CSIRO PUBLISHING

Marine and Freshwater Research, 2015, **66**, 1045–1056
http://dx.doi.org/10.1071/MF14102

Significant genetic structure despite high vagility revealed through mitochondrial phylogeography of an Australian freshwater turtle (*Chelodina longicollis*)

K. Hodges[A,B], *S. Donnellan*[B,C] *and A. Georges*[A,D]

[A]Institute for Applied Ecology *and* Collaborative Research Network for Murray–Darling Basin Futures, University of Canberra, ACT 2601, Australia.
[B]Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide, Adelaide, SA 5005, Australia.
[C]South Australian Museum, North Terrace, Adelaide, SA 5000, Australia.
[D]Corresponding author. Email: georges@aerg.canberra.edu.au

Abstract. Restriction to the freshwater environment plays a dominant role in the population genetic structure of freshwater fauna. In taxa with adaptations for terrestriality, however, the restrictions on dispersal imposed by drainage divides may be overcome. We investigate the mitochondrial phylogeographic structure of the eastern long-necked turtle (*Chelodina longicollis*), a widespread Australian freshwater obligate with strong overland dispersa\l capacity and specific adaptations to terrestriality. We predict that such characteristics make this freshwater species a strong candidate to test how life-history traits can drive gene flow and interbasin connectivity, overriding the constraining effects imposed by hydrological boundaries. Contrary to expectations, and similar to low-vagility freshwater vertebrates, we found two ancient mitochondrial haplogroups with clear east–west geographic partitioning either side of the Great Dividing Range. Each haplogroup is characterised by complex genetic structure, demographically stable subpopulations, and signals of isolation by distance. This pattern is overlaid with signatures of recent gene flow, likely facilitated by late Pleistocene and ongoing anthropogenic landscape change. We demonstrate that the divergent effects of landscape history can overwhelm the homogenising effects of life-history traits that connect populations, even in a highly vagile species.

Additional keywords: dispersal, freshwater biogeography, mitochondrial DNA, Murray–Darling Basin, Pleistocene refugia.

Received 12 April 2014, accepted 5 November 2014, published online 22 April 2015

Introduction

Freshwater organisms are collectively limited by barriers such as marine and terrestrial habitats, and this restriction plays a dominant role in their genetic structure at the broad level of whole river drainage basins. Relationships among freshwater populations also often reflect the dendritic structure of streams, and the nested hierarchy of tributaries and rivers within catchments. This stream hierarchy predicts that freshwater populations will have high connectivity and low genetic structure in populations within, but not among, catchments, for a particular river basin (Hughes *et al.* 2009). For obligate freshwater fauna, drainage divides present barriers to population connectivity (Banarescu 1990) and many studies have implicated their influence in shaping the evolution of freshwater faunal lineages. Inability to disperse across drainage divides creates isolated populations that over time provide opportunity for differentiation, divergence, and ultimately allopatric speciation. For example, studies in Australia highlight the eastern uplands of the Great Dividing Range that separate inland and coastal bioregions as a driver of allopatric speciation in freshwater cod (*Maccullochella* spp.) (Rowland 1993; Nock *et al.* 2010), and phylogenetic divergence in multiple species of fish (Unmack 2001; Hammer *et al.* 2007; Faulks *et al.* 2008, 2010; Unmack and Dowling 2010), freshwater crustaceans (Murphy and Austin 2004), and a low-vagility turtle (Hodges *et al.* 2014). In such cases, genetic divergence is a function of isolation by limited hydrological connectivity, rather than isolation by distance *per se*.

There are exceptions to population connectivity being driven by contemporary drainage divides and the dendritic and hierarchical nature of freshwater systems. Changes in stream organisation in recent geological history have facilitated enduring or intermittent interbasin connectivity through drainage reversals (Burridge *et al.* 2007; Unmack *et al.* 2012), exposure of the continental shelf (Ruzzante *et al.* 2011) and flooding at low-relief drainage divides (Masci *et al.* 2008). Furthermore, species with tolerance of saline conditions can move between drainage basins via a coastal marine corridor or infrequent freshwater

