene flow within this system. Though such incongruence between nuclear and mitochondrial datasets may suggest male biased gene flow and/or female philopatry to nesting sites (Toews & Brelsford 2012), both of which are reported in freshwater turtles elsewhere (e.g. Sheridan *et al*. 2010), neither is sufficient to explain the observed concentration of divergent haplotypes within this single location. Also, though all individuals were collected within impounded waters of the Barambah Dam, contemporary impoundments have also not been in place long enough to cause such divergence in this long-lived species, and many other such impoundments exist throughout its range. A possible explanation involves the anthropogenic translocation of turtles into the dam from elsewhere. Introgression of a divergent mitochondrial lineage into the native population through interbreeding could have increased its population frequency, while the dam prevented divergent haplotypes from being dispersed throughout the catchment. Though analysis of mtDNA from related subspecies did not reveal a likely source population, sampling was geographically restricted. Available samples of *E. m. macquarii* were from the Murray-Darling drainage, west of the GDR, though *E. macquarii* is also widespread in populated coastal areas south of the Mary River. Samples from these drainages would be necessary to further investigate the potential for a translocated origin of the divergent third lineage within *E. m. krefftii*.

Hybridisation

Mitochondrial and microsatellite data revealed a potential hybrid zone between northernmost *E. m. krefftii* populations (Normanby and Mulgrave-Russell drainages) and *E. tanybaraga*, a poorly known member of the northern *Emydura* complex. Seven individuals with mtDNA sequences identical to *E. tanybaraga* had microsatellite genotypes intermediate between the two species, implying first generation *E. tanybaraga* ♀ x *E. m. krefftii* ♂ hybrids. *Emydura tanybaraga* is thought to occupy coastal rivers of the Northern Territory and Queensland west

of the GDR, and is distinct from other *Emydura* electrophoretically (Georges & Adams 1996). However, it is not readily distinguishable in the field and its taxonomic status remains uncertain (Georges & Thomson 2010). Hybridisation may reflect recent contact between these species, via range expansion by *E. tanybaraga* across the GDR, which separates headwaters of the Staaten and Mitchell Rivers (occupied by *E. tanybaraga*) flowing west into the Gulf of Carpentaria, from those of the Normanby and Mulgrave-Russell Rivers flowing to the east. Since hybrids were sampled in two drainages, hybridisation may not be a one-off event.

Turtles appear especially prone to hybridisation (Karl *et al*. 1995; Fritz *et al*. 2008). Accumulating reports suggest it is also common among Australian chelids in the wild (Georges *et al*. 2002; Georges *et al*. 2007; Alacs 2008). The current data appears to be the first reported case within the genus *Emydura*, though is the second case of hybridisation in turtles from the Normanby River: *Chelodina canni* and *C. rugosa* hybridise extensively with introgression here and in several other areas of range overlap across northern Australia (Alacs 2008). *Chelodina canni* also freely hybridises with *C. longicollis* (a southern species) to yield fertile offspring in a region of range overlap in the Styx River east of the Burdekin-Fitzroy drainage divide, making the reported hybridisation in *Emydura* also the second example between northern and southern sub-generic groups of Australian chelid. The extent, direction and evolutionary implications of hybridisation at the northern range limit of *E. m. krefftii*, and Australian turtles more broadly, warrant further investigation.

Subspecies relationships

Genetic data presented herein do not support the genetic distinctiveness of the four *Emydura macquarii* subspecies as currently recognised, at least using mtDNA. Earlier allozyme work corroborates this finding, and found no fixed allozyme differences among the subspecies, which even share rare alleles (Georges & Adams 1996). Uncorrected ND4 *p*-distance between northern and southern *E. m. krefftii* clades, at 2.23%, is within the range typically observed among congeneric chelonian species (range 1.5% to 18.3%; see reviews in Vargas-Ramírez *et al*. 2010; Fritz *et al*. 2012). Nevertheless, contemporary gene flow clearly occurs where their ranges abut. Despite restricted geographical sampling of the remaining three subspecies, the haplotype network clearly shows *E. m. macquarii* and *E. m. nigra* are nested within the southern *E. m. krefftii* lineage (Fig. 5.2). Only *E. m. emmotti* appears distinct genetically. Chelonian subspecies designations often reflect subtle morphological variation among non-overlapping geographic entities, and it is not uncommon for these to be unsupported by molecular data (e.g. Fritz *et al*. 2008, 2012). Deep lineage structure across the broader *Emydura macquarii* complex likely represents phylogeographic substructure within a single widespread species, though it is not necessarily in line with current subspecies designations. Range-wide investigation of genetic

variation in *Emydura macquarii* using both mitochondrial and sensitive nuclear markers (e.g. microsatellites) is warranted (including detailed morphological analysis).

Nevertheless, haplotype relationships provide preliminary insights into biogeographic relationships between *E. m. krefftii* and the remaining subspecies, and tantalising evidence of historical relationships among major biogeographic regions. The close relationship of *E. m. nigra* (Fraser Is) with *E. m. krefftii* haplotypes from the Mary River reflects similar patterns reported in freshwater invertebrates and fishes (Bentley *et al*. 2010; Page *et al*. 2012) suggested to indicate historical fluvial connectivity at times of low sea level (Fraser Is is adjacent the coast directly from the mouth of the Mary River). *Emydura m. macquarii* sampled from the Murray-Darling basin, which is west of the GDR and considered a distinct zoogeographic province (Unmack 2001), shows an equally close relationship to haplotypes from the Burnett River. Genetic evidence from several freshwater fishes implies historical dispersal (primarily westwards) across the GDR in the vicinity of the Burnett River, where the Eastern Highlands are relatively subdued (Thacker *et al*. 2007, 2008; Jerry 2008; Faulks *et al*. 2010). Though *E. m. emmotti* (Cooper Ck) is obviously distinct, it appears most closely related to *E. m. krefftii* in the Burdekin River (northern lineage). Again, a similar relationship is reported for freshwater fishes and may indicate historical dispersal across the Eastern Highlands in one of several areas of low drainage divide (Unmack & Dowling 2010; Thacker *et al*. 2007).

CONCLUSIONS

Range-wide genetic structure and molecular diversity in a freshwater turtle, *E. m. krefftii*, primarily reflects isolation by drainage divides, though with evidence of occasional overland dispersal. Genetic data newly identified a biogeographic barrier in the form of an ancient drainage divide between the geographically extensive Burdekin and Fitzroy basins that appears significant in structuring freshwater biodiversity along Australia's east coast. Data suggest *Emydura m. krefftii* persisted in a region of severe historical aridity through multiple glacial cycles, though population bottlenecks may be responsible for very low contemporary genetic diversity. Data presented in Chapter 5 also highlight the importance of fine-scale geographical sampling in analysing the role of biogeographic barriers and the utility of integrating mitochondrial sequences and sensitive nuclear datasets for inferring evolutionary history.

Genetic relationships within the broader *Emydura macquarii* complex appear more complicated than previously appreciated. Members of the complex exhibit a strong biogeographic signature, highly concordant with co-distributed taxa. This taxon may be an especially valuable model for unravelling biogeographic complexity in south-eastern Australia, and may help answer several long-standing questions regarding historical patterns of freshwater connectivity and dispersal, including across the Eastern Highlands.

CHAPTER 6 – GENERAL DISCUSSION

Image: Adult female Krefft's turtle, *Emydura macquarii krefftii*, from the Connors River within the Fitzroy basin, showing typical macrocephaly seen in older animals. Photo by Blanche D'Anastasi.

CHAPTER 6 – GENERAL DISCUSSION

Work presented in this thesis reports on the development and application of molecular genetic tools to explore evolutionary histories of two riverine turtle taxa. Freshwater turtles are poorly researched but important components of Australia's endemic freshwater fauna, and their utility as models for biogeographic inference was examined to enhance understanding of historical influences on freshwater biodiversity evolution in Australia. Australia's eastern coastal margin was a region of particular focus, because of its complex geological and climatic history and complicated freshwater biogeography. The two turtle taxa examined are both primarily riverine but have important ecological differences, being either more generalist (*E. m. krefftii*), or more specialist (*Elseya* spp.), in their habitat requirements. Habitat differences between these two taxa were expected to engender differences in dispersal ability, and therefore, phylogeographic structure.

Comparison across turtle taxa

Generally, ecological specialisation, where a species exploits a narrow niche more effectively, is an important factor affecting dispersal capacity because suitable habitat for specialist species should be patchier than that which can be utilised by generalists. It follows that gene flow may be more restricted, and populations more genetically subdivided, in species with specialised ecologies (reviewed in Futuyma & Moreno 1988). Despite habitat differences, species from both turtle taxa were found to exhibit a strikingly similar pattern of genetic structure that fundamentally reflects contemporary and historical isolation across drainage divides. Analyses presented in Chapters 3 through 5 demonstrate that for both *E. m. krefftii* and *Elseya* spp., dispersal is largely unconstrained within hydrologically connected systems (i.e. drainage basins), though each basin contains a discrete genetic unit. Hierarchical genetic structure observed across the range of *E. m. krefftii* (Chapter 5) and *E. albagula* (Chapter 3) is consistent with changes to drainage architecture over geological (i.e. evolutionary) timescales, where there has been physical connectivity across some drainages but long-term isolation of others.

Genetic structure by drainage divides is also reported for riverine turtles in the Americas (Roman *et al*. 1999; Souza *et al*. 2002; Pearse *et al*. 2006; Escalona *et al*. 2009). Despite their ability to move overland, watershed boundaries apparently present significant dispersal barriers to riverine turtles generally. Semi-aquatic turtle species and those more adapted to swampy or estuarine habitats often show much less genetic structure (Walker *et al*. 1998; Hauswaldt & Glenn 2005). Therefore, although Krefft's turtles (*E. m. krefftii*) have broad niche requirements that enable them to inhabit rivers plus permanent waterbodies of the floodplain, their population genetic structure is that of a primarily riverine species.

There was, however, evidence for very infrequent (presumably overland) dispersal only by *E. m. krefftii* across a long-term drainage boundary common to both turtle taxa. Recent dispersal between divergent northern and southern Krefft's turtle lineages across the Burdekin-Fitzroy drainage divide was indicated by mtDNA and microsatellite data in three headwater locations (Pioneer and Proserpine Rivers, and an upstream Fitzroy basin location) (Chapter 5). There was also haplotype sharing and weak genetic structure for this subspecies across drainages north of the Burdekin, where demographic tests of population expansion were significant and evidence was found for hybridisation between Krefft's turtles and *Emydura tanybaraga*. Together, this indicates recent cross-drainage dispersal and population expansion in the far north of this subspecies' distribution.

As Krefft's turtles regularly occupy peripheral permanent freshwater habitats within the wider catchment area, they are better placed to exploit opportunities for cross-drainage dispersal. Greater propensity for dispersal across drainage divides in the habitat generalist may account for disparity in the degree of divergence observed for *E. m. krefftii* and *Elseya* spp. across the Burdekin-Fitzroy drainage boundary, despite both taxa being recorded from fossils within early Pliocene deposits in the north-west Burdekin drainage (Thomson & Mackness 1999). Further evidence for a greater long-term dispersal potential in *E. m. krefftii* is its extensive longitudinal distribution in coastal Queensland, which if the broader *E. macquarii* complex is considered (Fig. 5.1), represents one of the most extensive geographic distributions for any freshwater turtle in Australia.

For *Elseya* spp., which are confined largely to permanent flowing riverine habitat, long-term dispersal opportunities appear to depend largely on changes to drainage architecture over geological time, and there was evidence for a role of low sea level confluences (e.g. close genetic affinity of adjacent Burnett and Mary River populations in *E. albagula*), and potential river capture events (e.g. close relationship of *E. lavarackorum* with *Elseya* spp. on the north-east coast). Burridge *et al*. (2008) also found that dispersal across watershed boundaries may be predicted to some extent by species' ecology. Four freshwater fish species, differing in their ecology, were investigated for genetic evidence of inter-drainage dispersal across a young, low-relief drainage divide in New Zealand (thus controlling for historical drainage geometry). Phylogeographic relationships based on mtDNA were consistent with ecological expectations of inter-drainage dispersal capacity: the two obligate riverine taxa exhibited reciprocal monophyly between catchments, indicating genetic isolation, while the two facultative swamp dwellers were paraphyletic and shared haplotypes between drainages, indicating contemporary inter-drainage gene flow.

Overall, the more generalist *E. m. krefftii* may be better adapted to the cyclic climatic variability that characterises Australia's recent history, while riverine specialist turtles have struggled to adjust. Species of *Emydura* share similarly broad ecologies, are widely distributed, and are very closely related genetically based on studies of allozymes (Georges & Adams 1996). If the genus *Emydura* does represent a relatively recent radiation, this implies a more flexible response to the challenges presented by Australia's recently harsh climatic history. For riverine specialist turtles, like *Elseya* spp., but also *Rheodytes leukops* and *Elusor macrurus*, which currently represent monotypic genera that occupy single drainages, fossil evidence implies much broader historical distributions than the present day. Chapter 4 presents a dated phylogeny for the genus *Elseya* which implies they diversified initially in the Miocene, or earlier in the case of *R. leukops* (*E. macrurus* samples were unavailable for analysis), when Australia was yet to experience widespread aridity.

Turtles as model organisms for Australian freshwater biogeography

Turtles have proven sensitive biogeographic models in the Northern Hemisphere for inferring how historical geological and climatic factors influence contemporary patterns of biodiversity. Work presented in this thesis demonstrates the utility and value of riverine turtles for inferring biogeographic processes in an Australian context. Both turtle taxa examined exhibit strong genetic structure at multiple hierarchical scales, consistent with the underlying geological and climatic history of the continent. Their utility as phylogeographic models is outlined below, with respect to three key aspects of genealogical concordance proposed by Avise (1998). The discussion below draws on previous research into Australian freshwater biogeography, highlighting concordance with other taxa and offering new insights that clarify and extend our current knowledge of the field.

i) Concordance in significant genealogical partitions across multiple unlinked loci within a species

Concordance across multiple unlinked loci establishes observed genetic partitions as true intra-specific phylogenetic partitions. Modern phylogeographic investigations no longer rely solely on mitochondrial DNA as the bridge between phylogenetics and population genetics (Avise *et al*. 1987). Routine inclusion of multiple nuclear markers like microsatellites has dramatically improved the power of phylogeographic inference (Avise 2009; Edwards & Bensch 2009; Andrew *et al*. 2013). The utility of the microsatellite markers developed in Chapter 2 for phylogeographic inference is demonstrated in Chapters 3 and 5. A recent study also demonstrates the utility of the developed markers for genetic paternity studies in *E. albagula* (Todd *et al*. 2013c).

There was strong agreement among the genetic signatures inferred from mtDNA and microsatellite datasets within studies of *E. albagula* (Chapter 3) and *E. m. krefftii* (Chapter 5). Within each species, both marker types agreed as to the primary genetic division, which in *E. albagula* occurs between populations inhabiting the Fitzroy and Burnett-Mary drainages, and in *E. m. krefftii* occurs across the Burdekin-Fitzroy drainage divide. MtDNA and microsatellite markers were also consistent in estimating low versus high genetic diversity among major genetic lineages, and in identifying further genetic structure among drainage basins.

Discordance in genetic patterns observed at nuclear and mitochondrial data is not uncommon and can reflect demographic disparity among nuclear and mitochondrial genomes or sex-biased asymmetries in dispersal characteristics. For example, higher F_{ST} values are typical for mtDNA, due to haploid inheritance, lower effective population size, and higher fixation rate for the mitochondrial genome (Karl *et al*. 2012), but can also indicate male-biased dispersal (Toews & Brelsford 2012). For *E. albagula* (Chapter 3), higher F_{ST} values for mtDNA may reflect the tendency for female turtles to be philopatric to nesting sites (Freedberg *et al*. 2005). Strong discordance in biogeographic patterns is less commonly observed between nuclear and mtDNA markers, but can imply adaptive introgression of mtDNA, hybrid zone movement, or human introductions (reviewed in Toews & Brelsford 2012). Evidence for the latter was uncovered for *E. m. krefftii* in Chapter 5. The divergent matrilineage detected within the upstream Burnett River, despite free gene flow indicated within this drainage at microsatellite loci, cannot easily be explained by sex-biased dispersal. Anthropogenic translocation of turtles, followed by introgression of divergent haplotypes into a population now isolated by an artificial impoundment, would explain the observed discordance given the popularity of *E. macquarii* in the pet trade and its common occurrence in many urban areas. Further geographic sampling of *E. macquarii* populations is necessary to identify a potential source population.

ii) Concordance in the geographical positions of significant genetic partitions across multiple co-distributed taxa

Concordance in phylogeographic patterns described across co-distributed taxa strongly implies shared historical biogeographic factors shaping observed phylogenies, typically relevant on a regional scale. Remarkable concordance was observed in several aspects of phylogeographic structure between *Elseya* spp. and *E. m. krefftii* where they co-occur in eastern Australia, and between these taxa and other freshwater groups.

Most significantly, both turtle taxa exhibited deep genetic division across the Burdekin-Fitzroy drainage divide (Chapters 4 and 5). Past studies of widespread freshwater fishes (Wong *et al*. 2004; Jerry 2008) and platypus (Gongora *et al*. 2012) uncovered a similar genetic break in this region. However, insufficient sampling of Burdekin populations meant the underlying

biogeographic cause could not be accurately established. The Burdekin-Fitzroy drainage divide has not previously been considered an important biogeographic feature in eastern Australia. Genetic data for turtles presented in this thesis demonstrates its potential biogeographical significance in structuring eastern Australian freshwater communities (discussed under point *iii*, below), and the importance of appropriate fine-scale geographic sampling in accurately identifying, and differentiating between, multiple potential biogeographic drivers of divergence.

Further phylogeographic concordance was observed among *E. m. krefftii* (Chapter 5) and *E. albagula* (Chapter 3) in terms of the close genetic affinity of populations sampled across the Burnett and Mary drainages. Chapter 3 develops the hypothesis that a recent confluence of the Burnett and Mary rivers during low sea levels, which bathymetric and climatic data suggest was severed only 12,000 years ago, is responsible for current genetic similarity observed between populations of *E. albagula* and other freshwater taxa examined in this region to date. In Australia, this may in fact be a rare example of genetic evidence from diverse freshwater species corroborating a predicted low sea level drainage confluence. For example, in Chapter 4, the shallow, brackish waters of Lake Carpentaria in northern Australia were implicated as a habitat barrier for riverine specialist *Elseya* spp., despite genetic evidence for widespread dispersal in other freshwater taxa better adapted to lacustrine conditions. Unexpectedly high levels of genetic structure are also reported for freshwater galaxids (*Galaxiella* spp.) (Unmack *et al*. 2012) and pygmy perch (*Nannoperca* spp.) (Unmack *et al*. 2013) inhabiting drainages surrounding Bass Strait, despite extensive drainage coalescence predicted via the formation of Bass Lake within the last glacial cycle. Genetic evidence from freshwater gudgeons (*Philypnodon* spp. and *Hypseleotris* spp.) also contradicts expectations of population connectivity during low sea levels in south-eastern Australia, where levels of genetic structure actually increase with continental shelf width and thus potential for drainage coalescence (Thacker *et al*. 2007, 2008). Whether or not low sea level drainage confluence promotes genetic exchange among coastal freshwater populations may depend largely on species ecology and regional climatic scenarios. Heightened aridity during glacial low-stands conceivably reduced river outflow such that some downstream connections were made unsuitable for many freshwater taxa. Clearly, genetic structure cannot necessarily be predicted from expected patterns of drainage coalescence during historical low sea level events.

Chapter 4 presents a phylogeny and evolutionary timeline for the genus *Elseya* that is relevant over a much broader temporal and geological scale than is accessible with microsatellite data. Estimated timing of major divergence events within the genus *Elseya* are concordant with those inferred for other freshwater groups (e.g. *Cherax* crayfish), but also for terrestrial taxa (reviewed in Byrne *et al*. 2008, 2011). Therefore, patterns of divergence within the genus *Elseya* are consistent with molecular research to date that confirms major regional patterns of

diversity were established early, at the onset of major aridity in the Mid-to-Late-Miocene, and later reinforced and extended during Plio-Pleistocene climatic cycling (Byrne *et al*. 2008, 2011; Chapter 4). There was also strong concordance in patterns of connectivity estimated for *Elseya* spp. and other taxa across the periodically exposed land bridge connecting Australia with New Guinea. The detailed phylogeographic analysis of snapping turtles presented in Chapter 4 provides a critical freshwater perspective that adds strength to current hypotheses regarding the establishment of regional patterns of diversity for fauna and flora across Australia and New Guinea (Cracraft 1991; Unmack 2001; Crisp *et al*. 2004).