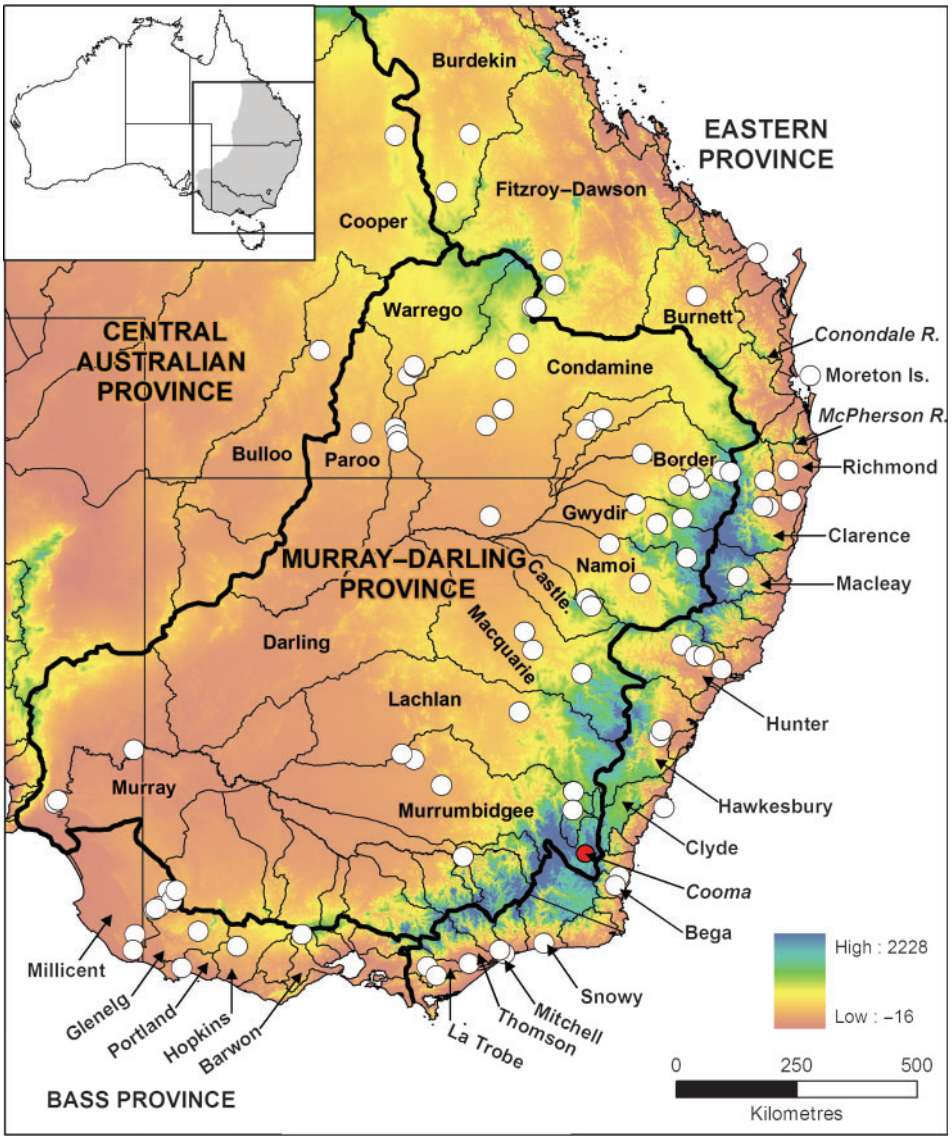

Fig. 1. Distribution of *C. longicollis* (shaded region in inset) and collection localities (white circles). Thick black lines delineate major freshwater biogeographic regions as per Unmack (2001), thin black lines delineate drainages, red circles indicate major cities and localities referred to in the text. Underlying colour indicates elevation in metres. The Great Dividing Range occurs at the interface of the inland Murray–Darling Basin and the eastern seaboard drainages. Note that the central sections of the Great Dividing Range are high elevation and the north and south are of lower elevation.

plumes that connect neighbouring catchments (Jerry and Cairns 1998). Dispersal capacity including flight ability, desiccation resistance, temperature tolerance, and propensity for overland migration can also determine whether freshwater taxa overcome drainage divides (e.g. Šlechtová *et al.* 2004; Craw *et al.* 2008). Each of the above abiotic (geological history) and biotic (life history) processes can leave genetic signatures in populations that contradict assumptions of population divergence based on traditional biogeography and hydrological architecture. Here we use mitochondrial phylogeography of an obligate freshwater turtle with strong terrestrial dispersal capacity to test the extent to which its vagile life-history traits mitigate the otherwise dominant influence of drainage divides.

The eastern long-necked turtle (*Chelodina longicollis*) is one of Australia's most widespread and ubiquitous species of chelid. It is continuously distributed throughout four major freshwater biogeographic regions (Unmack 2001): the Bass Province, the Eastern Province, the Murray–Darling Basin (MDB), and the Cooper and Bulloo drainages in the Central Australian Province (Fig. 1). The MDB and the Central Australian Province are large inland semiarid river basins characterised by low elevational gradients and braided distributary channels. These inland regions are separated from the coastal Bass Province and Eastern Province by the Great Dividing Range, which formed in the Cretaceous, ~90 million years ago (Wellman 1979). Compared with mountain ranges of other continents, the Great

Dividing Range has a subdued character with low to moderate elevational gradients throughout much of its length. It is particularly subdued at the drainage boundary between the MDB and the Bass Province and at the boundary between the MDB the Fitzroy–Dawson and Burnett drainages. Exchange of freshwater taxa across the Great Dividing Range has been long recognised (Musyl and Keenan 1992, 1996; McGlashan and Hughes 2001; Unmack 2001; Murphy and Austin 2004; Cook *et al.* 2006; Hammer *et al.* 2007; Thacker *et al.* 2007; Jerry 2008; Faulks *et al.* 2008, 2010; Unmack and Dowling 2010; Hodges *et al.* 2014) and we expect *C. longicollis* to easily transverse regions with low to moderate elevational gradients.

Chelodina longicollis occupies a broad suite of freshwater habitats throughout its range. The species occurs in greatest abundance in shallow ephemeral wetlands and disconnected water bodies, including artificial environments such as farm dams and irrigation channels, with an abundance of slow-moving invertebrate prey (Chessman 1984*a*, 1988). It is active in water temperatures as low as 12°C (Kennett *et al.* 2009) and displays the greatest cold tolerance of any Australian turtle, with nesting populations at montane sites such as in the vicinity of Cooma, New South Wales. The species belongs to the family Chelidae and, unlike the Testudinidae, uses a 'gape and suck' method of predation (Parmenter 1976), which renders it and all members of the family obligate freshwater species. Despite this, *C. longicollis* is a strong disperser at the landscape scale with a high propensity to utilise terrestrial environments (Roe and Georges 2007, 2008).

Terrestrial forays are reported in 91% of males and 75% of females in some populations, with individual movements up to 1470 m over the course of 1 year (Roe and Georges 2007). Average time spent in the terrestrial environment before returning to a wetland is ~2 months, though terrestriality of up to 16 months is possible (Roe and Georges 2008). This propensity for terrestrial migration enables *C. longicollis* to exploit highly productive disconnected ephemeral systems (Kennett and Georges 1990), and to find permanent water to escape periodic drought conditions (Roe and Georges 2007). To cope with extended periods of terrestriality *C. longicollis* has evolved specific water-conserving adaptations (Roe *et al.* 2008). It is able to draw its head, neck, limbs and tail tightly within the shell to both reduce exposure to predation and minimise evaporative water loss (Chessman 1984*b*). To limit desiccation the species has the capacity to store and reabsorb water from the cloacal bladder, adjust uric acid excretions, limit cutaneous water loss, and drink pooled water from terrestrial leaf litter (Rogers 1966; Chessman 1984*b*; Roe 2008).

Chelodina longicollis is the most vagile of Australian chelids. The related and broadly sympatric broad-shelled turtle (*C. expansa*), however, has no specific adaptions for terrestriality and is restricted to permanent water bodies connected to main river channels (Bower and Hodges 2014). As a consequence, mitochondrial phylogeographic structure in *C. expansa* is dictated by long-standing drainage divides. Mitochondrial nucleotide divergence between the MDB and Eastern Province bioregions in this species is 2.41%, and deeper divergence of 4.61% is found between the Mary and Brisbane drainages in the Eastern Province itself (Hodges *et al.* 2014). Eastern Province drainage divides similarly dictate deep phylogeographic structure in the Australian snapping turtle (*Elseya albagula*) and Krefft's river turtle (*Emydura macquarii krefftii*) (Todd *et al.* 2013, 2014). Compared with *C. longicollis*, *C. expansa* and *Em. m. krefftii* have poor dispersal capacity, and *Els. albagula* can be considered sedentary (Todd *et al.* 2013). Given that phylogeographic structure is closely tied to species vagility, especially in freshwater taxa, we expect genetic patterns in *C. longicollis* to be quite unlike those described for other Australian chelids. Rather, we expect phylogeographic structure in *C. longicollis* to be comparable to that of the common snapping turtle (*Chelydra serpentina*) from eastern and central North America.

Chelodina longicollis is broadly similar to *Chelydra serpentina* in that they both have an extensive range, a degree of cold tolerance, and strong terrestrial dispersal ability (Obbard and Brooks 1981*a*, 1981*b*; Costanzo *et al.* 1995). *Chelydra serpentina* exhibits almost no mitochondrial sequence variation across its distribution in the south-eastern United States, suggesting that its dispersal capacity and cold tolerance either allowed it to resist population subdivision during Pleistocene glacial periods (Walker and Avise 1998), or facilitated its rapid expansion during interglacials from a single population. We expected to find a similar pattern of limited mitochondrial genetic diversity in *C. longicollis*, and any diversity that we do find we expect to be broadly dispersed throughout the range of the species and not limited by drainage divides and bioregional boundaries. We used mitochondrial nucleotide sequences to test the following: (1) that there is no genetic subdivision between the MDB, Eastern Province, Bass Province and Central Australian Province; and (2) that genetic structure is dominated by signals of panmixia. Our study builds on the phylogeographic dataset for sympatric freshwater taxa in eastern Australia (Hughes *et al.* 2013; Hodges *et al.* 2014) to test the extent of influence that freshwater bioregions and hydrological connectivity have on genetic structure. *C. longicollis* represents the maximum dispersal capacity of an obligate Australian freshwater vertebrate and can potentially highlight the upper limit beyond which freshwater bioregions have no effect on phylogeographic partitioning.