Overall, there was a striking degree of concordance in patterns of phylogeographic structure observed between riverine turtle taxa examined in this thesis, and between these turtles and other freshwater groups. Australian freshwater turtles are shown to be sensitive model organisms for inferring historical biogeographic processes shaping contemporary patterns of freshwater biodiversity.

iii) Concordance of molecular genetic partitions with geographical boundaries between traditionally recognised biogeographic provinces

Genealogical concordance between organismal phylogenies and species distributional patterns strongly implies that shared historical biogeographic factors have shaped regional community structure. There are several good examples from the Northern Hemisphere where extensive study of regional faunas has provided detailed insights into how historical processes of geography and climate influence not only regional species community composition, but the spatial structure of intra-specific genetic lineages (e.g. in the southeastern United States: Bermingham & Avise 1986; Avise 1992, 1996; Walker & Avise 1998). In Australia, although terrestrial vertebrate communities have been well-researched (see reviews by Byrne *et al*. 2008, 2011; Bowman *et al*. 2010), a consensus for freshwaters is still lacking.

Unmack (2001) proposed a comprehensive series of freshwater zoogeographic provinces and sub-provinces based on distributional patterns of Australian freshwater fishes. He observed striking patterns of endemism across the continent, and his framework of province boundaries and inter-relationships provide an extensive hypothesis base for testing with molecular genetic data. However, cases are accumulating where genetic data match poorly with these province boundaries (Fig. 1.1), both in fishes (e.g. Thacker *et al*. 2007) and other groups (e.g. freshwater turtles examined herein). Within the Northern and Eastern freshwater faunal provinces especially, a gradation of species ranges is evident even at the drainage scale that implies a lack of distinct faunal breaks or clear biogeographic barriers. In stark contrast, phylogeographic studies continue to uncover deep genetic divisions for freshwater taxa in these regions, with very few exceptions (e.g. Bostock *et al*. 2006).

Northern and Eastern freshwater provinces are currently delineated by the northern boundary of the Burdekin basin (Fig 1.1), where at least 13 northern freshwater fish species reach their southernmost distribution limit (Unmack 2001). However, this dramatic change in species composition appears primarily driven by the Burdekin's depauperate ichthyofauna, attributable to present and historical aridity, regional volcanism, and low habitat heterogeneity (Pusey *et al*. 1998). Data presented herein for riverine turtles finds the biggest genetic disjunction along Australia's east coast occurs across the Burdekin's southern boundary with the Fitzroy basin, which is also consistent with genetic disjunctions reported for other co-distributed freshwater taxa. Chapters 4 and 5 discuss geological and accumulating biological evidence that the Burdekin-Fitzroy drainage divide, which separates the two largest drainages on the eastern seaboard, is an ancient enduring boundary and a long-term barrier to gene flow for freshwater taxa. It apparently pre-dates factors responsible for the Burdekin's current low species diversity and may be more significant in defining freshwater bioregions of north-eastern and south-eastern Australia.

Plio-Pleistocene aridity within the Burdekin drainage (i.e. the Burdekin Gap) has nevertheless contributed to current regional patterns of freshwater biodiversity. Strikingly low genetic diversity within the northern clade of Krefft's turtles (Chapter 5), found throughout the Burdekin and further north, may reflect historical bottlenecks. Aridity in the Burdekin drainage has likely worked to reinforce the observed disjunction in genetic and species distributions for many aquatic taxa, by driving local extinction and preventing recolonisation. By contrast, the much greater levels of genetic diversity recorded within the southern Krefft's lineage across the Fitzroy, Burnett and Mary basins, supports the hypothesis, developed in Chapters 3 and 4 for *Elseya* spp., that these drainages represent enduring, stable aquatic habitat throughout multiple glacial cycles. Therefore, contrast in terms of genetic and taxonomic diversity between the neighbouring Burdekin and Fitzroy regions may reflect regional climatic stability within the Fitzroy compared to severe cyclic stress within the Burdekin basin. Interestingly, in phylogenies of freshwater species that occur throughout coastal and interior drainages, Fitzroy or Burnett drainage populations are often basal, indicating this region was an important source for colonisation (or recolonisation) of inland systems (e.g. Thacker *et al*. 2007; Jerry 2008; Unmack & Dowling 2010) that potentially experienced extinctions during arid periods. The history of colonisation by freshwater species between east coastal and interior drainage systems warrants further research.

Emydura m. krefftii, and the wider *Emydura macquarii* complex more generally, is an ideal model taxon for examining the response of a widespread aquatic species to recent (i.e. Plio-Pleistocene) climatic variability in Australia, as well as for exploring many long-standing hypotheses regarding biogeographic barriers and historical dispersal routes. Coalescence-based

analyses (e.g. Approximate Bayesian Computation, Migrate-n, BayesAss) permit more rigorous statistical testing of alternative biogeographic hypotheses and evaluation of recent dispersal patterns and population size fluctuations, and would be a valuable addition to future studies.

High regional taxonomic diversity and endemism for freshwater fauna within the Fitzroy, Burnett and Mary Rivers, and their potential as a freshwater refugium, highlights the conservation value of this area. Australia still lacks a strong conservation framework for freshwaters, despite boasting several regions of significant worldwide freshwater biodiversity (Abell *et al*. 2008; Buhlmann *et al*. 2009; Vörösmarty *et al*. 2010) and the growing pressure on freshwater resources in Australia (Stein *et al*. 2002). Molecular data may be especially valuable in unveiling cryptic patterns of biodiversity distribution across the landscape, necessary for accurate spatial classification of freshwater biodiversity (Cook *et al*. 2008).

Biogeographic stochasticity

Overall, phylogeographic studies of diverse Australian freshwater fauna have revealed complex, often unexpected, patterns of lineage structure that paint a much more intricate biogeographic picture than can be appreciated from species distributions alone. Despite many instances of phylogeographic concordance, there are also numerous examples of discordance that suggest freshwater biogeography in Australia may be more idiosyncratic than previously appreciated. Therefore, potential reasons for stochasticity in biogeographic patterns are briefly discussed. These include that stochasticity reflects inherent differences in species ecology and dispersal potential, together with Australia's highly variable climatic history.

In terms of species ecology, those species with more generalist niche requirements, and with life history traits that encourage rapid establishment in new habitats, generally show low genetic structure (Bostock *et al*. 2006). By constrast, species with specialist habitat requirements are often highly genetically structured across small geographic areas (Hughes *et al*. 1999, 2012). For example, Australia's most ubiquitous freshwater fish, the spangled perch *Leiopotherapon unicolor*, lacks any significant genetic substructure across eastern, northern, and central Australia (Bostock *et al*. 2006). The same is reported for the almost equally widespread empire gudgeon *Hypseleotris compressa* (McGlashan & Hughes 2001a). Both species are highly fecund and exploit a broad variety of freshwater habitats. Their genetic structures are suggestive of recent widespread dispersal and population expansion. The last interglacial is predicted to have been wetter than the current one (Hughes 2003), and greater freshwater connectivity may have promoted dispersal and colonisation opportunities for hardy freshwater species. Again, low genetic structure and significant tests for population expansion at the northerly range extent of Krefft's turtles may also reflect an ecological capacity to exploit periodically more favourable conditions.

Stochasticity in biogeographic patterns has already been noted for Australian terrestrial communities (Byrne *et al*. 2008). Phylogeographic signals of habitat refugia and population expansions are frequently taxon-specific in their geographical location and extent, especially for arid adapted taxa (Byrne 2008; Lanier *et al*. 2013). This is in contrast to much more predictable patterns of refugia and expansions documented for Northern Hemisphere species (Hewitt 2000), where glaciations were prominent. Widespread glaciations are obviously overwhelmingly catastrophic to biotic communities. By contrast, cyclic aridity conceivably allowed for greater variability in the response of individual species to the challenges (e.g. extinction in some lineages in some areas during drought) and opportunities (e.g. for dispersal and range expansion during more favourable conditions) presented during each climatic cycle. For freshwater communities along Australia's eastern coastal margin especially, cyclic aridity, sea level fluctuations, and geological complexity have acted concurrently to shape a particularly complex history of drainage interconnectivity. With the inherent variability in ecological tolerances and dispersal abilities of the component species, this has produced a complex freshwater biogeography that deserves further research attention.

CONCLUSIONS

In order to uncover common biogeographic constraints on dispersal and drivers of divergence, traditional comparative phylogeography has focused on studying taxonomically diverse species with comparable niche characteristics and dispersal abilities. Molecular data presented in this thesis collectively demonstrate the importance of comparing phylogeographic patterns among co-distributed taxa with variable ecological tolerances and dispersal abilities. Doing so enables study of how differences in species ecology and dispersal potential determine individual species' responses to common landscape features and climatic pressures. This thesis demonstrates the utility of freshwater turtles for inferring historical processes influencing biodiversity across a continent with a unique climatic history. Riverine turtles show remarkable concordance in the position of phylogeographic breaks and may be especially suited to illuminating complex biogeographic processes. The current work not only highlights the value of further studies of ecologically diverse freshwater turtles for uncovering aspects of Australia's biogeographic history, but also provides a comprehensive molecular toolkit for doing so.

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APPENDIX 2A

PCR primer details for perfect microsatellite repeats identified within 454-squence reads of *Emydura macquarii krefftii* and *Elseya albagula*, including a unique sequence ID for each read, repeat motif, number of tandem repeats, forward and reverse primer sequences (with unique names), expected PCR product size, and whether or not the primers have been empirically tested. GenBank accession numbers are given for published sequences.

GenBank no.	Sequence ID	Motif	No. of repeats	F Primer ID	F Primer Sequence	R Primer ID	R Primer Sequence	PCR product size (bp)	[1]Empirical testing result (blank if untested)
Emydura macquarii krefftii									
	F7SYSBQ01A2DTW	AC	17	Ek001_F	CAATCTGAAGGGAGTGTCCTAAG	Ek001_R	CTTTGCTTCAGAGCCCAGTC	242	
	F7SYSBQ01AVY33	AC	17	Ek002_F	TTGACTTCAGTGGGTGTTGG	Ek002_R	AAAGTACACCGTCCAAGATAAAGG	143	
	F7SYSBQ01BQ9A2	TG	17	Ek003_F	GTGTCCTGTAATGCCAATGC	Ek003_R	TTTCCTTCATCAAAGACCACG	168	
	F7SYSBQ01D4KU5	TG	17	Ek004_F	GTGGGCATAAGCTTTCATGG	Ek004_R	TTAGTCACTGGCCCGTCCTC	158	
	F7SYSBQ01AXBSH	AC	15	Ek005_F	GATGTAGCCACACGTTGCAG	Ek005_R	TGCAAGCAAGCAGACTAAGG	150	
	F7SYSBQ01BW7E4	AC	15	Ekref16_F	AAGCGGACTTAACCTCATGC	Ekref16_R	GACAATGTGTACATACCAGGCG	110	no amplification
HQ690770	F7SYSBQ01BZWKQ	CA	15	Ekref17_F	TTCAAATGCACCTTCACTGC	Ekref17_R	CGCAGTCACACTCTCACACC	147	characterised
	cons74_2_A	CA	15	Ek008_F	ATCACAATCTCACACGCACC	Ek008_R	GACGAATTAGGGTAGGAAGCG	159	
	cons80_2_A	CA	15	Ek009_F	TCCCTTGTGCACTCACATTC	Ek009_R	CAGTCTAGATGGGAGGTCGC	176	
	F7SYSBQ01A2NC5	CA	14	Ek010_F	ACCGCTTTGCTCTAGATTGC	Ek010_R	AGACAGGGTGGAAACAGTGG	141	
	F7SYSBQ01BK5GH	AC	13	Ek011_F	CACGCCACTGAGTACAGCAC	Ek011_R	CGTGGTGTTGAATGATCAGC	205	
HQ690771	F7SYSBQ01BXI9K	GT	13	Ekref18_F	GAGCATTCATGCGTGGAAC	Ekref18_R	GCAGAGAAATGAGGAAAGGATG	160	characterised
	F7SYSBQ01C7YKF	GT	13	Ek013_F	AAGCGCATCAGGAAGTGAG	Ek013_R	TTCATCTGCCATGGTGAAAG	202	
	F7SYSBQ01B0H94	CA	12	Ek014_F	GTTCCTCAGGCCCAGAATC	Ek014_R	GTGGCCTATGAGGGTGTCAG	116	
	F7SYSBQ01BUAV2	GT	12	Ek015_F	GTGTGGTCATGGATGGATTG	Ek015_R	AACCACCCTGAACAGCTCTC	128	
	F7SYSBQ01D0JDO	GA	12	Ek016_F	ACGATGAGTTTGGGATGAGC	Ek016_R	GCTGAGTGTGGCGTCTTAAC	101	
	F7SYSBQ01D67I8	CA	12	Ek017_F	AGGAGTTGGCTTAGTGCTCG	Ek017_R	AAACTACCCTGGTATAGAAATGGC	190	
	F7SYSBQ01DP271	TA	12	Ekref19_F	AACCAAAGTGCTGTAACTCAGTATG	Ekref19_R	TGAATGGCCCTGATTGTATG	173	polymorphic, not characterised
HQ690772	F7SYSBQ01DX8BB	AC	12	Ekref20_F	AATTGTCAGTGCAGAAGGGTG	Ekref20_R	ACAAAGGACACAGTCCCTGC	134	characterised
	F7SYSBQ01EFC11	TC	12	Ek020_F	GGCATGAGATTGGAATAGGG	Ek020_R	ATGTTGCTTTGCAGATGCC	131	
	F7SYSBQ01A64CW	AG	11	Ek021_F	CGTCTTAGGTGAAAGAGTAGAGAAAG	Ek021_R	TGCATTCAAAGGAGGACTGG	108	
HQ690774	F7SYSBQ01A9AFC	CA	11	Ekref22_F	AGAGAAGTGGCTTCGGTGTG	Ekref22_R	GCTGAAAGATGAGGTGTGGG	337	characterised
HQ690773	F7SYSBQ01APXVX	GT	11	Ekref21_F	GAGTGTTCCGCAGCATATTG	Ekref21_R	TGAAGGATGCACAACCCAC	160	characterised
	F7SYSBQ01B8TBV	AC	11	Ek024_F	GGTTCTCTGAGTTGAGTGTACAAAG	Ek024_R	TCACTAATCCCTCCTGCCTG	117	
	F7SYSBQ01BAHZT	TC	11	Ek025_F	GAATGTAAGGAATGGCCACG	Ek025_R	ATCTGCATGTCAGCGAGAAC	116	
	F7SYSBQ01BL95S	AC	11	Ek026_F	GTTTCTGTGTCTGGGTTGGG	Ek026_R	GGGAGACTGTGATGAGCACG	183	
	F7SYSBQ01CXSVE	AC	11	Ek027_F	CTCCAGCTGTAGCAAATAGCC	Ek027_R	CCAGGTTCTGAGCTTTCAGG	306	
	F7SYSBQ01DM968	CA	11	Ek028_F	CTGGCACGAAGAGCTAAAGG	Ek028_R	ACTGGGAGAAGACATGCAGC	140	
	F7SYSBQ01EKKRB	TC	11	Ek029_F	GATTGACGCCAGTGTACCG	Ek029_R	CCCGAAGTAGGGAAGGAAAC	193	
	F7SYSBQ01A0RY9	TG	10	Ek030_F	GCAGAAGCCCATGAAGAGTC	Ek030_R	GCCCAACTTCAATTCATTCC	112	

F7SYSBQ01A2K3O	AG	10	Ek031_F	TGAAAGAAGCAGCTGCAGAG	Ek031_R	CTTGATCCTCAGCGAGTGTG	249
F7SYSBQ01ANR4L	GA	10	Ek032_F	CGAGGTGGATGTGATTCTCC	Ek032_R	TACTTGAGCACCAACAAGGC	115
F7SYSBQ01B61H7	CA	10	Ek033_F	CTCATCCTGCAGCCTCACTG	Ek033_R	CTGATGCAACCTCAGCTGTC	103
F7SYSBQ01C33K6	TG	10	Ek034_F	TGCATCAGAGTGATTCAGCG	Ek034_R	GTGCAACCATTGATCCACAG	306
F7SYSBQ01CNP9W	AG	10	Ek035_F	GGCTGCTTCTCCTGGTAGTG	Ek035_R	TTGTGCTGCTCTGTGACCTG	219
F7SYSBQ01CRJHP	AC	10	Ek036_F	GGCAGTATTCGTGATGCAG	Ek036_R	TTTAGTGTGTTGTATGTGCTCCC	117
F7SYSBQ01DI7C0	AT	10	Ek037_F	TCTTGGGCACCTCTATGTCC	Ek037_R	TCCCTTATCAGTTTCCCAGG	121
F7SYSBQ01DKSWF	CT	10	Ek038_F	CAGTTCTGGTGGTTGCTCAG	Ek038_R	ATTAGTGCCAGCTGGTCTGC	144
cons46_2_A	CA	10	Ek039_F	GAGGGCTGGGTCATAACAAG	Ek039_R	TGTCTTCATGTAAATGGGCG	249
F7SYSBQ01A7FK1	AT	9	Ek040_F	AATCCCTTAGCCAGCCCTC	Ek040_R	CCTCAGGAACGTCTCAGGAC	181
F7SYSBQ01AY0DE	TG	9	Ek041_F	GATGGGACAGCCTAGTTTGG	Ek041_R	CCAGCCAGATATCCAGGAAG	147
F7SYSBQ01AY46R	TG	9	Ek042_F	GACTGCCGGCTATTTGAATG	Ek042_R	TGGTGGCTTGTGACTTTGAC	288
F7SYSBQ01B371D	AC	9	Ek043_F	TACACCGCAAGACAGAGGTG	Ek043_R	ATCTGTCTGTTTCATCCGCC	283
F7SYSBQ01BFZ9Z	GA	9	Ek044_F	GCTTAACTACAGTGGTGATGGATG	Ek044_R	AAGCTCCAAATTAATCCCTCG	263
F7SYSBQ01BKDZ1	TG	9	Ek045_F	AGCTATTGCAGTGAAAGCAGG	Ek045_R	AGGTGATGTCCTCCATTTGC	100
F7SYSBQ01CBHR3	TG	9	Ek046_F	TGCAGTACAGGACTGGGATG	Ek046_R	CTCCCACTCTCCTGCGTAAG	287
F7SYSBQ01COTTH	AT	9	Ek047_F	TCGACACTAGGATGGTTTGC	Ek047_R	GTTGGTGGAAGCATTTCTGG	207
F7SYSBQ01D94MW	GT	9	Ek048_F	CAGGAAGAGGCTCTGGAATG	Ek048_R	GATATTGGGAGAAGGGACGG	225
F7SYSBQ01DT2IB	AG	9	Ek049_F	TTGCATTGTGTTGTTGGCTC	Ek049_R	AACCCTACGTTTCCCTTTCC	252
cons47_2_A	AT	9	Ek050_F	CTTCTCCTGCACTGGGAAAC	Ek050_R	GCCCATTCAGGTGGTAGATG	217
F7SYSBQ01A0J5O	CA	8	Ek051_F	TAGCCAGTGGGCATTAGAAG	Ek051_R	AGTGTCCATTGGAGCTGTCC	252
F7SYSBQ01A2G11	AT	8	Ek052_F	TCCTACATGCCACTGACACC	Ek052_R	CAGGTGCAGAGAAGGGCTAC	191
F7SYSBQ01A5G9I	TG	8	Ek053_F	TGCAGCATATAGGCTAATGACC	Ek053_R	TCACATCTCAGGCAAAGTGC	134
F7SYSBQ01A6Y2H	TG	8	Ek054_F	TCTCTGTGCTCCATGCTGTC	Ek054_R	CAAAGGTGCCCAATGTTTG	129
F7SYSBQ01AGCTA	CA	8	Ek055_F	GTGGTCCAGATTCATGGCTG	Ek055_R	TGTCAAGTCACAAGGACCAG	122
F7SYSBQ01AQZ2B	GA	8	Ek056_F	TTTCAGGGTGCAAGATAAAGG	Ek056_R	GGGACAAGGGCTGAAGTTTAC	102
F7SYSBQ01AT5PB	AT	8	Ek057_F	AAAGGGCTTTCCTTGTCCTG	Ek057_R	TGCAGTGGCACTATTTCTGG	182
F7SYSBQ01AXFSV	TA	8	Ek058_F	CAAGCATGGGCAATATTTGG	Ek058_R	CTCCCAGTTCTTCATGACACC	140
F7SYSBQ01B4VCX	AG	8	Ek059_F	GTTGAAGCCATTCCACTTTG	Ek059_R	TGCAGGAGTTCAATTTGCAG	177
F7SYSBQ01B880Y	TG	8	Ek060_F	GAATGGGAATGGGAACAGTG	Ek060_R	CCAACAGTGGAACGTAAGGC	137
F7SYSBQ01BFLAN	AG	8	Ek061_F	TTGGCTCTGCTTATCAATGC	Ek061_R	AAATTCAGGCCCAGTTGAC	141
F7SYSBQ01BMKLC	TA	8	Ek062_F	AAGCCATCGAATTTCACCC	Ek062_R	TTGCGGTCCAACTCTTAAAC	239
F7SYSBQ01BO4NO	TC	8	Ek063_F	GTTTAGGTGGCTGAAGGCAG	Ek063_R	TTGGAAAGGCATGGGAATAG	141
F7SYSBQ01BSTRW	AC	8	Ek064_F	TTTATTTAGGGCGACAAGGC	Ek064_R	TGGCTGGTAAACTAACACAAGC	149
F7SYSBQ01BU506	CT	8	Ek065_F	TCTTGGAGTTAGGTCCCTGG	Ek065_R	TGCACTTACACAGTGCCTTTC	176
F7SYSBQ01BXLPL	CT	8	Ek066_F	TGCCTGGGTAGCAGGAATG	Ek066_R	ACAGCCCTGTTGTCTGGTTC	144
F7SYSBQ01BYLTU	CT	8	Ek067_F	ATACAAAGGCACGGATGAGG	Ek067_R	TGAATTGGGCTGAGGAAGTC	141
F7SYSBQ01BYPB6	GT	8	Ek068_F	CTTGTAGGAAGGAAGGGCAC	Ek068_R	AAATCCAAGTGGGTGTCTGG	146
F7SYSBQ01C0OFT	AT	8	Ek069_F	GAATGGACATGAGCAAGCAC	Ek069_R	TGCAGGTCCCTAGGTTTCTC	160
F7SYSBQ01C3HWO	GT	8	Ek070_F	AGCCGTGTTTGCTTCTGTG	Ek070_R	TGGTAGCCCTTTGGCATTAC	220
F7SYSBQ01C51BF	AT	8	Ek071_F	TCCTCCCAACAGAGTTAGCAC	Ek071_R	TGCAAGCAGAATAAAGTCCAG	110
F7SYSBQ01CBV8N	TC	8	Ek072_F	CTGGGACATCCCATACCAAC	Ek072_R	AACCTGATACGAGGGTCACTG	178
F7SYSBQ01CHSY5	AG	8	Ek073_F	AATTTCCCTTATGCTCAGCG	Ek073_R	CTGCACGTTGATAGAGCAGC	122
F7SYSBQ01CO6DJ	AT	8	Ek074_F	GGGATACACATTCACAGGGC	Ek074_R	TGCTTTGATCTGCATTGTCC	136
F7SYSBQ01CTVS0	GA	8	Ek075_F	TGATGCAATCCAAGGACAAC	Ek075_R	GGTGCCAAACAGGATCTCAG	161
F7SYSBQ01D2HX0	AC	8	Ek076_F	TTTCCGTTGCCTCTTCCC	Ek076_R	GAACTGGCTCCTTCACTTGC	110
F7SYSBQ01D3AXR	AG	8	Ek077_F	ATCAAATGAGGGTTGAGGAGG	Ek077_R	ACCTTCTACCAGATCAAGCTGC	100
F7SYSBQ01D8GV2	AC	8	Ek078_F	CTCAGCGGGACTACTGAAGG	Ek078_R	CTTTGCTTTGGTGTGGAGTG	170
F7SYSBQ01DBLHN	TG	8	Ek079_F	AAGGTCAATGTCGGTTCCAC	Ek079_R	TAGGCCTCATGTCATTGCTG	195