Materials and methods

Sampling

We obtained tissue samples from 274 *C. longicollis* from 94 localities across 33 drainages throughout the geographic range of the species (Fig. 1). Skin samples were obtained from the webbing of the clawless digit on the hind foot and immediately placed in 95% ethanol for transport and storage. Sample details and collection localities are provided in 'Specimens examined' of the Supplementary material. Taxonomy follows that of Georges and Thompson (2010).

The mitochondrial regions examined and the procedures for DNA extraction, PCR amplification and PCR product purification follow those for *C. expansa* (Hodges *et al.* 2014) and thus the two studies are directly comparable. Briefly, we targeted a 630-bp fragment of the mitochondrial *ND4* gene, and a 470-bp fragment of mitochondrial *control region* including $tRNA^{Pro}$ (hereafter collectively referred to as *control region – CR*). Sequencing was performed in both directions using an ABI

3730XL DNA automated sequencer by Macrogen (Seoul, South Korea) and sequences were edited, assembled, and consensus sequences determined using Geneious Pro 5.3.4 (BioMatters Inc.). Sequences were aligned using ClustalX 1.81 (Thompson *et al.* 1997) to yield final edited alignments of 1042 bp, comprising 595 bp of *ND4*, 68 bp of $tRNA^{Pro}$ and 379 bp of *control region* (GenBank accession numbers for *ND4* haplotypes are KM581393–KM581420; GenBank accession numbers for *CR* haplotypes are KM581421–KM581448). Four methods, described in greater detail in Hodges *et al.* (2014), were used to confirm the genuine mitochondrial origin of the sequences and minimise the chance of undetected inclusion of nuclear paralogues in our analyses. Specimens examined in the Supplementary material details the two samples used in the present study for the mitochondrial enrichment procedure.

Population genetic structure

A median-joining haplotype network was constructed on concatenated *ND4* and *CR* sequences using NETWORK 4.610 (Fluxus Technology Ltd) with $\varepsilon = 0$ and maximum parsimony postprocessing. Molecular divergence indices were estimated in DnaSP 5 10.01 (Librado and Rozas 2009) using the average number of nucleotide substitutions per site between groups (D_{xy}) with 1000 bootstrap replicates. A molecular dating analysis was implemented using the Bayesian approach in BEAST 1.6.1 (Drummond and Rambaut 2007) to estimate time to most recent common ancestor for major genetic groups. Models of evolution were specified in ModelTest 3.7 (Posada and Crandall 2001): $tRNA^{Pro}$ K80, *control region* TrN+G, *ND4* HKY+G. Domains were tested for clocklike evolution in PAUP* (Swofford 2002) and $tRNA^{Pro}$ and *ND4* were estimated under a strict molecular clock model and *control region* was estimated under a relaxed uncorrelated log-normal clock. We applied a mitochondrial divergence rate of 0.895% per million years (Zamudio and Greene 1997; Rabosky *et al.* 2007) scaled per lineage per million years and modelled under a normal distribution. This divergence rate is consistent with a rate estimated from fossil chelid turtles by Georges *et al.* (2014) (0.86% per million years) and has been applied successfully elsewhere (Hodges *et al.* 2014; Todd *et al.* 2014). MCMC chains were run for 40 million generations with sampling every 1000 steps yielding a total of 40 000 trees. Convergence was checked and parameters assessed using Tracer 1.5 (Rambaut and Drummond 2007).

Correlation between geographic and genetic distance (isolation by distances) was assessed using Mantel tests implemented in GenAlEx 6.41 (Peakall and Smouse 2006) and significance tests were carried out using 9999 permutations. We also performed analyses of molecular variance (AMOVA) from haplotype frequencies using 1000 bootstrap replicates in Arlequin 3.5.1.2 (Excoffier and Lischer 2010) to investigate partitioning of genetic variation based on the four major freshwater biogeographic regions defined *a priori*. We first combined all individuals by drainage and performed AMOVAs with three levels: within drainages, among drainages within regions, and among regions. Four analyses were performed: (1) among all four freshwater biogeographic regions; (2) the MDB versus Eastern Province; (3) the MDB versus Bass Province; and (4) the Bass Province versus Eastern Province. Because of insufficient samples from the Central Australian Province, we excluded this region from paired analyses of variation.