F7SYSBQ01DZKPN	TG	8	Ek080_F	GAACATCCAGTGGAACGGTG	Ek080_R	TTAGCTACCTCCTGGTTTATGG	107
F7SYSBQ01EC6GA	AG	8	Ek081_F	GAAGGAAAGCCAAGTGAAGG	Ek081_R	TGGTGATAAGGCCCAGAAAC	136
F7SYSBQ01EDGWD	GT	8	Ek082_F	TGAATCGAAAGGTTGATCCC	Ek082_R	CCCAGGGTCAAATTTAGTGG	148
cons27_2_A	TC	8	Ek083_F	GATGCACACCCAAATTCCTC	Ek083_R	CCCTCCACTTTCTGGGACAG	100
F7SYSBQ01A0E89	CA	7	Ek084_F	TCAAGCTGTAGGAAAGCATGG	Ek084_R	AAGCTGCGGTATTAACCCTG	121
F7SYSBQ01A40W8	TA	7	Ek085_F	AGTGCTTTGGTTCAGATGCC	Ek085_R	TCATTTGACACAGTGACCACC	127
F7SYSBQ01A6F3A	AG	7	Ek086_F	TGGTGGAAGAGATCCTTTGG	Ek086_R	AGCAGGAAGACATCAGAGCC	105
F7SYSBQ01AC8PT	AG	7	Ek087_F	TCATCAGACACTTTCTGGGC	Ek087_R	TTTAACTGCATCCCAAAGGG	222
F7SYSBQ01AH2Y3	AT	7	Ek088_F	CCTCCTAATTGCTCAGACGC	Ek088_R	AATCCACAGCTGCATGTGAC	190
F7SYSBQ01AHS34	GA	7	Ek089_F	GCTGCACCTGAAGTGTAGACC	Ek089_R	GGCTTCCTACTTGACCCTCC	170
F7SYSBQ01ALT32	CT	7	Ek090_F	CTAAGGGCTCTGAATGGCTG	Ek090_R	GGTCACTAGAGCAAGCGACTG	129
F7SYSBQ01ASI52	TG	7	Ek091_F	TTCATACGGAAGGAAGTCGG	Ek091_R	GTGGGAAACTTGTCAAACGG	163
F7SYSBQ01AUQGX	CA	7	Ek092_F	TAAGCTTCCTCCCACTTCCC	Ek092_R	TCATTCTAAACCACTCGGGC	114
F7SYSBQ01AWYOQ	GA	7	Ek093_F	GCAGATAACCACGTCTGCAC	Ek093_R	AACTCCTGGCCACTACAAGC	184
F7SYSBQ01AXW8R	CA	7	Ek094_F	TGACCCTTCCACTTCCAGAC	Ek094_R	CCAAACCTGAAATGGTCCTG	124
F7SYSBQ01B03I1	CA	7	Ek095_F	GGCAATATGCCTACAATGGG	Ek095_R	GAGGCTTGTCTTCCATGAGC	164
F7SYSBQ01B4NP1	CT	7	Ek096_F	CTCAGGTCTGTGAGGACACG	Ek096_R	CAGTAGCACCATCTGCAACG	105
F7SYSBQ01B5IN3	GT	7	Ek097_F	CCTTAAGGGCTGACTGCAAG	Ek097_R	AGCACATCATAGCTCTGCCC	228
F7SYSBQ01B5Q12	TC	7	Ek098_F	TGGATGAAACAGCAAACCTG	Ek098_R	TTCCCTAGCTACTTCCTTGGC	137
F7SYSBQ01B6QCN	GA	7	Ek099_F	ACGTACCTGCTCAAGCTGC	Ek099_R	ATCCCTGGGCCTAGAGTGAG	144
F7SYSBQ01B8900	GT	7	Ek100_F	GCATTTGTGGAAACGTTGTG	Ek100_R	ATCACTTTGGCATAGCAGGC	250
F7SYSBQ01B9DEM	GA	7	Ek101_F	CTAGGCCTGGTCCATACTCG	Ek101_R	CTTTGTGCCATGATGCTAGG	147
F7SYSBQ01BEZ6B	TC	7	Ek102_F	TTAAGCCTACTTCTTCTCCTTAGACC	Ek102_R	AGCTATCCCAGGTTTGATTACAAAG	100
F7SYSBQ01BF943	TG	7	Ek103_F	TTTGCCGATGTAGATGGATG	Ek103_R	TCAGAATTGCACCTGACTGG	234
F7SYSBQ01BGM12	AC	7	Ek104_F	AAATGTGGGCACTAAGGCAC	Ek104_R	TGAAACGCTTTGTCTTCCAG	182
F7SYSBQ01BJ7GA	GA	7	Ek105_F	CCAAGTGATCAGAATGGTGC	Ek105_R	TAATTCCCATGCCCTCTCAC	141
F7SYSBQ01BN5MD	AG	7	Ek106_F	TCCTGCCACTTCTACCCATC	Ek106_R	CGCTCTATTTCTCACTCTTCCC	102
F7SYSBQ01BRQZF	TG	7	Ek107_F	GTTGCCCTGCTGTGCTCTAC	Ek107_R	GCACTGCAGTAGGACTTAGAAGC	214
F7SYSBQ01BSCHT	TG	7	Ek108_F	CGAATGAGGAGCCAGAACAG	Ek108_R	TGCTGTCGTGCTAGATGCTG	236
F7SYSBQ01BV4MA	TG	7	Ek109_F	AGAAATTCCAGAAGGGAGCC	Ek109_R	AGGCACAGCATATTCCTTGG	168
F7SYSBQ01BWXN0	AT	7	Ek110_F	TGGGTGGCCTTATCTCTTTG	Ek110_R	TGTGACTATGAACCACCTGC	214
F7SYSBQ01BYM8S	GT	7	Ek111_F	TCTGCGGATGTGCATCTAAG	Ek111_R	GCATTGTTACACATGGTGCC	228
F7SYSBQ01BZ2AJ	TG	7	Ek112_F	TGGGTAATTGTATTGAAAGGGC	Ek112_R	CACCTGTGTTCAGACCCAAG	140
F7SYSBQ01C14TM	GT	7	Ek113_F	TACCATTTCTCGGAGTATGCC	Ek113_R	GATGCAGGAAACTGGTATGTTATG	100
F7SYSBQ01C21JO	AG	7	Ek114_F	TGCCCTCATTAGTGGACCTC	Ek114_R	GGTGTTTCACTTCCCAGGTG	169
F7SYSBQ01C2BPD	AG	7	Ek115_F	AAACCTTGATATTCCTGGAGACAG	Ek115_R	CACTCAGCCTTACATGCTCG	144
F7SYSBQ01C6JP1	AG	7	Ek116_F	TGCTGAAGCTGTTCCACTG	Ek116_R	TTTGACTCCGTCTCCTGTCC	123
F7SYSBQ01C83LY	CA	7	Ek117_F	GTGCAACAGTTCCATCTGGG	Ek117_R	TGCAAATTCTATCATCTGGGC	131
F7SYSBQ01C8KZO	AC	7	Ek118_F	TGGCATGTAAGAGGGATTGG	Ek118_R	ACTAATGGGCGTGGTGTGAG	173
F7SYSBQ01C9GJ4	CA	7	Ek119_F	TCAGTGGTTCCCAACCTTTC	Ek119_R	GTTTATGGCCAGGTCAGGG	113
F7SYSBQ01CB4QU	TC	7	Ek120_F	TATCTACTTATGTGAGGCTGAGCAATG	Ek120_R	TTGTGTATCCCACATTGACCG	100
F7SYSBQ01CB517	TC	7	Ek121_F	AACACAGCCAGGAACTGAGG	Ek121_R	AACATAATGCACTAGGGCTGG	126
F7SYSBQ01CBR0R	CA	7	Ek122_F	TTCTACATTTGCATTGGCTCAC	Ek122_R	GGTGCATGGAAATTAGTGGG	102
F7SYSBQ01CCRBR	TG	7	Ek123_F	CTTCCTGCAGCTTCCTATGC	Ek123_R	GTGCAGCAGGCATCACTAGG	210
F7SYSBQ01CE8ZU	CT	7	Ek124_F	CTTCTAACCCTGCGGTTCTG	Ek124_R	CGCAGACGTACTGAGCAAAG	218
F7SYSBQ01CF9HZ	AC	7	Ek125_F	CCCATAGTTTGCAGCAGAGG	Ek125_R	CTCTAAGAGGGAGCAATGGG	208
F7SYSBQ01CIA47	TC	7	Ek126_F	AGTGGCACTGACTCTTTCCTG	Ek126_R	TATCCCTCACCATCACTCCC	102
F7SYSBQ01CO7GK	AC	7	Ek127_F	GGCCAGCACAGCTTATTTCTC	Ek127_R	AGGCAGCTTTGCTGACTG	117
F7SYSBQ01COGHO	TA	7	Ek128_F	CCTTTGACCCTTCCTTAATGC	Ek128_R	GGAGCACAGAATCCATAGCC	101

	F7SYSBQ01COHBR	AC	7	Ek129_F	TTCCATCCAATAGAGGGCAG	Ek129_R	CTCCATTGTGCTAGGTGCTG	216	
	F7SYSBQ01COR5Q	TG	7	Ek130_F	ACATCTCCTGGGTGTGAAGC	Ek130_R	AGCCCTGATCCTGCAAATAG	175	
	F7SYSBQ01CPTWQ	CT	7	Ek131_F	TGTGGACCCTATCCTTCCTG	Ek131_R	AACTGCTGGGTCGTCAATG	131	
	F7SYSBQ01CSZMT	TA	7	Ek132_F	CTTGGTTAGCACTTGCCCTC	Ek132_R	AAATTCCTGCGGGCATAAG	138	
	F7SYSBQ01CU1K2	AT	7	Ek133_F	TGAAGCACCATCATCAGACC	Ek133_R	CCCTAGCCAGACTCATCTGC	345	
	F7SYSBQ01CWPEE	TG	7	Ek134_F	CATCTGTCCATCCCACAGC	Ek134_R	CAGGCTCTTGAGTGTTCTGC	113	
	F7SYSBQ01CX234	GT	7	Ek135_F	GGGAGGGAAATCTGATTGTG	Ek135_R	CTACGATCACTCCTCCTGCC	102	
	F7SYSBQ01CYF5H	GA	7	Ek136_F	ATGTTCCCATCCTGCTGAAC	Ek136_R	CAGCCAATAACCTAGTGGTTAGG	251	
	F7SYSBQ01CYPMF	TG	7	Ek137_F	TGTGTCTGCTTGAAGGAAAGTG	Ek137_R	CCTCACCACTTACAGAATGCC	100	
	F7SYSBQ01D0V0J	CA	7	Ek138_F	AGGAGAAAGACTCCCTTCCG	Ek138_R	GGAGTAAGACTGGCCTGGTG	200	
	F7SYSBQ01D1DDQ	GA	7	Ek139_F	GTATATGAGGCCTTGGGAGG	Ek139_R	GGGAGATTATTAGCAGAGAAGCC	119	
	F7SYSBQ01D4FW8	CT	7	Ek140_F	CATGGAAAGCACATGGTGAC	Ek140_R	CAGGAATTAACTGGGTCTGTTC	167	
	F7SYSBQ01D7RKO	AT	7	Ek141_F	TCGTGGATTCAACTGTCGTG	Ek141_R	GTCATTGCTGTGATTGACGC	162	
	F7SYSBQ01DAIOS	GC	7	Ek142_F	CGAAGGAATACAGCTCGCTC	Ek142_R	AGCAGCAAGTGGGATCTGAG	167	
	F7SYSBQ01DB8SN	AT	7	Ek143_F	TCTACAAGGAACCCACAGGG	Ek143_R	GCCTGGACATCTTTCTACCG	228	
	F7SYSBQ01DCQUP	GT	7	Ek144_F	TGCCCTGACAAAGCAAATG	Ek144_R	AAAGGCTCTTGGCTGGATG	102	
	F7SYSBQ01DETN1	GA	7	Ek145_F	TCCATTTCAGAGTGACCAACAC	Ek145_R	CAATGCTGTGCTGTATTTGC	131	
	F7SYSBQ01DG0Q0	GA	7	Ek146_F	ATGGAGACCTGAGTTGGGTG	Ek146_R	GGACGGGAAGTGTAAAGCTG	118	
	F7SYSBQ01DGJ0K	GT	7	Ek147_F	ACCTGCTCCTGCTGCTACTG	Ek147_R	GTACCGATATGGCAGGAAGC	156	
	F7SYSBQ01DJZT7	AC	7	Ek148_F	AGTTCTGCCCATTTCCACTG	Ek148_R	TGGTTCTGACTCCTTTCCTTTC	148	
	F7SYSBQ01DPSZL	TG	7	Ek149_F	TGGGTTGTTTATTGGTTGGG	Ek149_R	CAAGTGAGAGGCACAACTGC	115	
	F7SYSBQ01DSLRT	TC	7	Ek150_F	ATTTCCTCCTCTCCTGGGAC	Ek150_R	TTGAGCCTGAATTTGAACCC	120	
	F7SYSBQ01DUK19	GT	7	Ek151_F	AACCGAACAGGGCTCAGTG	Ek151_R	AGTCATTTGGATCAGTCCCG	105	
	F7SYSBQ01DW92E	AC	7	Ek152_F	AGCACAAGTCTCTTTCATGGG	Ek152_R	TGTTTGATGTCTCAGTGCTGC	115	
	F7SYSBQ01DXK77	AG	7	Ek153_F	AAGTCACCCTGATTAGGCCC	Ek153_R	CATTGGAGGAGGGTGGTTTC	142	
	F7SYSBQ01DYQXR	TA	7	Ek154_F	ATGCAATTGTGGTAGGCTGG	Ek154_R	TTTGTAGCTGGCCTTTGAGG	155	
	F7SYSBQ01EAAYB	GT	7	Ek155_F	GCGATGGATGGATATGTTCC	Ek155_R	CAATAGCGAGGTGTGAGTGC	238	
	F7SYSBQ01EBOHJ	AT	7	Ek156_F	TTGCTGCTATAGAAGGGACAC	Ek156_R	GGAATCATCATGGTACAACAGG	116	
	F7SYSBQ01EGZIF	AC	7	Ek157_F	AGTACCCTGGCTACACCTGG	Ek157_R	AACTGCGTGTTCCTCATGG	193	
	F7SYSBQ01EHJ4Y	AC	7	Ek158_F	CAGAATGGTAGACACTACACCATC	Ek158_R	TGAACCCTGCAATTTGTAACTG	108	
	F7SYSBQ01EHOEY	GA	7	Ek159_F	GTTCCAGCCAGACTCAGGTC	Ek159_R	TCATGCAAGGCAGAATGAAG	158	
	F7SYSBQ01EL7KC	CT	7	Ek160_F	TGAATCTCAGCAGGCACAAG	Ek160_R	AGTAGGCGAGGTCTGCTTTG	136	
	F7SYSBQ01ELTPT	TC	7	Ek161_F	ATGCAGATTTGCTGCTGAAC	Ek161_R	CATCCACAAGGTGTTCTCCC	173	
	F7SYSBQ01EOBIT	AG	7	Ek162_F	ATAGCCAAGCAAAGCAGAGG	Ek162_R	TTGTTCCGTAGGTGGAATGG	110	
	cons21_2_A	TC	7	Ek163_F	TCACCAATGTAGCAGCTTCC	Ek163_R	CACCCAGAACGTTAGCTTCC	252	
	cons29_2_A	AT	7	Ek164_F	TCTCACACCAAAGACAACGC	Ek164_R	GCAGTTTATTACACCACTGCC	171	
	cons60_2_A	GA	7	Ek165_F	GAATCCCGTCAGCCTATCAC	Ek165_R	ACTTGGTTCCCATGCTGAAG	149	
	cons66_2_A	TC	7	Ek166_F	TGTTTCCCATACCTTGAGCC	Ek166_R	GGACAGTGGTGTACTGACGC	267	
	cons67_2_A	CA	7	Ek167_F	GAATAGAAGGGAAGTCACACGC	Ek167_R	TAGCCATCAGAAATGCTGCC	117	
	cons77_2_A	TA	7	Ek168_F	TGTGAACTGTTCCTTGCATTAC	Ek168_R	ACCCATAGCACTAGCTTGCC	106	
HQ690765	F7SYSBQ01CD3ED	CAG	13	Ekref10_F	ATGCTGCTGAAGCAGGTGTC	Ekref10_R	ATGCTCGTTGAGGCTGTAGG	157	characterised
HQ690766	F7SYSBQ01CYWN8	ATT	11	Ekref12_F	AGGCCACCCAGTTTACACC	Ekref12_R	TCTTTCAATGAGCTCCACCTG	178	characterised
	F7SYSBQ01D023S	TCT	11	Ekref11_F	ATGGTGAGGAGCCTAGAGCC	Ekref11_R	TCAAAGGGATACAGATTCAGCC	196	unclear peak profile
HQ690767	F7SYSBQ01DPATB	TAA	10	Ekref13_F	GCTGAATGGCAATGTAACCC	Ekref13_R	GCATTTCAAAGAGACTGCCC	125	characterised
HQ690769	F7SYSBQ01C3SOW	TAA	9	Ekref15_F	GCTTTCTCAGACGGGAGGC	Ekref15_R	AGGGCAGATAGCTACCACAG	287	characterised
HQ690768	F7SYSBQ01DG8MK	GCT	9	Ekref14_F	AGAGCCTAGAAGGAATGGGC	Ekref14_R	CAAGGGAAGTGAAACAGTGG	103	characterised
	cons41_2_A	GAA	9	Ek174_F	GAAACGCTAGCCTCCTGTTG	Ek174_R	GAAGTAGAGGGAAGGAGGCG	194	
	F7SYSBQ01BHQWL	ATC	8	Ek175_F	GAGCCACAAGGGCTAGAAAC	Ek175_R	AAACCCACTTCGTCAAATGC	212	