Historical population demography

Number of segregating sites (S), haplotype diversity (hd), nucleotide diversity (π), and average number of nucleotide differences (k) were calculated in DnaSP. Tajima's (1989) *D* statistic, *Fs* (Fu 1997), and R2 (Ramos-Onsins and Rozas 2002) were calculated to test whether populations were conforming to models of neutral evolution and demographic stability, or were departing from these states owing to population expansion. All tests were performed in DnaSP and significance was estimated using the distribution of random samples generated by 10 000 coalescent simulations.

Results

Haplotypic relationships

Twenty-eight mitochondrial haplotypes were recovered from concatenated *ND4* and *CR* mitochondrial sequences from 274 *C. longicollis* representing the geographic range of the species. Haplotypes fall into two major haplogroups, A and B (Fig. 2), separated by 44 mutational steps, and D_{xy} sequence divergence of 4.38%. Bayesian dating analysis estimated that the most recent common ancestor of the two haplogroups occurred 6.53 million years ago (95% highest posterior density (HPD) = 4.89–8.26 million years ago) in the late Miocene, and the time to most recent common ancestor for each haplogroup in the early Pleistocene –Haplogroup A: 1.29 million years ago (95% HPD = 0.71–1.95 million years ago); Haplogroup B: 1.5 million years ago (95% HPD = 0.89–2.17 million years ago). Haplogroups A and B comprise 13 and 15 haplotypes respectively. Divergence within each haplogroup is low, with an average of 1.8 substitutions separating haplotypes in Haplogroup A, and 3.3 separating haplotypes in Haplogroup B. Despite low divergence, the haplotype network reveals a complex genetic structure with little evidence of star-like patterns (i.e. many recently evolved haplotypes), common haplotypes not always located centrally, and a large number of mutational steps separating terminal haplotypes in Haplogroup B.

Phylogeographic relationships

The two major haplogroups do not strictly correspond to freshwater biogeographic regions defined *a priori*; however, there is clear geographic structure in their distributions (Fig. 3). Haplogroup A tends to have an easterly distribution associated with the eastern uplands of the MDB and coastal Eastern Province drainages from Moreton Island in the north to the southern boundary of the Eastern Province. Haplogroup B tends to have a westerly distribution associated with the entire MDB, the Bass Province, and the north-western drainages of the Eastern Province. Haplogroups A and B both occur in the Eastern Province but their ranges do not overlap nor do they occupy the same drainages. In the MDB, however, the two haplogroups are sympatric in the eastern uplands of the Border Rivers, Namoi, and Castlereagh drainages. The drainage with the highest haplotype diversity in the Eastern Province is the Hunter ($n = 16$ individuals and 5 haplotypes, Hd = 0.76) and the