	F7SYSBQ01EDZCO	GTT	8	Ek176_F	TTCATGTGAACGTGGTAGAGG	Ek176_R	GAGTTCATTGACCTGGGCTC	305	
	F7SYSBQ01EW2CE	GTG	8	Ek177_F	ATCCTCTGCCGAAGGTTTG	Ek177_R	TGCTGAGTGGCTTGGAAAG	106	
	cons31_2_A	TTA	8	Ek178_F	GCAAGAAGTAAACTATGAGTAGTGGG	Ek178_R	TTCAGTTTGGCACAGTTTGG	178	
	F7SYSBQ01BIQWM	ATT	7	Ek179_F	TCTTCCAGCTCAAGCTCTAAATTAC	Ek179_R	CAAACAAGTCTGAGCTAATGTGC	126	
	F7SYSBQ01BYV1S	CTT	7	Ek180_F	TCGAACTTGAATTTGTCTGGC	Ek180_R	AAGCACTGGAATGGCTGAAG	115	
	F7SYSBQ01C0ZNJ	ATT	7	Ek181_F	CCTGCCAGAGCCTTCATTAC	Ek181_R	CCTCCTCACCAGCCATAGAC	222	
	F7SYSBQ01C2OIJ	GCA	7	Ek182_F	GTGTGGGTGATTGTGGACTG	Ek182_R	CATCTCAATGGTCCTCTGCTC	166	
	F7SYSBQ01CCI9Z	ATT	7	Ek183_F	GGCTTAGCTTCTTACGCAGG	Ek183_R	ACTAGATGAAACATGCCGCC	152	
	F7SYSBQ01DF3SK	GCT	7	Ek184_F	GGGCTCTGGTTATTGCTCAG	Ek184_R	TCTTGCCCAGGAAACTCAAC	109	
	F7SYSBQ01DKKN0	ATT	7	Ek185_F	TATGCAAGATGCCAGTGGAC	Ek185_R	AGCATTATACTGTGGCACTTGAG	138	
	F7SYSBQ01DSF6L	AAT	7	Ek186_F	GTCCTGGTGGCCTTGTGTAG	Ek186_R	CCTCAGAGGGATGGTGTGAG	116	
	F7SYSBQ01DXK77	TTA	7	Ek187_F	TGTATTGGTTACTTGTGATCAGCTC	Ek187_R	ACAGGCCTGATTCAACTTCC	192	
	F7SYSBQ01EAROG	TGC	7	Ek188_F	GAGAGTTGACCAGTCCAGGG	Ek188_R	GATCGTCCATAGGCAACCAC	103	
	F7SYSBQ01EQII1	AGC	7	Ek189_F	ATATGCCTTTGCCAGAGCAG	Ek189_R	ACGTTTCACCTACGCATTCC	152	
	F7SYSBQ01ESDLY	TAA	7	Ek190_F	CTTTACACCTGTTCCCGGAG	Ek190_R	AGCGCTTTAAGATCCGCTG	154	
	cons55_2_A	GTG	7	Ek191_F	CAGTGACATCAGCAAGCAGG	Ek191_R	AGCTCCTGGTGATTCTGCTC	139	
	cons72_2_A	TGC	7	Ek192_F	GAGTATAGCTACTGCGCGGG	Ek192_R	ACAGCAGCATCTTCCGAGTG	140	
	F7SYSBQ01A639V	TTG	6	Ek193_F	CCACAGGCAACCACCTTTC	Ek193_R	ATATGCCATGAGCTGGGTTC	100	
	F7SYSBQ01B3RBE	ATT	6	Ek194_F	TGCCCTTTAGAATTTCATGC	Ek194_R	GCTGCCTATGAGACTTTGGG	106	
	F7SYSBQ01B447Z	CAA	6	Ek195_F	GCTGTTGCTAGGGCTAATGG	Ek195_R	ACTCACTCAAGTGCCACTGC	108	
	F7SYSBQ01B7B5W	TGA	6	Ek196_F	CCCTAAAGCTTGTGGGACTG	Ek196_R	TTGGTCTTGAGGAGGCTACC	131	
	F7SYSBQ01BSSPU	CAA	6	Ek197_F	AGCTGCAACGCCCTAAAG	Ek197_R	CCACACTGATATTGTAATGCTTCTG	181	
	F7SYSBQ01BZBBB	CAT	6	Ek198_F	AGGGAGAATGGATACCTCGC	Ek198_R	GCATGTGATTCTTGGCAGTG	148	
	F7SYSBQ01BZC65	GCA	6	Ek199_F	GCTAAAGTATGGGAACCCAGC	Ek199_R	AAGACTGTCCTTGGCACCAC	123	
	F7SYSBQ01C0C37	GCT	6	Ek200_F	CTCCAGGAATTCAAAGGTGC	Ek200_R	TTCCTGCTGAACCACACAAC	176	
	F7SYSBQ01CGYE2	GTT	6	Ek201_F	AGGATTGTCTGTCTGCTGGC	Ek201_R	TGGAATATGAGAGCACAGGC	285	
	F7SYSBQ01CIKWH	CAG	6	Ek202_F	ACTTTGGGATCGTCTTCACG	Ek202_R	AAAGAACAAGACGCTGGAGG	308	
	F7SYSBQ01CQ3KR	TTA	6	Ek203_F	AAGGCCAGGTCACCTATTCC	Ek203_R	AATTCTTGCTTGATCCTGCG	214	
	F7SYSBQ01CRXK3	GCA	6	Ek204_F	GCAAGTCCCTCCTACAGTGG	Ek204_R	CACAATTTGGTGAATGGCTG	136	
	F7SYSBQ01CSC4O	TCC	6	Ek205_F	TTCATTCCCTCTGAAGCACC	Ek205_R	GAAGTGGGACAAGGATCGAG	167	
	F7SYSBQ01D14K5	TAT	6	Ek206_F	CATTATTCCTCTGAGACTGCTGC	Ek206_R	GAGAGTGAGAGTAAGGGCCTG	102	
	F7SYSBQ01D3EC8	GGA	6	Ek207_F	TTCTGGTGAGCCCTGAAATC	Ek207_R	TTGCCTTATTGGGTACTGCC	270	
	F7SYSBQ01D5AAT	TGC	6	Ek208_F	GCACCATAAGACAGTGTGAGC	Ek208_R	GGTGTGGGTGTGACTGTGAG	113	
	F7SYSBQ01DCXWE	TTA	6	Ek209_F	CGAGGAGTCATCCACCAGAC	Ek209_R	CCTGATGCTTAGGCTGCTTC	180	
	F7SYSBQ01DSG4Y	TAA	6	Ek210_F	GGATCGTGGTCCATGACTG	Ek210_R	TCATCAGATCCTACCCTTTCTG	102	
	F7SYSBQ01DT50M	TGA	6	Ek211_F	TGGACCTTTGCTGTCTAGGTG	Ek211_R	GACCTTACAGCCTCACTGGG	181	
	F7SYSBQ01DVEIB	TTA	6	Ek212_F	AACTGGGAATCGGTCAGAAC	Ek212_R	TCTTGCATAGCACCAAGCAC	117	
	F7SYSBQ01EC45J	AAG	6	Ek213_F	TTCGGTGGCACAGTATCATC	Ek213_R	CATCGTTATTGTACAAGGTGAGC	134	
	F7SYSBQ01EGQ1Z	TTG	6	Ek214_F	CCAATCTCCATTTACCAGGC	Ek214_R	CGACCCACGACTAATTGACC	212	
HQ690761	F7SYSBQ01CBHSI	TGTT	9	Ekref06_F	GCCCTGTCTTTCCAATTCAG	Ekref06_R	GCCAGTTTCTATGTTTGCAGC	152	characterised
HQ690762	F7SYSBQ01C2E3D	AGAT	8	Ekref07_F	AGCACTGTTAAGACTTCCTACG	Ekref07_R	TGGAGGCATCCTTGTGACC	344	characterised
	F7SYSBQ01B6LA1	TCTT	7	Ek216_F	CTCGCACAATTGAACAGGTC	Ek216_R	TGTTCCACTGTGGGTAAAGG	101	
	F7SYSBQ01CDQL1	TTAT	7	Ek217_F	TTCAGCAATAAACACAGCCC	Ek217_R	AGGAGCTACTGCAGTGAATGG	145	
HQ690763	F7SYSBQ01CNV2H	TGCC	7	Ekref08_F	GTTCTGGGTGAGGGTGTGG	Ekref08_R	TGTCCCAAAGAACAAGGCTC	181	characterised
HQ690764	F7SYSBQ01CY5HP	GGAG	7	Ekref09_F	TGGCCTATCCTAGAGGAGGTG	Ekref09_R	GAGTCATCCCATGTTCCAATTC	150	characterised
	F7SYSBQ01AJZP0	ATTA	6	Ek219_F	ACACTGACTATCTTCCAAAGACTTC	Ek219_R	CTATTGTTTCTTCGCAGCCC	140	
	F7SYSBQ01B7YFP	AAAC	6	Ek220_F	ACATAATTAGGTTGGGCGGG	Ek220_R	CGCATAAGAAGAAACCCAGC	142	
	F7SYSBQ01BDXDN	AAAC	6	Ek221_F	ACGGACCCACACGTCACTAC	Ek221_R	GAGGTGATGGGAAATTGTGC	134	
	F7SYSBQ01BKKZ9	AGAT	6	Ek222_F	TATGCTCAGATACCATGGCG	Ek222_R	GTGGTTGCAACTCCAGTGAC	116	

	F7SYSBQ01BOPSD	TAAA	6	Ek223_F	CCCTGGATAAGCAGAAGCAG	Ek223_R	GTCGAGTCTTAATACTACTCTTTGGTG	112	
	F7SYSBQ01CJWXL	CCTG	6	Ek224_F	GCTGGGAAGGGCTAATAAGG	Ek224_R	TTTGCTTAAGGCCTGCTACTG	110	
	F7SYSBQ01CNM8T	CTGA	6	Ek225_F	ACAATATCTTCTGGGCAGCG	Ek225_R	CTGAGGAGTCCAGAGCCATC	274	
	F7SYSBQ01CWCNR	AAAT	6	Ek226_F	TGACTGTTGGTCCCTCTTGG	Ek226_R	GCTGTTTCAGAACCAAACCC	305	
	F7SYSBQ01DURHR	ATTT	6	Ek227_F	CAGCATTTGTGCATATTGGC	Ek227_R	AAGGAAATCACCCTGCAGTC	153	
	F7SYSBQ01EMO67	TTCA	6	Ek228_F	AAACAAAGGCAATCTTTGCG	Ek228_R	TCTGCAGGCATGTAGGATTC	176	
	cons36_2_A	GGAG	6	Ek229_F	TGGCCTATCCTAGAGGAGGTG	Ek229_R	TTGCTCTTGACTTAGGGTGC	314	
	F7SYSBQ01A4AJE	CATT	5	Ek230_F	CTCCAAGGGTTAGTGCTTGC	Ek230_R	CCCTACACTTTGCAGGGTTG	187	
	F7SYSBQ01B5J9X	CAAA	5	Ek231_F	GATTCTCCAGCAGCCAGTG	Ek231_R	ATCCTATGCTCCACCTGCTC	137	
	F7SYSBQ01BAOD4	TTAA	5	Ek232_F	TGTGAGGCATCAACTTGCTC	Ek232_R	CCATTTAAAGAGGAGTATTTCCCAC	145	
	F7SYSBQ01BF6NM	TATT	5	Ek233_F	GTGATTTCCGTGAGAGGAGC	Ek233_R	AGCAAGGACAGAAACACCAAC	206	
	F7SYSBQ01BT8VW	TCTG	5	Ek234_F	TTTACACCTGCCCAGACTCC	Ek234_R	GAGCATGGTACGAAAGCCTG	272	
	F7SYSBQ01BZI6N	CTGA	5	Ek235_F	GACCTGCATATTTATTTCTGTGCTG	Ek235_R	GAATGCTCAACTAGGGCTGG	170	
	F7SYSBQ01C9TMY	ATGG	5	Ek236_F	ACCATGAGACGTTCCACTGC	Ek236_R	TTGCTTCCTGTTAATCTTCCC	132	
	F7SYSBQ01CCOGK	CCTC	5	Ek237_F	TTTCTCACAGGTTTGGCAAC	Ek237_R	ATTGTACGGCCGTCTCTGC	103	
	F7SYSBQ01CEZZC	ATCT	5	Ek238_F	TCCATTCACCTTTCATCTGTC	Ek238_R	GACACAGCAAATGTGCCAAC	105	
	F7SYSBQ01CJWRL	AACA	5	Ek239_F	GAGAAGGATGGTTTAAGGATGC	Ek239_R	TTCGTGGGAATCATGAATGTAG	187	
	F7SYSBQ01D2BDK	TCAT	5	Ek240_F	TAATTCATCTCCCTGTGCCC	Ek240_R	TGTCGGCCATCCAAATAATC	145	
	F7SYSBQ01D2R20	GATA	5	Ek241_F	CTCATCTGCCCAGATTCTGAC	Ek241_R	TGCCACTCAGACCTTGCTTAC	256	
	F7SYSBQ01DGUOL	GAAA	5	Ek242_F	GTGTGGCTGTGATGAGCAAC	Ek242_R	GCAAAGCTACACTCAGATTCCC	115	
	F7SYSBQ01DR96U	GTTT	5	Ek243_F	TCCTTGATATCCATCCCTGG	Ek243_R	ATGCTAACCACGAGACCACC	138	
	F7SYSBQ01EARFH	GACA	5	Ek244_F	GCTTAATGCAATGCAAACATGC	Ek244_R	CCAATCTATTATCATCCCTCCC	136	
	F7SYSBQ01ED7Q5	TCTT	5	Ek245_F	ACCATTCACAGTGTTGCCAG	Ek245_R	TTGTTCACAACCAGGAGTGC	174	
	F7SYSBQ01EFOAA	TTAT	5	Ek246_F	ACCCATCAGATTGCACCAAG	Ek246_R	GCCTCCCTCTCCGTTAAATTAG	315	
	F7SYSBQ01EOSW1	TGGG	5	Ek247_F	GACGCAGTTGAAGGGTATAAATC	Ek247_R	GAGTCTGGGACTTCTCGTGC	119	
	F7SYSBQ01EQUH5	ACTC	5	Ek248_F	CTTGGCCAAATGGCTGAAC	Ek248_R	CCTTCAATCGGATTTATTCCC	115	
	F7SYSBQ01EUDMG	GATA	5	Ek249_F	TCTGGTGGTTGGATTTCAGG	Ek249_R	GGAGTGAAAGAAGCAGAGGG	169	
	cons3_3_A	CATT	5	Ek250_F	CACCCAAATACAGCCATCATC	Ek250_R	TGGAGCAGACTGAATGAATG	104	
	F7SYSBQ01B1O39	GGCTA	12	Ekref01_F	TAGGAGAAGAAGCGGAGTGG	Ekref01_R	AGTATGTGGCAGTGGTTGCTC	112	unclear peak profile
	F7SYSBQ01BVHXA	GCTGG	8	Ekref03_F	TGTCCTGTCCCAGTCACCTG	Ekref03_R	ACCTCACTGTTTGTGCAGGC	246	no amplification
HQ690760	F7SYSBQ01CLILZ	TTCTA	8	Ekref04_F	CCTGATTTATTTCCTACGCTCAG	Ekref04_R	GGTGACAAGGTTGGTACAAGAAC	159	characterised
	F7SYSBQ01BHD54	CTGCC	6	Ek254_F	GGCGGAGTATCTACAGGAAGG	Ek254_R	CCTGGCATCATCTCAGTTCC	155	
	F7SYSBQ01BM2F9	GTTTT	5	Ek255_F	CATCCTTTACCAGCAGGAGC	Ek255_R	TGAATTGGGAAGAGTCCACC	167	
	F7SYSBQ01C4ER6	GGGAG	5	Ek256_F	CCTCGAAGCTGGAAACTGAC	Ek256_R	ACCACTAAACACCACTGCCC	155	
	F7SYSBQ01C6ZZZ	GCAGA	5	Ek257_F	TCTCAAGCATGACCCGAGG	Ek257_R	CTTTGTTGCTGGACTCGCTC	255	
	F7SYSBQ01EJXZX	TTCTA	5	Ek258_F	CAAGCTAGGGTGAGGAATGG	Ek258_R	GGAAGGCTGGATGAATGTTG	241	
	cons1_2_A	AGGCC	5	Ek259_F	CTCCTCGCTGTCTAGGGTTG	Ek259_R	GAGATTCTGGGATGAGGCTG	234	
	F7SYSBQ01CX7YU	TTGGGC	5	Ek260_F	AATTAAACACCATGGCTGGC	Ek260_R	AGCTCTGAAACCCAGTGGAC	161	
	F7SYSBQ01EENX3	GGCATC	5	Ek261_F	ACAGCAGGTTTGCTGTGTTG	Ek261_R	ATGTAAGACACCGAGACGGG	170	
Elseya albagula									
	F7SYSBQ01EHV8R	GT	17	Ea001_F	AACTGTACATGAACACTGTGCG	Ea001_R	ATGCAGGTCGTGTCTTCC	108	
HQ690753	F7SYSBQ01B0E82	AC	15	Ealb15_F	GGTTCACTGATGTTGTTGAAACTG	Ealb15_R	TGGTTCCCATTGCCTAAGAG	158	characterised
	F7SYSBQ01BFN63	CA	15	Ealb16_F	TGGATACATCATCTGCTGCC	Ealb16_R	TTTGTCACTGCTTTGGTTGG	199	no amplification
	F7SYSBQ01EE6WB	CA	14	Ea004_F	CTTCTCTTGGCAGGGAACAG	Ea004_R	CAATGTGGCTTTGTTCATGG	185	
	F7SYSBQ01BW4OG	TG	14	Ea005_F	CCTAGGTGTGAAATCCTGGC	Ea005_R	GATCCCTACTAGCATACCTCCC	126	
	F7SYSBQ01BRFUM	TG	13	Ea006_F	TAAGAAGGGAAGAAATGCGG	Ea006_R	TTTACGTCCTCTCAGCCACC	127	