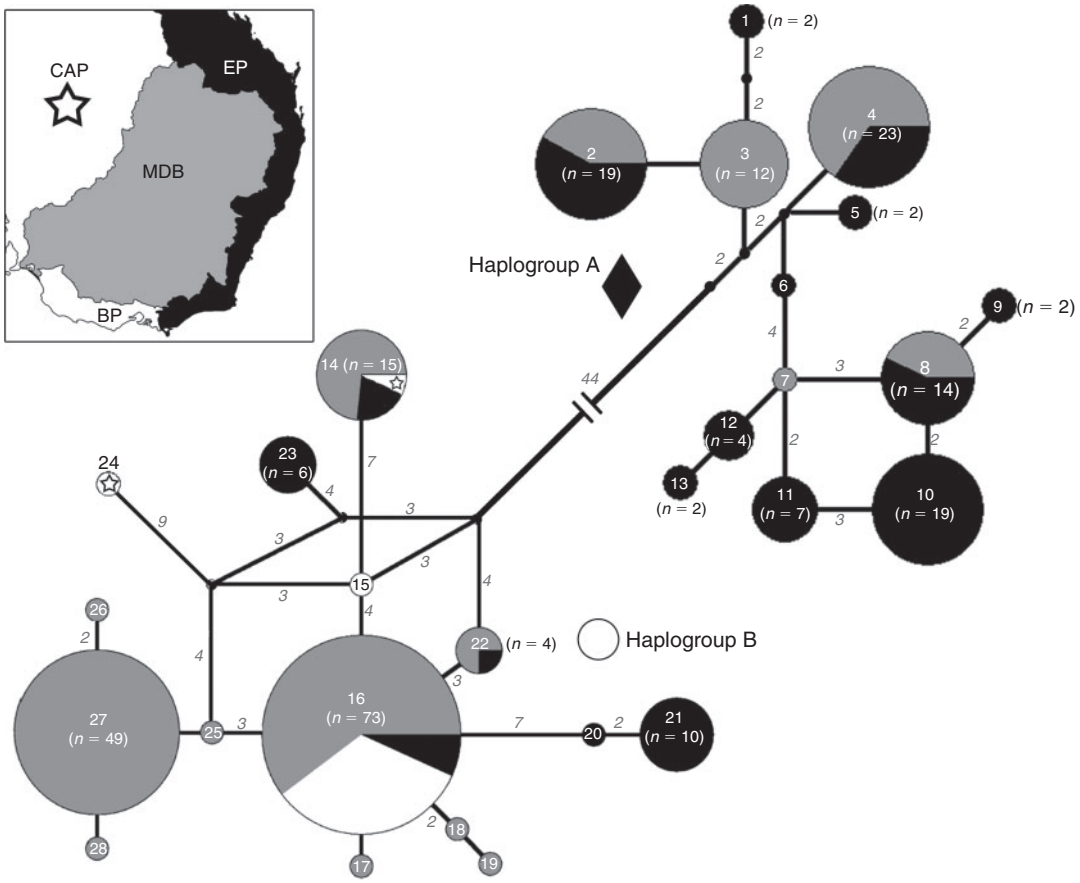

Fig. 2. Median-joining haplotype network of the 28 *C. longicollis* haplotypes. Number of mutational steps (>1) in the haplotype network are indicated in grey, circle area is proportional to the number of individuals sharing a haplotype, haplotype number is given inside the circle, number of individuals (>1) is indicated in parentheses. Haplotypes are coloured by representation of individuals from each of the four major freshwater biogeographic regions: grey indicates the Murray–Darling Basin (MDB), white indicates the Bass Province (BP), black indicates the Eastern Province (EP), star indicates the Central Australian Province (CAP). Black diamond (Haplogroup A) and white circle (Haplogroup B) are consistent with symbology presented in Fig. 3.

highest haplotype diversity in the MDB is in the Namoi ($n = 9$ individuals and 4 haplotypes, Hd = 0.75). Haplotype sharing among bioregions is moderate, with six of the 28 haplotypes (21.4%) being found in more than one major freshwater bioregion. Overall, there are seven broad locations where haplotype sharing occurs between freshwater bioregions (Fig. 3). Haplotype frequencies for each drainage division are available in Table S1 of the Supplementary material.

Analysis of molecular variance

Analysis of molecular variance (Table 1) among the four freshwater biogeographic regions apportioned 12.48% of total genetic variation among regions ($P < 0.05$), 52.4% among drainages within regions ($P < 0.001$), and 35.12% within drainages ($P < 0.001$). Genetic differentiation was significant between the MDB and the Eastern Province, with only 6.54% apportioned between regions ($P < 0.05$), 56.06% among drainages within regions ($P < 0.001$), and 37.4% within drainages ($P < 0.001$). Between the MDB and the Bass Province 20.04% of variation was shared between regions ($P < 0.05$), 48.13% among drainages within regions ($P < 0.001$), and 31.83% within drainages ($P < 0.001$). Finally, between the Eastern Province and the Bass Province 29.88% of variation was shared between regions ($P < 0.001$), driven primarily by the widespread Haplotype 16 and a lack of diversity in the Bass Province; 41.18% was apportioned among drainages within regions ($P < 0.001$), and 28.94% within drainages ($P < 0.001$).

Isolation by distance

Results from Mantel tests on each haplogroup imply significant positive correlation between genetic and geographic distance. Haplogroup A yields a strong signal of isolation by distance (correlation coefficient of the Mantel test, $R_{xy} = 0.565$; $P < 0.001$), and a moderate signal characterises Haplogroup B ($R_{xy} = 0.23$, $P < 0.001$). We also tested for isolation by distance across the entire range of *C. longicollis* (94 collection localities) and again a significant but weaker signal of isolation by distance was found ($R_{xy} = 0.118$, $P < 0.001$).

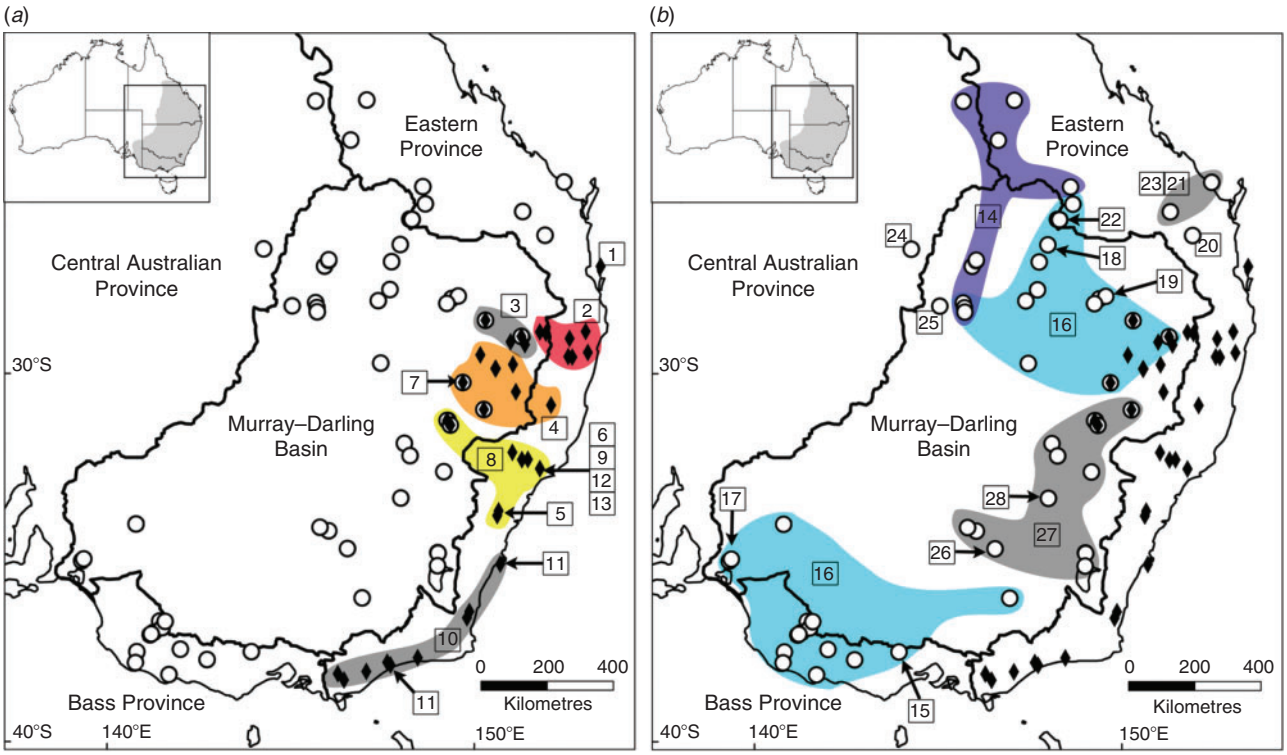

Fig. 3. Phylogeographic structure of the two major haplogroups in *C. longicollis*. Each panel indicates the location of individuals in Haplogroup A (black diamonds) and Haplogroup B (white circles). Note that Haplogroups overlap at some sites. Panel A and B highlight the distribution and number of Haplogroup A and B haplotypes respectively. Coloured groups encompass all individuals with the same haplotype, and grey shaded groups indicate haplotypic distributions that do not cross freshwater province boundaries. Numbers presented in boxes indicate haplotype number, and black lines indicate boundaries of the four major freshwater biogeographic regions.

Table 1. Hierarchical analysis of molecular variance (AMOVA) results for *Chelodina longicollis* mitochondrial haplotype frequency data
SS, sum of squares; MDB, Murray–Darling Basin; EP, Eastern Province; BP, Bass Province

Source of variation	d.f	s.s.	Variance components	Variation %	Fixation index	*P*
Among all biogeographic regions						
among regions	3	15.01	0.06	12.48	$F_{CT} = 0.125$	0.003
among drainages within regions	30	64.35	0.25	52.40	$F_{SC} = 0.599$	0.000
within drainages	240	39.99	0.17	35.12	$F_{ST} = 0.649$	0.000
total	273	119.35	0.47			
Between the MDB and EP						
between regions	1	7.13	0.03	6.54	$F_{CT} = 0.065$	0.034
among drainages within regions	25	63.56	0.27	56.06	$F_{SC} = 0.600$	0.000
within drainages	220	39.32	0.18	37.40	$F_{ST} = 0.626$	0.000
total	246	110.01	0.48			
Between the MDB and BP						
between regions	1	6.29	0.10	20.04	$F_{CT} = 0.200$	0.020
among drainages within regions	15	37.91	0.23	48.13	$F_{SC} = 0.602$	0.000
within drainages	163	24.61	0.15	31.83	$F_{ST} = 0.682$	0.000
total	179	68.8	0.47			
Between the BP and EP						
between regions	1	8.84	0.17	29.88	$F_{CT} = 0.299$	0.001
among drainages within regions	18	26.24	0.24	41.18	$F_{SC} = 0.587$	0.000
within drainages	97	16.05	0.17	28.94	$F_{ST} = 0.710$	0.000
total	116	51.13	0.57			