	F7SYSBQ01B6MVZ	AC	13	Ea007_F	GCAAATTGCCTGGGTACTTC	Ea007_R	GGTGGGAATGCTACCTGTTC	190	
	cons29_2_A	GA	12	Ea008_F	AGTGATCTGGCAGTCCCATC	Ea008_R	TGCCTCTCCATGTAGGAAAGTAG	208	
HQ690755	F7SYSBQ01B4G04	AC	12	Ealb18_F	TCCATTCTTCTTTGTGAACCG	Ealb18_R	GACCTGGCGTCGTTGTATG	174	characterised
HQ690754	F7SYSBQ01BH6M3	AC	12	Ealb17_F	GGGCGATGTAGTACGTGTGG	Ealb17_R	TGTGTACTTCTTGTAGGGTTAAAGAGC	103	characterised
	F7SYSBQ01CSD82	AC	11	Ea011_F	GAACTAGCACTGGCTTGTTACTG	Ea011_R	CTAAGTGGCTTTCCCTGTGG	215	
	cons16_2_A	AC	11	Ea012_F	TGGAAGGCATAGGTTATCCG	Ea012_R	ATCGGTCGCCTTATTGATTG	196	
	F7SYSBQ01CCC6E	CA	11	Ea013_F	GCACTAGCATCTAGGAGCCC	Ea013_R	TTCTTTAGGGCAGCAAGTGTC	131	
	F7SYSBQ01DFYFK	GT	11	Ea014_F	CTATTTCTCAAGGCAGCACG	Ea014_R	TATCCCTAAACTGCCCATGC	127	
	F7SYSBQ01DB3KY	TG	11	Ea015_F	GTTGGTCCTGCATTGTGGG	Ea015_R	CAGGGCTTGTACAGAATGTGC	127	
	F7SYSBQ01C3ZGL	CT	11	Ea016_F	AGCTGGAGATTTGGTGTTGG	Ea016_R	CTTTGACTGGGCCTGAGAAG	106	
HQ690756	F7SYSBQ01DFB7R	GA	11	Ealb19_F	TCCCTCTGCACAAAGTGCC	Ealb19_R	GTGCTAGGAACTGCCTGTGG	103	characterised
	F7SYSBQ01BQPSI	AG	11	Ea018_F	GGGCTTTGGTTAGGTCCTTC	Ea018_R	GTGGCAGAGGCACTTTATGC	318	
HQ690757	F7SYSBQ01AXSLW	CT	11	Ealb20_F	AGCCACTGGAGGTGATTGTC	Ealb20_R	CCACTGAGACACCATTGAGC	111	characterised
	F7SYSBQ01D8BL4	AC	10	Ea020_F	TTAAGAGCCACCCAAACAGC	Ea020_R	TTTGACTCAACACAATCGGTG	153	
	F7SYSBQ01DF6J8	AC	10	Ea021_F	TCTTTCAGCCTGATCACACC	Ea021_R	GGGAGAAGCAAACTGAGCTG	108	
	F7SYSBQ01C2NGN	CA	10	Ea022_F	TCAACATTCAAATAGACGGTGG	Ea022_R	AGGATCTCTGTCAAGCACGG	122	
	F7SYSBQ01EOK9N	CA	10	Ea023_F	AAAGAGGGTCAGGCCAAATC	Ea023_R	AGATGCTACAAGCCGTGGTC	250	
	F7SYSBQ01DZ0NC	GT	10	Ea024_F	GAGGTTGTGCACCTCTGCTC	Ea024_R	CACAGGCCCTTGAGAATG	121	
	F7SYSBQ01C605T	TG	10	Ea025_F	TGGGACAATTGTTTCCGAAG	Ea025_R	CCTCTCCTAGTTTGCCACACC	100	
	F7SYSBQ01DRCI3	TG	10	Ea026_F	AATTTCCTAGCTTGGGCCAG	Ea026_R	AGTTCCCTTAGCTTGGAGCC	198	
	cons56_2_A	TG	10	Ea027_F	CCCAACAATAACTTGGGCAC	Ea027_R	CGTTTACTCCCTTAACTATTTCCC	271	
	F7SYSBQ01A7N1A	AC	10	Ea028_F	CCTCGGCAATACATGCAATAG	Ea028_R	TTGGTATGATGGTTTGGAACAG	304	
	F7SYSBQ01ANJ2U	AC	10	Ea029_F	TTTCTGCCCTGGCTATATCAC	Ea029_R	GGACTCGGGAACTATACCTCG	117	
	F7SYSBQ01BO3NX	TG	10	Ea030_F	CTTGAACTGGATTTGGAAATGG	Ea030_R	GAAGCTCTTAAATATCCTGTAGTGTCC	100	
	F7SYSBQ01BLNSP	AG	10	Ea031_F	TTGTTGGGAGGGTTTCTCAG	Ea031_R	CTACATCCCAGCTGAGTGCC	109	
	F7SYSBQ01AT6GE	TC	10	Ea032_F	ACAGAGACGCTCTTTCCAGG	Ea032_R	CACCATCATGCACTGACAGC	235	
	F7SYSBQ01DHD03	AC	9	Ea033_F	GGATTATCCCTACCTCAGCAAAC	Ea033_R	CAGGCTGATAATGTGACCCAG	126	
	F7SYSBQ01CAWJF	GT	9	Ea034_F	AAAGCAGAACACCCATGCTC	Ea034_R	AACTTGTCTCTTGAATATCCTGGG	100	
	F7SYSBQ01CZRZE	AG	9	Ea035_F	AGGAGAGGGTTTGTGGTGTG	Ea035_R	CTTGGACTCTCACCTGCACC	110	
	F7SYSBQ01D4X6K	CT	9	Ea036_F	ATCTCTGGGATGGACTGTGC	Ea036_R	GTCAGCCATGTCTGCTGGAG	173	
	F7SYSBQ01DBWHD	CT	9	Ea037_F	TGGGCCCTTGATATTCATTG	Ea037_R	CCAACAGCTAAGAAGGTGGAG	146	
	F7SYSBQ01DDROC	TC	9	Ea038_F	TTTGCTACTCCATCCTCCTACC	Ea038_R	AATACCCTGCTCCAAAGAAGC	100	
	F7SYSBQ01ATYBY	TG	9	Ea039_F	TCTACGGAGTCATGCCACAC	Ea039_R	TCGTAACTGACACAGCGAGC	196	
	F7SYSBQ01BG1NO	AG	9	Ea040_F	CTGCTCTAGCCATTTCCCAG	Ea040_R	TCTGGCCCAATGATTACTTATG	245	
	F7SYSBQ01BR09H	CA	8	Ea041_F	TGCATCTCGGGTAAGAAATAGC	Ea041_R	GCACCCTAGCTATCTTCAGCTTC	120	
	F7SYSBQ01DV9M3	CA	8	Ea042_F	GAGCACAGCACTGACAACAC	Ea042_R	GCCATCTCAGCAGCTAAGGG	102	
	F7SYSBQ01BY7F3	GT	8	Ea043_F	ATCTGTTTGGTGCAAGGGAG	Ea043_R	GGTCTGGCCACCTGTTTATG	130	
	F7SYSBQ01ESYYG	GA	8	Ea044_F	AGATCGTACGGCCACAGAAG	Ea044_R	GCAGCCATTTGATGTGTTTG	152	
	F7SYSBQ01C38BG	TC	8	Ea045_F	TCTGAGCAGAAGGTCCTGAG	Ea045_R	CCTTACAGTTGGACACTGCG	163	
	F7SYSBQ01CK5U4	TC	8	Ea046_F	ATGTTCACTCCATGCTGGC	Ea046_R	ACCAGCACTCTGAACCCAAG	101	
	F7SYSBQ01ESPA2	TC	8	Ea047_F	TCTGTTGGGCTACTTCTTCG	Ea047_R	CCCAGCTTAGTGCTTCCTTG	176	
	F7SYSBQ01BUVIK	AT	8	Ea048_F	GCTGATGGTTGTGCAGTAGC	Ea048_R	AGGCCCAAATAATCAGACCC	175	
	F7SYSBQ01AC4MA	AC	8	Ea049_F	ATTGTTCTGACCAAGCGTCC	Ea049_R	TTAACTCACTGGGTGGGAGG	151	
	F7SYSBQ01AWXVE	AC	8	Ea050_F	AGATGTTTAGACACCTCTAGTTACCC	Ea050_R	TGTGCTTCTCAGCACACCTC	114	
	F7SYSBQ01AGNP5	CA	8	Ea051_F	TGCCTCAAGTAGGACAGATCC	Ea051_R	AGTCAGCTCTGTGGGACCTC	100	
	F7SYSBQ01B7LEG	CA	8	Ea052_F	AGCACTGAAATTTGACAAGTGG	Ea052_R	TATCCCTTGAGCCAGTCAGC	141	
	F7SYSBQ01AXIMQ	GT	8	Ea053_F	ACAAGCCTGTGTAAGCACCC	Ea053_R	TGCAATTGAAGGCATGATTG	170	
	F7SYSBQ01A8UZX	CG	8	Ea054_F	TCGTACCTGTCCGACTCCTC	Ea054_R	GAACAGCTCCTACCTGTCCG	197	
	F7SYSBQ01CAULI	AC	7	Ea055_F	CCAGAAGTTCATGGGACTGG	Ea055_R	AGTAGCCCTAGCTGGAAGGC	194	

F7SYSBQ01D2XJC	AC	7	Ea056_F	ATGTCTACATTGCAGCTGGG	Ea056_R	GTGGGCATCTACTCAGGCAG	128
F7SYSBQ01DLS3J	AC	7	Ea057_F	CAACCTCAATCTCCTCACTCC	Ea057_R	TCTGCACTGTATGTTTGAGGG	128
F7SYSBQ01DV9AD	AC	7	Ea058_F	CCTTGCAAATCACAATTCCC	Ea058_R	GTCCTGGACCTGAAAGATGC	101
F7SYSBQ01EHEOQ	AC	7	Ea059_F	CTGGCCGCCTTATCTTCTC	Ea059_R	TTTACTATTGAGGGAAAGCAGG	105
F7SYSBQ01D5XR3	CA	7	Ea060_F	CAAGTCCGTTGATATTCTGGC	Ea060_R	TACTCGAAACCCAGCAAATG	155
F7SYSBQ01DA0ZE	CA	7	Ea061_F	CCCGACTTATGCTTGAACTTG	Ea061_R	AGATCGGTGGTGTGAAGGAG	112
cons34_2_A	CA	7	Ea062_F	TCCAATGAAGTGGATAGGGC	Ea062_R	TGTTAACACGCTATGCCAGC	166
F7SYSBQ01BS79S	GT	7	Ea063_F	AGGTGACACAGCCTGTAAACTG	Ea063_R	GGTTTCAGATGGAGGAGGG	101
F7SYSBQ01CGUPF	GT	7	Ea064_F	TGCACTATAATTATGGCAGTGG	Ea064_R	TCAGATGAGCCATTTCATAACG	138
F7SYSBQ01CIF6N	GT	7	Ea065_F	ACTGTGCTTTGCACCAACTG	Ea065_R	TCTATAAGGCTGCAGCAGGAC	180
F7SYSBQ01D25MZ	GT	7	Ea066_F	CCTCCTCTTCCAACCCTCTC	Ea066_R	AGGCACCTAGGGTTTGATCC	232
F7SYSBQ01D2ISG	GT	7	Ea067_F	ATGTGTAAGGCTGCTCTGGAGTC	Ea067_R	TTCCGACATCCCAACATGAC	100
cons53_2_A	GT	7	Ea068_F	CACCGATGTTTATCAGTGTAGGG	Ea068_R	CAAAGGAAATACTGGCGTCC	284
cons72_2_A	GT	7	Ea069_F	TGGTGTTCAGTTGTCTTTCCTTC	Ea069_R	CCTTTATTCATGGTTCGTGC	140
F7SYSBQ01C99I1	TG	7	Ea070_F	AATTGCAACACCTGTAGCCC	Ea070_R	CTTCTGCATAACGAGGAGCC	190
F7SYSBQ01CQF19	TG	7	Ea071_F	TGGCCAGTGGTGTTCTATTG	Ea071_R	GCTGCTGACACAAGGAGTTAAG	156
F7SYSBQ01DSTIJ	TG	7	Ea072_F	AGGCCACCAATACGAGTGTC	Ea072_R	GCCAGTGTGAATTGAGTTGG	101
F7SYSBQ01EO1SN	TG	7	Ea073_F	CATCATTTGGCATGGATCAG	Ea073_R	GACGCATAATTTATTGTGGCATCTC	102
cons23_2_A	TG	7	Ea074_F	TGTCCACCAGTTGTCTGTCATC	Ea074_R	GCCCTATGAGGTACAGAGGC	137
F7SYSBQ01BSEUD	AG	7	Ea075_F	AGGAGCACACCTTTGATTCAG	Ea075_R	TCCTGACCGTAGTTTGGGAG	119
F7SYSBQ01EX6VQ	AG	7	Ea076_F	TTGGCCTTAGTGGGAGAATG	Ea076_R	ATCCTTAGGCCTGCTTGTCC	177
F7SYSBQ01CE59Z	CT	7	Ea077_F	TGGCTTAACTAGAAGGAATCTGC	Ea077_R	GTCTTCCTGGAGAGCCTGAG	117
F7SYSBQ01CEJ6N	CT	7	Ea078_F	GTTCTCGCTTCTGTTCTGGG	Ea078_R	TTAGCATGAGATGGAGGCTG	182
F7SYSBQ01DC7VC	CT	7	Ea079_F	CATCTGCCTCCCACAGCTAC	Ea079_R	ACATGCACTGCCTGAATGTC	135
F7SYSBQ01CA77N	GA	7	Ea080_F	GAGCGCATGCTCCTTTAGAC	Ea080_R	TTGTGTAATCATTCCCATCTGC	105
F7SYSBQ01CYP6N	GA	7	Ea081_F	AACGGATCCTAAGGTCCCAC	Ea081_R	TCCCTTGTGTCTTTGTTTGC	189
F7SYSBQ01DARB9	GA	7	Ea082_F	CAAGTGAGTATGTGTATTGGCG	Ea082_R	CACAGGGTGAAATACCTGGTC	104
F7SYSBQ01BYDK0	TC	7	Ea083_F	ATCCTCTGCGGTCCAAGTAG	Ea083_R	TGGGAAGAAGGTGAAAGGTG	276
F7SYSBQ01C8TYK	TC	7	Ea084_F	TATCCCTACAGGGCATGGAC	Ea084_R	GAGGACAGGAAATGAAAGGG	112
F7SYSBQ01DEEVG	TC	7	Ea085_F	CTTTGAGTGAGAATGACGGC	Ea085_R	CTCCGAAATCCATCCAATG	236
F7SYSBQ01DJTYK	TC	7	Ea086_F	CTCACCTCCTGTTGGTGTTG	Ea086_R	CCTAGGATACATCTGAGGTCCAG	100
F7SYSBQ01EFX6V	TC	7	Ea087_F	AATTGGGTTATGACAGCACG	Ea087_R	ACTACAGCGATGGGTTCCAC	133
cons37_2_A	TC	7	Ea088_F	ATTGGAACCAAGAGACTGGG	Ea088_R	TCCAACAACTGGGCTATGTG	163
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F7SYSBQ01A47J9	AC	7	Ea093_F	GAAAGCACAGGAATGCAGAAC	Ea093_R	TACCCTATCGGTGTTGCCAG	296
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F7SYSBQ01B62VH	GT	7	Ea096_F	TGGTCCCACAGGAGTAGGAG	Ea096_R	AGTTGCAGAGGGATCACGAC	115
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F7SYSBQ01BFQSE	TG	7	Ea098_F	CATCATCAGATTCATTTCACCC	Ea098_R	TTGCCTAGATTGTTGTTTGCC	115
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	F7SYSBQ01AVHPZ	TTC	8	Ealb13_F	TGCTCCTACCTCCTTCATCG	Ealb13_R	TGTCAGCCTCACCTCCTAGC	162	no amplification
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	F7SYSBQ01BHOZ7	AAC	6	Ea129_F	CAATGAAAGCTGAGATTGCC	Ea129_R	GCAGGAGGCAGCCACTATAC	262	
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	F7SYSBQ01BOISO	CGG	6	Ea135_F	TACGGTACATCACGGCAAAG	Ea135_R	GGGTGCGTCTTAGTACAGTGG	117	
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[1]Characterised refers to markers (n = 29) developed for population genetic analyses in Chapter 2 and published in Todd *et al*. (2011).

APPENDIX 2B

A list of mitochondrial sequence fragments identified within 454-sequence data from *Emydura macquarii krefftii* and *Elseya albagula*, presented in FASTA format. Each sequence identifier contains the contig or sequence name, followed by the corresponding fragment name, as per Fig. 2.3 and 2.4.

Emydura macquarii krefftii

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CTTGCTGACTCACCCCACTAATAATCCTAGCAAGTCAAAATCACCTGATAACTGAGCCAACCCTACGAA
AACAAATCTTTATCTCTACAGCCGTTTTCTTACAAGCCTCCCTAATTATAGCATTCTCCGCCACAGAATT
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TTGGCTCGGGTGTACTACTAAAACTAGGTGGATACGGCATTATCCGAGTATCCTTAATTATAGACCCAC
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CCTCTACATTCGTGCAAACCCAATGAGCACTCACAGGGGCTATCACCCTTATAATTGCCCACGGTTTAA
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>MID9_100bp_c844_FragEa11
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CGCTGGCGTGAAGTTGTCTGGGTCTCCTAGTAGGTTTGGGTAGAATAGGGTCAGGRTGAGCAGGCATGT
GATTATTAGAATGAGGCCTAGTAGGTCCTTGTAGGAGAAGTAGGGGTGGAACGGGATTTTGKCACAGT
TTGAGTTCAGTCCTGTTGGGTTGTTTGATCCTGTTTCGTGTAGCAGTAGGAGGTGTAGTATGGTTATTCC

TAGGATGGCGAATGGGATTATAAAATGGAATGTGAAGAATCGGGTTAGGGTGGCGTTGTCTACAGAGA
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APPENDIX 2C

Annotation results from functional sequence homology searches (BLASTx) using large (> 500 bp) contigs. Data are presented for unique genes, with the corresponding contig name/s given for each, along with the relevant GO groups (P = biological process; F = molecular function; C = cellular process), number of associated GO ID's (with an example), and an example GO term.

Contig name/s (length in bp)	Hit description	GO groups	No. of GO IDs (example)	GO term example
Emydura macquarii krefftii				
MID8_100bp_c9535 (831)	aarf domain containing kinase isoform cra_a	P, F, C	5 (GO:0005576)	extracellular region
MID8_100bp_rep_c54 (558)	accs protein	F	1 (GO:0003824)	catalytic activity
MID8_100bp_c3038 (510)	acetyl-coenzyme a cytoplasmic-like	P, F, C	7 (GO:0005730)	nucleolus
MID8_100bp_c8047 (675)	acidic mammalian chitinase-like	P, F	3 (GO:0004553)	hydrolase activity, hydrolyzing O-glycosyl compounds
MID8_100bp_c8799 (1880)	af355752_1reverse transcriptase	P, F	13 (GO:0003723)	RNA binding
MID8_100bp_rep_c10210 (2221)				
MID8_100bp_rep_c10224 (515)				
MID8_100bp_rep_c10225 (912)				
MID8_100bp_rep_c10228 (846)				
MID8_100bp_rep_c10239 (1307)				
MID8_100bp_rep_c10256 (1474)				
MID8_100bp_rep_c10264 (786)				
MID8_100bp_rep_c10274 (503)				
MID8_100bp_rep_c10276 (1015)				
MID8_100bp_rep_c10314 (710)				
MID8_100bp_rep_c10315 (1199)				
MID8_100bp_rep_c10318 (1080)				
MID8_100bp_rep_c10344 (1206)				
MID8_100bp_rep_c10348 (827)				
MID8_100bp_rep_c10349 (891)				
MID8_100bp_rep_c10355 (513)				
MID8_100bp_rep_c10359 (881)				
MID8_100bp_rep_c10365 (848)				
MID8_100bp_rep_c10370 (1187)				
MID8_100bp_rep_c10385 (686)				

MID8_100bp_rep_c10390 (1408)
MID8_100bp_rep_c10396 (1207)
MID8_100bp_rep_c10400 (761)
MID8_100bp_rep_c10402 (797)
MID8_100bp_rep_c10418 (1895)
MID8_100bp_rep_c10426 (1638)
MID8_100bp_rep_c10428 (790)
MID8_100bp_rep_c10435 (895)
MID8_100bp_rep_c10442 (751)
MID8_100bp_rep_c10450 (805)
MID8_100bp_rep_c10453 (1133)
MID8_100bp_rep_c10464 (622)
MID8_100bp_rep_c10487 (904)
MID8_100bp_rep_c10495 (818)
MID8_100bp_rep_c10501 (763)
MID8_100bp_rep_c10509 (759)
MID8_100bp_rep_c10516 (648)
MID8_100bp_rep_c10522 (733)
MID8_100bp_rep_c10563 (1347)
MID8_100bp_rep_c10588 (555)
MID8_100bp_rep_c10591 (884)
MID8_100bp_rep_c10597 (666)
MID8_100bp_rep_c10630 (518)
MID8_100bp_rep_c10635 (813)
MID8_100bp_rep_c10671 (629)
MID8_100bp_rep_c10690 (557)
MID8_100bp_rep_c10701 (628)
MID8_100bp_rep_c10709 (671)
MID8_100bp_rep_c1194 (1247)
MID8_100bp_rep_c1221 (767)
MID8_100bp_rep_c3540 (1749)
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MID8_100bp_rep_c5535 (673)
MID8_100bp_rep_c6452 (530)
MID8_100bp_rep_c6695 (677)
MID8_100bp_rep_c7055 (1653)
MID8_100bp_rep_c8100 (668)
MID8_100bp_rep_c9226 (1216)
MID8_100bp_rep_c9369 (632)

MID8_100bp_rep_c9434 (680)				
MID8_100bp_rep_c9612 (782)				
MID8_100bp_rep_c9682 (1140)				
MID8_100bp_rep_c9928 (702)				
MID8_100bp_rep_c211 (1153)	af442732_3reverse transcriptase ribonuclease h methyltransferase	F	GO:0016740	transferase activity
MID8_100bp_rep_c1552 (519)	alcohol dehydrogenase 1-like	P, F, C	5 (GO:0005737)	cytoplasm
MID8_100bp_c7760 (562)	aminotransferase class i and ii	F	1 (GO:0016740)	transferase activity
MID8_100bp_rep_c10279 (925)	ankyrin unc44	P, F	4 (GO:0003723)	RNA binding
MID8_100bp_rep_c10529 (741)				
MID8_100bp_rep_c10572 (791)				
MID8_100bp_c332 (585)	aoc2 protein	P, F	5 (GO:0005507)	copper ion binding
MID8_100bp_rep_c9705 (596)	at5g12010 f14f18_180	C	2 (GO:0005886)	plasma membrane
MID8_100bp_c1626 (514)	beta 1-like	P, F	3 (GO:0043169)	cation binding
MID8_100bp_c1421 (527)	calpain 7-like	P, F, C	3 (GO:0005634)	nucleus
MID8_100bp_rep_c10587 (772)	camp and camp-inhibited cgmp -cyclic phosphodiesterase	P, F	3 (GO:0003723)	RNA binding
MID8_100bp_c1352 (501)	cd34 antigen	P, F, C	5 (GO:0005737)	cytoplasm
MID8_100bp_c3395 (504)	cdc2l5 protein kinase	F	1 (GO:0016301)	kinase activity
MID8_100bp_c4831 (557)	coiled-coil domain containing 88a	P, F, C	21 (GO:0030027)	lamellipodium
MID8_100bp_c6739 (601)	complement component (3d epstein barr virus) receptor 2	P, F	2 (GO:0006955)	immune response
MID8_100bp_c10102 (512)	connectin titin	P, F, C	14 (GO:0031430)	M band
MID8_100bp_c5662 (511)	ctage member 5 isoform 1 variant	F	2 (GO:0005515)	protein binding
MID8_100bp_c9225 (691)	cysteine protease atg4b-like	P, F, C	6 (GO:0005737)	cytoplasm
MID8_100bp_rep_c91 (1012)	cytochrome b	P, F, C	8 (GO:0046872)	metal ion binding
MID8_100bp_c9525 (565)	cytochrome c oxidase subunit i	P, F, C	9 (GO:0009060)	aerobic respiration
MID8_100bp_rep_c263 (1312)	cytochrome c oxidase subunit ii	P, F, C	9 (GO:0070469)	respiratory chain
MID8_100bp_c7188 (544)	cytochrome c oxidase subunit iii	P, F, C	4 (GO:0016021)	integral to membrane
MID8_100bp_c602 (736)	cytochrome oxidase subunit 1	P, F, C	9 (GO:0009060)	aerobic respiration
MID8_100bp_rep_c638 (927)	daz interacting protein 1	F, C	4 (GO:0005737)	cytoplasm
MID8_100bp_rep_c306 (630)	dna repair protein xrcc1-like	P, F, C	7 (GO:0005654)	nucleoplasm
MID8_100bp_c10181 (670)	dna-dependent protein kinase catalytic subunit	P, F, C	23 (GO:0045202)	synapse
MID8_100bp_c10285 (1316)				
MID8_100bp_c10427 (930)				
MID8_100bp_c10589 (697)				
MID8_100bp_c10713 (808)				
MID8_100bp_c4650 (736)				
MID8_100bp_rep_c10044 (601)				
MID8_100bp_rep_c10077 (674)				
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MID8_100bp_rep_c10170 (809)				

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MID8_100bp_rep_c10422 (765)

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MID8_100bp_rep_c10510 (801)
MID8_100bp_rep_c10511 (655)
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MID8_100bp_rep_c10534 (684)
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MID8_100bp_rep_c10711 (678)
MID8_100bp_rep_c10721 (774)
MID8_100bp_rep_c10722 (683)
MID8_100bp_rep_c10727 (521)
MID8_100bp_rep_c10730 (556)
MID8_100bp_rep_c1336 (1301)
MID8_100bp_rep_c1711 (513)
MID8_100bp_rep_c1814 (892)
MID8_100bp_rep_c2885 (871)
MID8_100bp_rep_c2972 (502)
MID8_100bp_rep_c3269 (893)
MID8_100bp_rep_c5696 (829)
MID8_100bp_rep_c5792 (1092)
MID8_100bp_rep_c6343 (612)
MID8_100bp_rep_c6384 (501)
MID8_100bp_rep_c717 (547)
MID8_100bp_rep_c761 (754)
MID8_100bp_rep_c8985 (884)
MID8_100bp_rep_c9450 (742)
MID8_100bp_rep_c9787 (791)

MID8_100bp_rep_c9865 (653)				
MID8_100bp_rep_c9929 (557)				
MID8_100bp_rep_c9997 (897)				
MID8_100bp_rep_c3311 (1196)	dnaj homolog subfamily c member 21- partial	P, F	5 (GO:0003723)	RNA binding
MID8_100bp_rep_c10325 (1075)				
MID8_100bp_rep_c10374 (980)				
MID8_100bp_rep_c10502 (911)				
MID8_100bp_rep_c10542 (709)				
MID8_100bp_rep_c10680 (801)				
MID8_100bp_rep_c10519 (611)	endonuclease-reverse partial	P, F	3 (GO:0003723)	RNA binding
MID8_100bp_rep_c10736 (663)				
MID8_100bp_rep_c9863 (818)				
MID8_100bp_c10043 (817)	endonuclease-reverse transcriptase	P, F, C	15 (GO:0030054)	cell junction
MID8_100bp_c10552 (777)				
MID8_100bp_rep_c10122 (788)				
MID8_100bp_rep_c10204 (1237)				
MID8_100bp_rep_c10222 (1651)				
MID8_100bp_rep_c10227 (589)				
MID8_100bp_rep_c10233 (1081)				
MID8_100bp_rep_c10266 (1322)				
MID8_100bp_rep_c10281 (622)				
MID8_100bp_rep_c10298 (1174)				
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MID8_100bp_rep_c10525 (599)				
MID8_100bp_rep_c10543 (771)				
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MID8_100bp_rep_c10586 (781)				
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MID8_100bp_rep_c10660 (530)				
MID8_100bp_rep_c10723 (620)				
MID8_100bp_rep_c1702 (912)				
MID8_100bp_rep_c1915 (770)				
MID8_100bp_rep_c2905 (897)				
MID8_100bp_rep_c4533 (817)				

MID8_100bp_rep_c696 (520)				
MID8_100bp_rep_c8148 (690)				
MID8_100bp_rep_c8998 (693)				
MID8_100bp_rep_c9298 (702)				
MID8_100bp_rep_c9739 (3152)				
MID8_100bp_c7841 (723)	envelope protein	C	1 (GO:0044423)	virion part
MID8_100bp_rep_c1212 (575)	enzymatic poly	F	1 (GO:0016740)	transferase activity
MID8_100bp_rep_c481 (779)				
MID8_100bp_rep_c544 (519)				
MID8_100bp_rep_c685 (797)				
MID8_100bp_rep_c8342 (700)				
MID8_100bp_rep_c887 (633)				
MID8_100bp_rep_c935 (903)				
MID8_100bp_rep_c9387 (536)				
MID8_100bp_c9223 (596)	ephrin type-a receptor 1-like	P, F, C	8 (GO:0005524)	ATP binding
MID8_100bp_rep_c10628 (908)	epidermal differentiation-specific	P, F	3 (GO:0003723)	RNA binding
MID8_100bp_rep_c9966 (515)	erythroid differentiation-related factor 1-like	P, C	2 (GO:0005634)	nucleus
MID8_100bp_c8689 (720)	family with sequence similarity member b-like	C	1 (GO:0016021)	integral to membrane
MID8_100bp_c3754 (508)	fbln1 protein	P, F, C	6 (GO:0005604)	basement membrane
MID8_100bp_rep_c5179 (684)	fyve and coiled-coil domain-containing protein 1-like	F	1 (GO:0016740)	transferase activity
MID8_100bp_c1178 (539)	g protein-coupled family group member a	P, F, C	4 (GO:0004872)	receptor activity
MID8_100bp_c8458 (509)	gag protein	F	1 (GO:0016787)	hydrolase activity
MID8_100bp_c915 (711)	gag_mmtvc ame: full=gag polyprotein contains: ame: full=matrix protein p10 contains: ame: full=phosphorylated protein pp21 contains: ame: full=protein p3 contains: ame: full=protein p8 contains: ame: full=protein n contains: ame: full=capsid protein p27 contains: ame: full=nucleocapsid protein p14	F	2 (GO:0005488)	binding
MID8_100bp_rep_c2352 (607)	gag-pro-pol polyprotein	F	2 (GO:0005488)	binding
MID8_100bp_c3705 (707)	gag-pro-pol protein	P, F, C	5 (GO:0044464)	cell part
MID8_100bp_c6072 (604)	gatad1 protein	P, F	4 (GO:0008270)	zinc ion binding
MID8_100bp_c10446 (704)	gfr receptor alpha 4	P, F	7 (GO:0003723)	RNA binding
MID8_100bp_rep_c10533 (587)				
MID8_100bp_rep_c10642 (631)				
MID8_100bp_rep_c220 (638)	glutamic pyruvate transaminase (alanine aminotransferase) 2	P, F, C	6 (GO:0005739)	mitochondrion
MID8_100bp_c1356 (613)	homeobox protein nkx-	P, F, C	13 (GO:0005634)	nucleus
MID8_100bp_c10439 (983)	hydroxyindole o-methyltransferase-like	P, F	2 (GO:0030187)	melatonin biosynthetic process
MID8_100bp_c3745 (561)	hypothetical protein LOC239151 [*Mus musculus*]	F	1 (GO:0005525)	GTP binding
MID8_100bp_rep_c10431 (805)	immunoglobulin-like receptor chir- partial	P, F	3 (GO:0003723)	RNA binding

MID8_100bp_c6220 (539)	inosine-5 -monophosphate dehydrogenase	P, F	2 (GO:0055114)	oxidation reduction
MID8_100bp_c905 (667)	inter-alpha-trypsin inhibitor heavy chain2	P, F	2 (GO:0030212)	hyaluronan metabolic process
MID8_100bp_c5034 (612)	junction plakoglobin-like	P, F, C	28 (GO:0030056)	hemidesmosome
MID8_100bp_rep_c243 (1908)	l _3	P, F	9 (GO:0003677)	DNA binding
MID8_100bp_rep_c150 (555)				
MID8_100bp_c948 (1908594				
MID8_100bp_c8981 (743)	low quality protein: slit homolog 2	P, F, C	17 (GO:0005615)	extracellular space
MID8_100bp_c590 (516)	low-density lipoprotein receptor-related protein 2-like	P, F, C	4 (GO:0016021)	integral to membrane
MID8_100bp_c7845 (814)	membrane-bound dehydrogenase domain protein	F	3 (GO:0020037)	heme binding
MID8_100bp_c2751 (501)	microtubule-associated protein 7	P, F, C	7 (GO:0005874)	microtubule
MID8_100bp_c1547 (523)	mitochondrial-processing peptidase subunit beta-like	P, F, C	4 (GO:0005759)	mitochondrial matrix
MID8_100bp_c8890 (845)	mitofusin 2	P, F, C	16 (GO:0005829)	cytosol
MID8_100bp_c8936 (771)	mitogen-activated protein kinase 8 interacting protein 3	P, F, C	19 (GO:0000139)	Golgi membrane
MID8_100bp_c5283 (521)	mosaic protein lr11	P, F, C	7 (GO:0006869)	lipid transport
MID8_100bp_c1905 (796)	myosin heavy chain	P, F, C	9 (GO:0030016)	myofibril
MID8_100bp_rep_c159 (731)	nadh dehydrogenase subunit 1	P, F, C	4 (GO:0016021)	integral to membrane
MID8_100bp_rep_c377 (1300)				
MID8_100bp_c3715 (673)	nadh dehydrogenase subunit 2	F, P, C	6 (GO:0006810)	transport
MID8_100bp_rep_c39 (1938)	nadh dehydrogenase subunit 5	P, C	2 (GO:0016020)	membrane
MID8_100bp_c222 (505)	neuroepithelial cell-transforming gene 1	P, F, C	3 (GO:0005622)	intracellular
MID8_100bp_rep_c625 (1145)	nuclease harbi1-like	C	3 (GO:0016020)	membrane
MID8_100bp_rep_c9999 (569)				
MID8_100bp_rep_c10263 (703)	nucleic acid binding protein	F	1 (GO:0016787)	hydrolase activity
MID8_100bp_rep_c1949 (583)	olfactory receptor	P, F, C	5 (GO:0016021)	integral to membrane
MID8_100bp_rep_c402 (571)				
MID8_100bp_rep_c6966 (540)				
MID8_100bp_c9822 (772)	olfactory receptor 4d9-like	P, F, C	4 (GO:0016021)	integral to membrane
MID8_100bp_rep_c1138 (589)	olfactory receptor 654	P, F, C	4 (GO:0016021)	integral to membrane
MID8_100bp_c9891 (547)	olfactory receptor chor12	P, F, C	3 (GO:0016021)	integral to membrane
MID8_100bp_rep_c9629 (704)	olfactory receptor mor20-1	P, F	2 (GO:0023052)	signalling
MID8_100bp_rep_c8771 (554)	ORF1 [*Platemys spixii*]	F	2 (GO:0016787)	hydrolase activity
MID8_100bp_rep_c8601 (541)				
MID8_100bp_rep_c4173 (552)				
MID8_100bp_rep_c10109 (1227)	ORF2 [*Platemys spixii*]	P, F	3 (GO:0003723)	RNA binding
MID8_100bp_c8202 (705)				
MID8_100bp_rep_c10234 (592)				
MID8_100bp_rep_c10401 (973)				
MID8_100bp_rep_c8987 (735)				

MID8_100bp_c3727 (520)	oxidation resistance protein 1-like	P, C	4 (GO:0005730)	nucleolus
MID8_100bp_c1470 (517)	pdz and lim domain protein 3 isoform 1	F, C	4 (GO:0005737)	cytoplasm
MID8_100bp_c7460 (662)	peptidyl-trna hydrolase 1 homolog	C	2 (GO: 0044444)	cytoplasmic part
MID8_100bp_c2646 (505)	phosphatidylinositol transfer membrane-associated isoform cra_a	C	1 (GO:0044444)	cytoplasmic part
MID8_100bp_c2531 (526)	phosphatidylinositol- -trisphosphate 5-phosphatase 2-like	P, F, C	24 (GO:0030027)	lamellipodium
MID8_100bp_rep_c9484 (686)	phospholipase d3	P, F, C	5 (GO:0004630)	phospholipase D activity
MID8_100bp_c1330 (655)	plectin 11 isoform 1	P, F, C	8 (GO:0005856)	cytoskeleton
MID8_100bp_rep_c7017 (807)	pol- partial	P, F	3 (GO:0003723)	RNA binding
MID8_100bp_c1061 (606)	pol polyprotein	F	2 (GO:0016787)	hydrolase activity
MID8_100bp_rep_c6835 (514)				
MID8_100bp_rep_c389 (671)	pol protein	P, F	4 (GO:0016787)	hydrolase activity
MID8_100bp_c8053 (585)				
MID8_100bp_rep_c317 (544)				
MID8_100bp_rep_c9980 (923)	pol-like protein	P, F	3 (GO:0003723)	RNA binding
MID8_100bp_c5556 (516)	potassium channel tetramerisation domain containing 15	P, F, C	4 (GO:0005515)	protein binding
MID8_100bp_c7569 (737)	potassium voltage-gated shaker-related member 10	P, F, C	5 (GO:0005515)	protein binding
MID8_100bp_c9808 (803)	protein argonaute-2-like	P, F, C	16 (GO:0005515)	protein binding
MID8_100bp_c9556 (509)	protein kiaa0284 homolog	C	1 (GO:0005856)	cytoskeleton
MID8_100bp_rep_c154 (507)	protein kinase domain containing protein	P, F	3 (GO:0004672)	protein kinase activity
MID8_100bp_rep_c456 (694)	protein kinase domain cytoplasmic homolog	P, F, C	10 (GO:0005794)	Golgi apparatus
MID8_100bp_c7628 (721)	protein tyrosine non-receptor type 3	P, F, C	9 (GO:0019898)	extrinsic to membrane
MID8_100bp_c2218 (529)	rab gdp dissociation inhibitor alpha	F, C	2 (GO:0005829)	cytosol
MID8_100bp_c10297 (1152)	reverse transcriptase	F, P, C	28 (GO:0005634)	nucleus
MID8_100bp_c6120 (599)				
MID8_100bp_c9848 (895)				
MID8_100bp_c9984 (604)				
MID8_100bp_rep_c10032 (720)				
MID8_100bp_rep_c10037 (568)				
MID8_100bp_rep_c10047 (539)				
MID8_100bp_rep_c10078 (771)				
MID8_100bp_rep_c10188 (952)				
MID8_100bp_rep_c10202 (1010)				
MID8_100bp_rep_c10208 (672)				
MID8_100bp_rep_c10209 (640)				
MID8_100bp_rep_c10211 (839)				
MID8_100bp_rep_c10212 (1108)				
MID8_100bp_rep_c10215 (1124)				
MID8_100bp_rep_c10216 (967)				
MID8_100bp_rep_c10230 (548)				

MID8_100bp_rep_c10243 (641)
MID8_100bp_rep_c10244 (1134)
MID8_100bp_rep_c10246 (1606)
MID8_100bp_rep_c10249 (1607)
MID8_100bp_rep_c10250 (728)
MID8_100bp_rep_c10255 (1192)
MID8_100bp_rep_c10258 (1068)
MID8_100bp_rep_c10259 (1215)
MID8_100bp_rep_c10269 (785)
MID8_100bp_rep_c10271 (1181)
MID8_100bp_rep_c10283 (2021)
MID8_100bp_rep_c10284 (838)
MID8_100bp_rep_c10287 (1448)
MID8_100bp_rep_c10290 (1105)
MID8_100bp_rep_c10296 (799)
MID8_100bp_rep_c10299 (862)
MID8_100bp_rep_c10303 (916)
MID8_100bp_rep_c10310 (763)
MID8_100bp_rep_c10312 (933)
MID8_100bp_rep_c10316 (754)
MID8_100bp_rep_c10323 (624)
MID8_100bp_rep_c10331 (977)
MID8_100bp_rep_c10332 (596)
MID8_100bp_rep_c10334 (768)
MID8_100bp_rep_c10336 (611)
MID8_100bp_rep_c10339 (803)
MID8_100bp_rep_c10352 (915)
MID8_100bp_rep_c10353 (1122)
MID8_100bp_rep_c10354 (1358)
MID8_100bp_rep_c10364 (744)
MID8_100bp_rep_c10367 (802)
MID8_100bp_rep_c10380 (691)
MID8_100bp_rep_c10381 (1254)
MID8_100bp_rep_c10384 (679)
MID8_100bp_rep_c10387 (1043)
MID8_100bp_rep_c10388 (858)
MID8_100bp_rep_c10392 (821)
MID8_100bp_rep_c10394 (812)
MID8_100bp_rep_c10395 (825)

MID8_100bp_rep_c10409 (769)
MID8_100bp_rep_c10416 (878)
MID8_100bp_rep_c10421 (1150)
MID8_100bp_rep_c10432 (567)
MID8_100bp_rep_c10445 (510)
MID8_100bp_rep_c10448 (842)
MID8_100bp_rep_c10456 (1263)
MID8_100bp_rep_c10459 (705)
MID8_100bp_rep_c10460 (848)
MID8_100bp_rep_c10461 (1002)
MID8_100bp_rep_c10469 (1260)
MID8_100bp_rep_c10470 (1218)
MID8_100bp_rep_c10483 (1290)
MID8_100bp_rep_c10484 (1291)
MID8_100bp_rep_c10497 (722)
MID8_100bp_rep_c10499 (1235)
MID8_100bp_rep_c10537 (1306)
MID8_100bp_rep_c10559 (849)
MID8_100bp_rep_c10562 (699)
MID8_100bp_rep_c10568 (760)
MID8_100bp_rep_c10583 (809)
MID8_100bp_rep_c10592 (540)
MID8_100bp_rep_c10595 (833)
MID8_100bp_rep_c10599 (778)
MID8_100bp_rep_c10611 (628)
MID8_100bp_rep_c10612 (917)
MID8_100bp_rep_c10622 (589)
MID8_100bp_rep_c10640 (630)
MID8_100bp_rep_c10641 (847)
MID8_100bp_rep_c10645 (693)
MID8_100bp_rep_c10658 (536)
MID8_100bp_rep_c10664 (864)
MID8_100bp_rep_c10665 (820)
MID8_100bp_rep_c10666 (542)
MID8_100bp_rep_c10684 (542)
MID8_100bp_rep_c10687 (528)
MID8_100bp_rep_c10697 (843)
MID8_100bp_rep_c10724 (949)
MID8_100bp_rep_c10725 (676)

MID8_100bp_rep_c122 (523)				
MID8_100bp_rep_c1235 (505)				
MID8_100bp_rep_c1928 (899)				
MID8_100bp_rep_c212 (2562)				
MID8_100bp_rep_c3951 (524)				
MID8_100bp_rep_c6079 (514)				
MID8_100bp_rep_c6672 (732)				
MID8_100bp_rep_c6738 (882)				
MID8_100bp_rep_c7903 (758)				
MID8_100bp_rep_c7921 (624)				
MID8_100bp_rep_c9095 (580)				
MID8_100bp_rep_c9329 (529)				
MID8_100bp_rep_c9478 (948)				
MID8_100bp_rep_c9530 (1004)				
MID8_100bp_rep_c9565 (655)				
MID8_100bp_rep_c9736 (814)				
MID8_100bp_rep_c9751 (724)				
MID8_100bp_rep_c9795 (894)				
MID8_100bp_rep_c9823 (628)				
MID8_100bp_rep_c9829 (697)				
MID8_100bp_rep_c9868 (721)				
MID8_100bp_rep_c9985 (969)				
MID8_100bp_rep_c1365 (953)	ribosomal large subunit pseudouridine synthase b	F	1 (GO:0016853)	isomerase activity
MID8_100bp_c9060 (635)	rin (ric-related gene expressed in neurons)	P, F, C	4 (GO:0016020)	membrane
MID8_100bp_c10020 (515)	s-adenosylmethionine synthase isoform type-1	P, F	9 (GO:0043531)	ADP binding
MID8_100bp_c3788 (581)	serine-threonine protein kinase	P, F	3 (GO:0004672)	protein kinase activity
MID8_100bp_c6605 (702)	signal cub and egf-like domain-containing protein 2-like	F	1 (GO:0005509)	calcium ion binding
MID8_100bp_c9948 (666)	sortilin 1	P, F, C	36 (GO:0006897)	endocytocis
MID8_100bp_rep_c996 (690)	suppressor of tumorigenicity 14	P, F, C	5 (GO:0006508)	proteolysis
MID8_100bp_c7583 (523)	tetratricopeptide repeat protein 39a-like isoform 2	F	1 (GO:0005488)	binding
MID8_100bp_c4451 (505)	tight junction protein 1 (zona occludens 1)	P, F, C	5 (GO:0005737)	cytoplasm
MID8_100bp_c1218 (532)	transcription initiation factor tfiid subunit 6-like	P, F, C	13 (GO:0071339)	MLL1 complex
MID8_100bp_rep_c10169 (616)	transmembrane protein 90b	P, C	2 (GO:0016021)	integral to membrane
MID8_100bp_rep_c210 (3175)	transposon tx1 uncharacterized 149 kda	P, F	3 (GO:0003723)	RNA binding
MID8_100bp_rep_c1083 (1020)				
MID8_100bp_c8407 (505)	twinkle mitochondrial-like	P, F, C	10 (GO:0042645)	mitochondrial nucleoid
MID8_100bp_c7605 (605)	uncharacterized protein c4orf29-like	C	1 (GO:0005576)	extracellular region
MID8_100bp_c9908 (828)	zinc finger protein 106 homolog	P, F, C	7 (GO:0005730)	nucleolus
MID8_100bp_c1224 (776)	zinc finger protein rfp-like	P, F, C	22 (GO:0005654)	nucleoplasm

Elseya albagula				
MID9_100bp_c6339 (507)	5 -3 exoribonuclease 1 isoform 1	P, F, C	5 (GO:0005737)	cytoplasm
MID9_100bp_c4177 (738)	actin-related protein 2 3 complex subunit 5	P, F, C	7 (GO:0005737)	cytoplasm
MID9_100bp_rep_c2500 (764)	af355752_1reverse transcriptase	P, F, C	9 (GO:0003723)	RNA binding
MID9_100bp_rep_c3713 (1663)				
MID9_100bp_rep_c458 (1492)				
MID9_100bp_rep_c4930 (837)				
MID9_100bp_rep_c5126 (641)				
MID9_100bp_rep_c6423 (1330)				
MID9_100bp_rep_c6894 (1124)				
MID9_100bp_rep_c7039 (1059)				
MID9_100bp_rep_c7170 (906)				
MID9_100bp_rep_c7172 (625)				
MID9_100bp_rep_c7219 (515)				
MID9_100bp_rep_c7287 (1065)				
MID9_100bp_rep_c7490 (1177)				
MID9_100bp_rep_c7570 (979)				
MID9_100bp_rep_c7582 (683)				
MID9_100bp_rep_c7588 (997)				
MID9_100bp_rep_c7592 (1655)				
MID9_100bp_rep_c7607 (559)				
MID9_100bp_rep_c7610 (926)				
MID9_100bp_rep_c7612 (1120)				
MID9_100bp_rep_c7624 (852)				
MID9_100bp_rep_c7637 (1039)				
MID9_100bp_rep_c7653 (622)				
MID9_100bp_rep_c7661 (656)				
MID9_100bp_rep_c7683 (1440)				
MID9_100bp_rep_c7711 (873)				
MID9_100bp_rep_c7716 (916)				
MID9_100bp_rep_c7718 (1129)				
MID9_100bp_rep_c7739 (712)				
MID9_100bp_rep_c7748 (978)				
MID9_100bp_rep_c7750 (518)				
MID9_100bp_rep_c7756 (674)				
MID9_100bp_rep_c7762 (714)				
MID9_100bp_rep_c7766 (818)				
MID9_100bp_rep_c7767 (1318)				

MID9_100bp_rep_c7776 (938)				
MID9_100bp_rep_c7782 (787)				
MID9_100bp_rep_c7786 (1221)				
MID9_100bp_rep_c7797 (581)				
MID9_100bp_rep_c7804 (1190)				
MID9_100bp_rep_c7831 (731)				
MID9_100bp_rep_c7847 (655)				
MID9_100bp_rep_c7853 (616)				
MID9_100bp_rep_c7863 (720)				
MID9_100bp_rep_c7881 (769)				
MID9_100bp_rep_c7884 (667)				
MID9_100bp_rep_c7899 (755)				
MID9_100bp_rep_c7900 (757)				
MID9_100bp_rep_c7913 (786)				
MID9_100bp_rep_c7921 (585)				
MID9_100bp_rep_c7936 (577)				
MID9_100bp_rep_c7967 (785)				
MID9_100bp_rep_c7971 (1000)				
MID9_100bp_rep_c7972 (805)				
MID9_100bp_rep_c7999 (663)				
MID9_100bp_rep_c8006 (850)				
MID9_100bp_rep_c8014 (519)				
MID9_100bp_rep_c834 (506)				
MID9_100bp_rep_c7109 (822)	af442732_3reverse transcriptase ribonuclease h methyltransferase	F	1 (GO:0016740)	transferase activity
MID9_100bp_c7080 (638)				
MID9_100bp_rep_c7708 (913)	ankyrin unc44	P, F	4 (GO:0003723)	RNA binding
MID9_100bp_rep_c7587 (680)				
MID9_100bp_rep_c6518 (562)				
MID9_100bp_c2369 (610)	atp-binding sub-family a member 3	P, F, C	7 (GO:0005624)	membrane fraction
MID9_100bp_c1763 (551)	c2 and ww domain containing e3 ubiquitin protein ligase 2	F	1 (GO:0016874)	ligase activity
MID9_100bp_rep_c7568 (641)	camp and camp-inhibited cgmp -cyclic phosphodiesterase	P, F	3 (GO:0003723)	RNA binding
MID9_100bp_c4447 (610)	camp-dependent protein kinase type i-beta regulatory subunit-like	P, F, C	13 (GO:0005626)	insoluble fraction
MID9_100bp_rep_c7379 (810)	cat eye syndrome chromosome candidate 5 homolog	P, F	2 (GO:0016787)	hydrolase activity
MID9_100bp_rep_c12 (1794)	collagen type iii alpha 1	P, C	2 (GO:0005576)	extracellular region
MID9_100bp_c844 (862)	cytochrome b	P, F, C	8 (GO:0070469)	respiratory chain
MID9_100bp_c6998 (507)				
MID9_100bp_rep_c200 (594)	cytochrome c oxidase subunit i	P, F, C	9 (GO:0009060)	aerobic respiration
MID9_100bp_rep_c51 (1965)	cytochrome c oxidase subunit iii	P, F, C	5 (GO:0016021)	integral to membrane
MID9_100bp_c1758 (603)	dna fragmentation alpha polypeptide	P, F, C	5 (GO:0005634)	nucleus

MID9_100bp_c1214 (502)	dna-binding protein satb2-like	P, F, C	4 (GO:0005634)	nucleus
MID9_100bp_c6986 (713)	dna-dependent protein kinase catalytic subunit	P, F, C	18 (GO:0005634)	nucleus
MID9_100bp_c7327 (685)				
MID9_100bp_rep_c1610 (511)				
MID9_100bp_rep_c187 (1106)				
MID9_100bp_rep_c1934 (552)				
MID9_100bp_rep_c2750 (952)				
MID9_100bp_rep_c349 (572)				
MID9_100bp_rep_c3789 (810)				
MID9_100bp_rep_c448 (894)				
MID9_100bp_rep_c4817 (974)				
MID9_100bp_rep_c5134 (1051)				
MID9_100bp_rep_c5499 (819)				
MID9_100bp_rep_c6213 (516)				
MID9_100bp_rep_c6763 (601)				
MID9_100bp_rep_c6873 (515)				
MID9_100bp_rep_c7102 (1205)				
MID9_100bp_rep_c7347 (568)				
MID9_100bp_rep_c7422 (528)				
MID9_100bp_rep_c7446 (871)				
MID9_100bp_rep_c7562 (582)				
MID9_100bp_rep_c7572 (536)				
MID9_100bp_rep_c7574 (755)				
MID9_100bp_rep_c7577 (728)				
MID9_100bp_rep_c7584 (907)				
MID9_100bp_rep_c7598 (878)				
MID9_100bp_rep_c7611 (665)				
MID9_100bp_rep_c7617 (799)				
MID9_100bp_rep_c7620 (538)				
MID9_100bp_rep_c7622 (901)				
MID9_100bp_rep_c7623 (684)				
MID9_100bp_rep_c7636 (784)				
MID9_100bp_rep_c7642 (649)				
MID9_100bp_rep_c7644 (826)				
MID9_100bp_rep_c7646 (724)				
MID9_100bp_rep_c7655 (730)				
MID9_100bp_rep_c7658 (740)				
MID9_100bp_rep_c7677 (505)				
MID9_100bp_rep_c7679 (959)				

MID9_100bp_rep_c7688 (717)
MID9_100bp_rep_c7691 (854)
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MID9_100bp_rep_c7706 (761)
MID9_100bp_rep_c7710 (941)
MID9_100bp_rep_c7719 (924)
MID9_100bp_rep_c7721 (659)
MID9_100bp_rep_c7730 (688)
MID9_100bp_rep_c7733 (697)
MID9_100bp_rep_c7735 (821)
MID9_100bp_rep_c7742 (636)
MID9_100bp_rep_c7761 (630)
MID9_100bp_rep_c7765 (860)
MID9_100bp_rep_c7772 (740)
MID9_100bp_rep_c7773 (899)
MID9_100bp_rep_c7778 (708)
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MID9_100bp_rep_c1602 (505)	dynein heavy chain axonemal- partial	P, F, C	8 (GO:0005737)	cytoplasm
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MID9_100bp_rep_c661 (1276)	nadh dehydrogenase subunit 6	F, C	3 (GO:0016020)	membrane
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MID9_100bp_rep_c2047 (744)	olfactory receptor 10a7-like	P, F, C	4 (GO:0016021)	integral to membrane
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MID9_100bp_c959 (523)	olfactory receptor 13g1-like	P, F, C	4 (GO:0016021)	integral to membrane
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MID9_100bp_rep_c7964 (547)				
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MID9_100bp_c787 (1212)	pol protein	F	1 (GO:0003824)	catalytic activity
MID9_100bp_c6868 (589)	polyprotein	F	2 (GO:0005488)	binding
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MID9_100bp_c7179 (559)	sodium potassium-transporting atpase subunit beta-1-interacting protein 2-like	C	1 (GO:0005886)	plasma membrane
MID9_100bp_c2699 (505)	solute carrier family 5 (iodide transporter) member 8	P, F, C	4 (GO:0016020)	membrane
MID9_100bp_c5156 (1237)	steroidogenic acute regulatory protein related isoform 1	P, F, C	8 (GO:0006869)	lipid transport
MID9_100bp_c3432 (535)	structural maintenance of chromosomes flexible hinge domain containing 1	P, F, C	4 (GO:0005694)	chromosome
MID9_100bp_c6821 (759)	superfast myosin heavy chain	F, C	4 (GO:0032982)	myosin filament
MID9_100bp_c153 (563)	testis-expressed sequence 15	P	6 (GO:0007126)	meiosis
MID9_100bp_rep_c459 (610)	tripartite motif-containing 21	P, F, C	4 (GO:0005737)	cytoplasm
MID9_100bp_c2092 (561)	unc-13 homolog b (elegans)	P, F, C	6 (GO:0042734)	presynaptic membrane
MID9_100bp_c2511 (587)	uncharacterized	F	1 (GO:0003676)	nucleic acid binding
MID9_100bp_c6701 (712)	uncharacterized protein	P, F	4 (GO:0003677)	DNA binding
MID9_100bp_c1265 (504)	vascular endothelial growth factor receptor 3-like	P, F, C	9 (GO:0005524)	ATP binding
MID9_100bp_c4053 (559)	very large inducible gtpase 1	F, C	3 (GO:0005829)	cytosol
MID9_100bp_c7096 (756)	wdfy family member 4	F, C	2 (GO:0016021)	integral to membrane
MID9_100bp_c3784 (614)	x-ray repair cross-complementing protein 5-like	P, F, C	17 (GO:0005694)	chromosome

APPENDIX 2D

Annotation details for a CR1-Like LINE sequence recovered from *Elseya albagula*, based on similarity to the published sequence, PsCR1, from the South American chelid turtle, *Acanthochelys spixii* (GenBank no. AB005891). Annotation is presented in GenBank format.

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DEFINITION  Elseya albagula retrotransposon CR1-like LINE, partial
       sequence.

SOURCE      Elseya albagula, contig: MID9_100bp_rep_c28_2169

  ORGANISM  Elseya albagula
              Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
              Testudines; Pleurodira; Chelidae; Elseya.
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//
```

APPENDIX 2E

A list of contigs with significant sequence similarity, and extensive coverage of the nuclear ribosomal DNA complex (see Table 2.7). Sequences are given in FASTA format.

Emydura macquarii krefftii

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Elseya albagula

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GTGAGGGTTAGGGTTCCGGGTGGAGGGGCGGGGGAGAGGCAGACCGGCCTCGGCCTTTCGGTCCCGACCGC
AGAGAGGGAGGCAGGGTAGCCCCTCTCCGTCCTGGGCTTTCCGTGGACGTCACGGGAGGGGGGSGCGCGTA
ACCGACGGGGGGCGTGGGCGTCCTCAGGCGTCCTTCCCCTCACGGCACTTACCGGCCCCCCCCGCCGCCGC
GGGCATCCACGGGCGGCCTCAGGCCGCACAAGTCTTTGAACCACCGCCTTCCCCGTGCCGTGGAGGCTCGC
GGAGAGCGCTAGGTACCTGGCAAATGGGGTGGAGGGAAACCGTTCCGAAACCCTCTGGGGCGTCTCCCCCC
CGCCGGCCACCTCCCGCCTCCGGAGAGGCGGGTGGCGGCGACGGACGGCACGCGGAGTGGTGCCCGGCAC

CCGCCAGCCGGCTCCGCGTGCCTCGGGGGGCATCGTTCCCAGAGGGCGCACCGAGAAACCGCTGCCGTGGC
CTCGCCCGCACCCAGGGATCCGGGGTTTCCCTCTGTTCCCGGCGTGCCTGGGAGGCGGACGTGGCTGGGGG
CCGCCCGGCCACAGGCCCCGGCGCACCATCCCGGCCCCACGCACCCACATCCGCAGCACCGGCGGTCGGTC
GCCCGAGCCGACCCCGGCCCCGTCGTCGCCTGCGCACTCACGGACGCC

>MID9_100bp_rep_c2 Length=2281 bp
AAGGGGCCCCGACGGTCCTCCTGCGTCCCTTCTGCCGTCCGCCGTCCGGGTGGCCCGGGCCGGTCTCGGTGG
GATCCGCCATCCCGCCGGCCTTCCGCCCAGGAGGGAAGGAGCAGGGGTCCGCTGGGSGGGGGTGCCCCCCA
GTCCTCGTGGTGGGGCCCCCCCGCTCCTCCTTCTGGAGCGCAGCTACCTGGTTGATCCTGCCAGTAGCATAT
GCTTGTCTCAAAGATTAAGCCATGCATGTTTAAGTACACACGGGCGTTACAGTGAAACTGCGAATGGCTCAT
TAAATCAGTTATGGTTCCTTTGGTCGCTCCAACCCTTACTTGGATAACTGTGGTAATTCTAGAGCTAATACAT
GCCGACGAGCGCTGACCTCCGGGGATGCGTGCATTTATCAGACCAAAACCAACCCGGGCTCGCCCGGCCGC
TTTGGTGACTCTAGATAACCTCGGGCCGATCGCACGCCCCCGTGGCGGCGACGATGCATTCGAATGTCTGCC
CTATCAACTTTCGATGGTACTTCCTGTGCCTACCATGGTGACCACGGGTAACGGGGAATCAGGGTTCGATTC
CGGAGAGGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCC
GACCCGGGGGAGGTACGTGACGAAAAATAACAATACAGGACTCTTTCGAGGCCCTGTAATTGGAATGAGTA
CACTTTAAATCCTTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCC
AATAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTTGGGATCGAGCTGGCGGTCCGCC
GCGAGGCGAGCTACCGCCTGTCCCAGCCCCTGCCTCTCGGCGCTCCCTTGATGCTCTTAACTGAGTGTCCTG
GGGGTCCGAAGCGTTTACTTTGAAAAAATTAGAGTGTTCAAAGCAGGCCGGTCGCCGGAATACTCCAGCTA
GGAATAATGGAATAGGACTCCGGTTCTATTTTGTTGGTTTTCGGAACTGGGGCCATGATTAAGAGGGACGG
CCGGGGGCATTCGTATTGTGCCGCTAGAGGTGAAATTCTTGGACCGGCGCAAGACGAACCAAAGCGAAAGC
ATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTCGGAGGTTCGAAGACGATCAGATACCGTCGTAG
TTCCGACCATAAACGATGCCGACTAGCGATCCGGCGGCGTTATTCCCATGACCCGCCGGGCAGCTTACGGG
AAACCAAAGTCTTTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGC
ACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAACCTCACCCGGCCCGGACACGGAAA
GGATTGACAGATTGATAGCTCTTTCTCGATTCTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCG
ATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTGGCATGCTAACTAGTTATGCGACCCCCGAGCGGTCG
GCGTCCAACTTCTTAGAGGGACAAGTGGCGTTCAGCCACCCGAGATTGAGCAATAACAGGTCTGTGATGCC
CTTAGATGTCCGGGGCTGCACGCGCGCTACACTGACTGGCTCAGCGTGTGTCTACCCTACGCCGACAGGTGC
GGGTAACCCGTTGAACCCCATTCGTGATGGGGATCGGGGATTGCAATTATTCCCCATGAACGAGGAATTCC
CAGTAAGTGCGGGTCATAAGCTCGCGTTGATTAAGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACC
GATTGGATGGTTTAGTGAGGTCCTCGGATCGTCCCTGCCGGGGTCGGTCACGGCCATGGTGGAGTGCCGAG
AAGACGGTCGAACTTGACTATCTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAA
GGATCATTAACGGTGGTGTCGCGCTCGGCCGGCTSGGGCCCCCCCCGACGGCGGCGCGGCTTACCCCGTCCC
ATCACGGGCGCACAGCCTCGGACGCCTCCCCACGCGCAGGATGGGACCCCCGCCGGCGGGGTCCCGGGCGT
GGGGAGGGCCGGGCGCCCCCTCCCCGCCTTCTCCCTCTCAGGAGGGCGGGACGCTGGCGGAGGGGGTCTCC
CTCCGCGCCGGGCTGCTGCCGGGCCGCCTCCGCGCCTTCCTCCGCGCGCGTACCCGAGCCACGCTC

APPENDIX 3A

Sampling locality information for *Elseya albagula* specimens used in genetic analyses presented in Chapter 3.

Drainage Sub-region	**Locality**	**Latitude**	**Longitude**	***N***
Fitzroy				
Fitzroy R	Alligator Ck Junction	-23.190	150.402	16
Fitzroy R	Alligator Ck Junction	-23.166	150.386	17
Fitzroy R	Marlborough Ck	-22.969	149.867	9
Mackenzie R	Riley's Crossing	-23.533	148.585	5
Dawson R	Moura boat ramp	-24.603	149.913	8
Dawson R	Moura Weir	-25.183	150.183	11
Dawson R	Theodore Weir	-24.960	150.073	6
Dawson R	Glebe Weir	-25.467	150.028	2
Dawson R	Wide Water, Taroom	-25.625	149.792	2
Dawson R	Hutton Ck, Warndoo	-25.770	148.736	1
Isaac-Connors R	Cardowan Station	-22.017	149.140	20
Burnett				
Upstream	Grahan's Crossing, Mingo	-25.419	151.689	2
Upstream	Grey's Waterhole, Gayndah	-25.536	151.658	3
Upstream	Beronne Road, Gayndah	-25.595	151.614	1
Upstream	Claude Wharton Weir, Gayndah	-25.615	151.592	3
Upstream	Museum Weir, Gayndah	-25.624	151.613	1
Upstream	Boyd Road, Gayndah	-25.627	151.631	1
Upstream	Thompson's Crossing, Barambah Ck	-25.685	151.778	18
Upstream	Canine Rapids, Barambah Ck	-25.691	151.774	5
Downstream	Ned Churchward Weir	-25.051	152.099	34
Mary				
Upstream	Borumba Dam, Yabba Ck	-26.526	152.565	16
Upstream	Imbil, Yabba Ck	-26.458	152.675	1
Upstream	Kenilworth	-26.585	152.731	5
Downstream	Banting	-25.627	152.610	2
Downstream	Pioneer's Rest	-25.700	152.573	1
Downstream	Petrie Park	-25.749	152.521	3
Downstream	Tinana Creek Barrage	-25.570	152.719	1
Downstream	Tallegalla Weir, Tinana Ck	-25.783	152.715	5
Kolan				
Kolan R	Bucca Weir	-24.861	152.072	2
Kolan R	Cascades, Goodnight Scrub	-25.035	151.879	1

APPENDIX 3B

Frequencies of CR and ND4 haplotypes analysed in Chapter 3 for *Elseya albagula*, by a) sampling location and b) drainage basin.

a)

Haplotype[1]	**Location and sample size**									
	Fitzroy Alligator Ck (32)	Fitzroy Marlborough Ck (9)	Fitzroy Mackenzie R (3)	Fitzroy Dawson R (30)	Fitzroy Connors R (20)	Burnett Upstream (34)	Burnett Downstream (33)	Mary Upstream (21)	Mary Downstream (8)	Kolan R (3)
CR										
1	11	-	1	4	2	-	-	-	-	-
2	13	3	2	20	13	-	-	-	-	-
3	2	-	-	-	1	-	-	-	-	-
4	6	6	-	-	-	-	-	-	-	-
5	-	-	-	3	3	-	-	-	-	-
6	-	-	-	1	-	-	-	-	-	-
7	-	-	-	1	-	-	-	-	-	-
8	-	-	-	1	-	-	-	-	-	-
9	-	-	-	-	1	-	-	-	-	-
10	-	-	-	-	-	33	25	1	7	1
11	-	-	-	-	-	1	8	-	-	-
12	-	-	-	-	-	-	-	15	1	-
13	-	-	-	-	-	-	-	5	-	-
14	-	-	-	-	-	-	-	-	-	2
ND4										
1	11	-	1	7	5	-	-	-	-	-
2	21	9	2	19	15	-	-	-	-	-
3	-	-	-	4	-	-	-	-	-	-
4	-	-	-	-	-	34	33	21	8	1
5	-	-	-	-	-	-	-	-	-	2

[1]Haplotypes represent 475 bp of control region (CR) and 680 bp of the NADH dehydrogenase subunit 4 (ND4), together with 70 bp of tRNAHis, 61 bp of tRNASer and the first 46 bp of tRNALeu (GenBank accession numbers: JX871334- JX871338, JX871340-JX871353).

b)

Haplotype[1]	**Location and sample size**			
	Fitzroy (94)	Burnett (67)	Mary (29)	Kolan (3)
CR				
1	18	-	-	-
2	51	-	-	-
3	3	-	-	-
4	12	-	-	-
5	6	-	-	-
6	1	-	-	-
7	1	-	-	-
8	1	-	-	-
9	1	-	-	-
10	-	58	8	1
11	-	9	-	-
12	-	-	16	-
13	-	-	5	-
14	-	-	-	2
ND4				
1	24	-	-	-
2	66	-	-	-
3	4	-	-	-
4	-	67	29	1
5	-	-	-	2

[1]Refer to Table a) for haplotype description.

APPENDIX 4A

Locality information for *Elseya* spp. specimens and outgroup taxa sampled for phylogenetic analyses presented in Chapter 4.

Species	Drainage (region)	Mitochondrial DNA			Nuclear DNA (R35)		
		Lat.	Long.	*N*	Lat.	Long.	*N*
New Guinea							
Elseya novaeguineae	Aer Besar R (Western NG)	-2.932	132.334	1			
	Missool R (Western NG)	-1.830	129.823	1			
	Aru Is (Western NG)				-5.769	134.416	1
	Sepik R (Northern NG)	-4.297	140.957	1			
	Tami R (Northern NG)	-2.694	140.980	1			
	Waren R (Northern NG)				-2.502	136.557	1
	Aika R (Southern NG)	-4.780	136.846	1			
	Kikori R (Southern NG)	-7.097	143.993	1			
Elseya branderhorsti	Merauke R (Southern NG)	-7.510	140.861	1			
	Fly R (Southern NG)	-8.294	141.910	1	-8.245	141.767	1
Australia							
Elseya dentata	Victoria R (Northern Territory)	-15.562	130.946	1			
		-15.559	130.942	2			
		-15.734	130.014	2			
	Roper R (Northern Territory)	-14.829	133.692	2			
	Limmen-Bight R (Northern Territory)	-15.935	135.585	3	-16.083	135.367	1
Elseya sp. Sth Alligator	Mary (Arnhem Land)	-12.889	131.643	1			
Elseya lavarackorum	Gregory-Nicholson R (Gulf)	-18.633	139.250	7			
		-18.702	138.484	21			
	Calvert R (Gulf)	-17.002	137.315	4			
		-16.655	137.415	6			
Elseya sp. Daintree R	Daintree R (Qld Wet Tropics)	-16.155	145.285	1			
		-16.017	145.302	4			
Elseya irwini	Johnstone R (Qld Wet Topics)	-17.593	145.872	3			
		-17.569	145.898	7			
		-17.522	145.912	16			
		-17.653	145.957	5			

Species	Drainage (region)	Mitochondrial DNA			Nuclear DNA (R35)		
		Lat.	Long.	*N*	Lat.	Long.	*N*
		-17.336	145.619	7			
		-17.355	145.631	3			
		-17.337	145.623	6			
	Burdekin (Qld Wet Tropics)	-20.914	148.427	15	-20.914	148.427	1
		-20.921	148.327	16			
Elseya albagula[1]	Fitzroy R (SE Qld)	-23.190	150.402	15			
		-23.166	150.386	17			
		-22.969	149.867	9			
		-23.533	148.585	3			
		-24.603	149.913	8			
		-25.183	150.183	11			
		-24.960	150.073	6			
		-25.467	150.028	2			
		-25.625	149.792	2			
		-25.770	148.736	1			
		-22.017	149.140	20			
	Burnett R (SE Qld)	-25.419	151.689	2			
		-25.536	151.658	3			
		-25.595	151.614	1			
		-25.615	151.592	3			
		-25.624	151.613	1			
		-25.627	151.631	1			
		-25.685	151.778	18			
		-25.691	151.774	5			
		-25.051	152.099	33			
	Mary R (SE Qld)	-25.749	152.521	2	-25.749	152.521	1
		-26.458	152.675	1			
		-26.585	152.731	4			
		-25.627	152.610	2			
		-25.700	152.573	1			
		-26.526	152.565	16			
		-25.783	152.715	3			
	Kolan R (SE Qld)	-24.861	152.072	2			

Species	Drainage (region)	Mitochondrial DNA			Nuclear DNA (R35)		
		Lat.	Long.	*N*	Lat.	Long.	*N*
		-25.035	151.879	1			
Outgroups							
Emydura macquarii krefftii	Fitzroy R (SE Qld)	-25.442	148.664	1			
		-23.190	150.402	1			
Emydura victoriae	Ord R (Western Australia)	-15.726	128.086	1			
Emydura subglobosa worrelli[4]	Gregory-Nicholson R (Gulf)	-17.956	139.257	1			
	Unknown						1
Myuchelys latisternum[2]	?			1			
	Burnett R (SE Qld)				-24.887	152.291	1
Myuchelys georgesi[2]	?			1			
Rheodytes leukops	Fitzroy R (SE Qld)	-23.190	150.402	1			
Chelodina longicollis[2,5]	?						
	Unknown						1
Chelodina rugosa[3]	Unknown			1			1

[1]Samples for *Elseya albagula* were available from Chapter 3. [2]Samples represent complete mitochondrial genomes for these species (A. Georges *et al*. unpubl. data). [3]Sequences for *Chelodina rugosa* were obtained from an available mitochondrial genome sequence (HQ172157, Wang *et al*. 2012) and R35 sequence (AY339641, Fujita *et al*. 2004), from individuals of unknown geographic origin. [4]R35 sequence of *Emydura subglobosa worrelli* was downloaded from GenBank (AY339632, Fujita *et al*. 2004), from an individual of unknown geographic origin. [5]R35 sequence of *Chelodina longicollis* was downloaded from GenBank (AY339636, Fujita *et al*. 2004), from an individual of unknown geographic origin.

APPENDIX 5A

Sampling locality information for specimens of *Emydura macquarii krefftii* and related subspecies used in genetic analyses presented in Chapter 5.

Drainage Sub-region	**Locality**	**Latitude**	**Longitude**	***N***
Emydura m. krefftii				
Normanby				
Normanby R	Jack's Lake	-14.877	144.433	6
Normanby R	Leichardt Hole	-15.259	144.613	1
Laura R	Crocodile Hole	-15.653	144.595	20
Mulgrave-Russell				
Alice R	Eubenangee Swamp	-17.409	145.982	35
Herbert				
Herbert R	Jourama Falls	-18.867	146.125	3
Alligator Ck				
Alligator Ck	Downstream pool	-19.309	146.765	8
Burdekin				
Burdekin R	Greenvale Station	-19.083	145.050	32
Bowen R	Urannah Causeway	-20.914	148.427	6
Bowen R	Cloverly Pool	-20.921	148.327	5
Bowen R	Eungella Dam	-21.145	148.385	32
Suttor R	Kennedy Billabong	-21.417	147.677	68
Suttor R	Mistake Cr, Laglan Rd crossing	-22.564	147.073	32
Pioneer				
Pioneer R	Mirani bridge	-21.159	148.860	33
Proserpine				
Proserpine R	Below Proserpine dam	-20.366	148.392	25
Fitzroy				
Fitzroy R	Alligator Ck Junction	-23.190	150.402	32
Fitzroy R	Marlborough Ck	-22.969	149.867	36
Mackenzie R	Nogoa R, Fairburn Dam	-23.660	148.077	32
Mackenzie R	Nogoa R, Van Dyke Ck	-24.074	147.761	17
Mackenzie R	Carnarvon Ck, Warrinilla Station	-24.924	148.600	16
Mackenzie R	Comet R, Carnarvon Ck	-25.060	148.230	1
Mackenzie R	Theresa Ck, Hoods Lagoon	-22.818	147.642	6
Dawson R	Korcha pump house	-25.440	148.664	4
Dawson R	Hutton Ck, Warndoo	-25.770	148.736	8
Dawson R	Moura boat ramp	-24.603	149.913	23
Dawson R	Glebe Weir	-25.467	150.028	26
Burnett				
Upstream	Barambah Dam	-26.305	151.979	32
Midstream	Wharton Weir, Gayndah	-25.615	151.592	28
Downstream	Wallaville Weir, Berrembea	-25.051	152.099	30
Mary				
Upstream	Borumba Dam	-26.526	152.565	27
Upstream	Kenilworth	-26.587	152.730	7
Downstream	Banting	-25.627	152.610	12
Downstream	Pioneer's Rest	-25.700	152.573	5

Drainage Sub-region	**Locality**	**Latitude**	**Longitude**	***N***
Downstream	Petrie Park, Tiaro boat ramp	-25.718	152.576	7
Kolan				
Kolan R	Smith's Crossing	-24.816	152.166	29
Emydura m. emmotti				
Cooper Ck	Cullyamurra Waterhole	-27.701	140.843	5
Emydura m. macquarii				
Murray-Darling	Murray R, Doctors Point Lagoon	-36.097	146.934	5
Emydura m. nigra				
Frazer Is	Lake Birrabeen	-25.507	153.063	5
Emydura tanybaraga				
Mitchell	Tea-tree Ck, Dunbar Station	-16.003	142.396	1
Staaten	Dorunda Station	-16.545	141.844	4

APPENDIX 5B

Frequencies of CR and ND4 mtDNA haplotypes analysed in Chapter 5 for *Emydura macquarii krefftii* by drainage basin.

Haplotype[1]	**Location and sample size**										
	Mary (57)	Burnett (90)	Kolan (29)	Fitzroy (206)	Pioneer (33)	Proserpine (25)	Burdekin (143)	Alligator Ck (8)	Herbert (3)	Mulgrave-Russell (33)	Normanby (22)
CR											
1	39	-	-	-	-	-	-	-	-	-	-
2	6	-	-	-	-	-	-	-	-	-	-
3	9	-	-	-	-	-	-	-	-	-	-
4	2	-	-	-	-	-	-	-	-	-	-
5	1	-	-	-	-	-	-	-	-	-	-
6	-	35	-	3	-	-	-	-	-	-	-
7	-	3	-	-	-	-	-	-	-	-	-
8	-	1	-	-	-	-	-	-	-	-	-
9	-	11	-	-	-	-	-	-	-	-	-
10	-	4	5	-	-	-	-	-	-	-	-
11	-	1	-	-	-	-	-	-	-	-	-
12	-	1	-	-	-	-	-	-	-	-	-
13	-	2	-	-	-	-	-	-	-	-	-
14	-	18	-	-	-	-	-	-	-	-	-
15	-	3	-	-	-	-	-	-	-	-	-
16	-	8	-	-	-	-	-	-	-	-	-
17	-	1	-	-	-	-	-	-	-	-	-
18	-	2	-	-	-	-	-	-	-	-	-
19	-	-	6	-	-	-	-	-	-	-	-
20	-	-	5	-	-	-	-	-	-	-	-
21	-	-	1	-	-	-	-	-	-	-	-
22	-	-	9	-	-	-	-	-	-	-	-
23	-	-	3	-	-	-	-	-	-	-	-
24	-	-	-	18	-	-	-	-	-	-	-
25	-	-	-	33	-	-	1	-	-	-	-
26	-	-	-	78	-	-	-	-	-	-	-
27	-	-	-	1	-	-	-	-	-	-	-
28	-	-	-	14	-	-	-	-	-	-	-
29	-	-	-	11	-	-	-	-	-	-	-
30	-	-	-	9	-	-	-	-	-	-	-
31	-	-	-	2	-	-	-	-	-	-	-

Haplotype[1]	Location and sample size										
	Mary (57)	Burnett (90)	Kolan (29)	Fitzroy (206)	Pioneer (33)	Proserpine (25)	Burdekin (143)	Alligator Ck (8)	Herbert (3)	Mulgrave-Russell (33)	Normanby (22)
32	-	-	-	1	-	-	-	-	-	-	-
33	-	-	-	1	-	-	-	-	-	-	-
34	-	-	-	5	-	-	-	-	-	-	-
35	-	-	-	4	-	-	-	-	-	-	-
36	-	-	-	1	-	-	-	-	-	-	-
37	-	-	-	3	-	-	-	-	-	-	-
38	-	-	-	14	-	-	-	-	-	-	-
39	-	-	-	2	-	-	-	-	-	-	-
40	-	-	-	1	-	-	-	-	-	-	-
41	-	-	-	1	-	-	-	-	-	-	-
42	-	-	-	3	-	-	-	-	-	-	-
43	-	-	-	-	33	24	1	-	-	-	-
44	-	-	-	-	-	1	-	-	-	-	-
45	-	-	-	-	-	-	42	-	-	-	-
46	-	-	-	1	-	-	89	-	1	-	-
47	-	-	-	-	-	-	1	-	-	-	-
48	-	-	-	-	-	-	1	-	-	-	-
49	-	-	-	-	-	-	1	-	-	-	-
50	-	-	-	-	-	-	6	-	-	-	-
51	-	-	-	-	-	-	1	-	-	-	-
52	-	-	-	-	-	-	-	8	2	32	3
53	-	-	-	-	-	-	-	-	-	1	-
54	-	-	-	-	-	-	-	-	-	-	4
55	-	-	-	-	-	-	-	-	-	-	14
56	-	-	-	-	-	-	-	-	-	-	1
ND4											
1	1	45	25	57	-	-	-	-	-	-	-
2	40	3	-	-	-	-	-	-	-	-	-
3	7	-	-	-	-	-	-	-	-	-	-
4	8	-	-	-	-	-	-	-	-	-	-
5	2	-	-	-	-	-	-	-	-	-	-
6	-	12	-	-	-	-	-	-	-	-	-
7	-	26	-	-	-	-	-	-	-	-	-
8	-	3	-	-	-	-	-	-	-	-	-
9	-	1	-	-	-	-	-	-	-	-	-
10	-	-	4	-	-	-	-	-	-	-	-
11	-	-	-	33	-	-	1	-	-	-	-

Haplotype[1]	Location and sample size										
	Mary (57)	Burnett (90)	Kolan (29)	Fitzroy (206)	Pioneer (33)	Proserpine (25)	Burdekin (143)	Alligator Ck (8)	Herbert (3)	Mulgrave-Russell (33)	Normanby (22)
12	-	-	-	25	-	-	-	-	-	-	-
13	-	-	-	10	-	-	-	-	-	-	-
14	-	-	-	15	-	-	-	-	-	-	-
15	-	-	-	2	-	-	-	-	-	-	-
16	-	-	-	43	-	-	-	-	-	-	-
17	-	-	-	1	-	-	-	-	-	-	-
18	-	-	-	4	-	-	-	-	-	-	-
19	-	-	-	1	-	-	-	-	-	-	-
20	-	-	-	2	-	-	-	-	-	-	-
21	-	-	-	1	-	-	-	-	-	-	-
22	-	-	-	1	-	-	-	-	-	-	-
23	-	-	-	7	33	25	1	-	-	-	-
24	-	-	-	1	-	-	101	-	1	-	-
25	-	-	-	-	-	-	1	-	-	-	-
26	-	-	-	-	-	-	6	-	-	-	-
27	-	-	-	-	-	-	17	-	-	-	-
28	-	-	-	-	-	-	16	-	-	-	-
29	-	-	-	-	-	-	-	8	2	33	22

[1]Haplotypes represent 439 bp control region (CR) and 670 bp NADH dehydrogenase subunit 4 (ND4), together with 70 bp tRNAHis, 63 bp tRNASer and the first 34 bp tRNALeu (GenBank accession numbers: KF181795-KF181850, KF181854-KF181882).