Table 2. Molecular diversity indices and tests for population stability

n, number of sequences; h, number of haplotypes; S, number of segregating sites; Hd, haplotype diversity; π, nucleotide diversity; k, and average number of nucleotide differences; *D*, Tajima's *D* (Tajima 1989); *Fs*, Fu's *Fs* (Fu 1997); R2, Ramos-Onsins and Rozas's R2 (Ramos-Onsins and Rozas 2002)

Haplogroup	*n*	h	S	Hd ± s.d.	π	k	*D* (95% CI)	*Fs* (95% CI)	R2 (95% CI)
A	108	13	16	0.864 ± 0.013	0.00389	4.06	0.92 (−1.62–1.95)	0.72 (−7.19–7.87)	0.13 (0.04–0.16)
B	166	15	23	0.71 ± 0.025	0.00289	3.00	−0.72 (−1.60–1.98)	−1.04 (−6.88–7.48)	0.06 (0.03–0.15)

Haplogroup demographic analyses

Haplotype diversity (Table 2) is higher in Haplogroup A (Hd = 0.86) than in Haplogroup B (Hd = 0.71). Estimates of Tajima's *D* and *Fs* are not significant for either haplogroup (A: $D = 0.92$, $P = 0.85$; $Fs = 0.72$, $P = 0.66$) (B: $D = -0.72$, $P = 0.23$; $Fs = -1.04$, $P = 0.41$), supporting the null hypothesis that the gene fragments associated with each lineage are selectively neutral and conform to a model of population size stability. The R2 statistic is not significant for either haplogroup, further supporting demographic stability.

Discussion

Turtles such as *C. longicollis* are intermediate in life-history traits, such as dispersal capacity and an ability to occupy a range of freshwater habitats, when compared with freshwater fish and low-vagility terrestrial mammals (Walker and Avise 1998). In contrast to expectations for other vertebrate freshwater obligates, in *C. longicollis* we predicted highly connected populations and insensitivity to traditional freshwater biogeographic boundaries. Instead, we found two divergent mitochondrial haplogroups with east–west geographic partitioning, genetic structure within each haplogroup, signals of historic demographic stability, and isolation by distance. These patterns are overlaid with signatures of recent population connectivity and haplotype sharing among bioregions.

East–west divergence

Chelodina longicollis mitochondrial haplogroups diverged ~6.53 million years ago in the late Miocene. The maintenance of this ancient signature in contemporary populations seems at odds with the species' dispersal capacity and its potential to traverse low-to-moderate-elevation regions of the Great Dividing Range. The processes that led to late Miocene mitochondrial divergence are uncertain as these signatures have been replaced with diversity acquired since the early Pleistocene. We cannot speculate on the Miocene–Pliocene distributions of ancestral Haplogroups A and B; however, the antiquity of each group suggests long-term demographic decoupling of mitochondrial lineages. We suggest that the barrier presented by the ancient and topographically complex Great Dividing Range drove independent evolution of the two mitochondrial lineages and has also maintained separate distributions of the two contemporary haplogroups at least since the early Pleistocene. A range of sympatric freshwater taxa, including fish, crustaceans and a turtle, also display intraspecific phylogeographic structure in varying extent and age as a result of the Great Dividing Range (Rowland 1993; Unmack 2001; Murphy and Austin 2004; Hammer *et al.* 2007; Faulks *et al.* 2008, 2010; Unmack and Dowling 2010; Hodges *et al.* 2014). Despite its often subdued character, this landscape feature is an important driver of evolutionary diversity in freshwater taxa, regardless of life history.

We acknowledge that phylogeographic breaks can arise without long-term barriers to gene flow (Irwin 2002) and that mitochondrial haplotypic relationships do not necessarily reflect the organismal history of a species. However, breaks without barriers are more likely to occur in low-vagility species. Also, there is evidence for an association between east–west mitochondrial divergence and morphological traits in *C. longicollis*. Cann (1998) recognised two morphological forms within *C. longicollis*: eastern-distributed specimens collected in the Eastern Province have long ovoid- to oblong-shaped carapaces (Cann 1998; Goode 1967), whereas the carapaces from western-distributed specimens in the Bass Province and the MDB are wider and 'more squat' (Cann 1998). Cann suggested that these two morphological forms may highlight distinct *C. longicollis* populations, and our mitochondrial genetic data support this claim, though in the absence of nuclear gene data we do not recognise the different haplogroups as requiring taxonomic recognition. Future work could investigate whether individuals from different haplogroups correspond to Cann's putative morphotypes, focussing especially on the site of distributional overlap.

The persistent influence of the Great Dividing Range is visible today in *C. longicollis*. Higher-elevation montane environments, such as those at the interface of the Murrumbidgee and Snowy drainages, appear to inhibit connectivity in south-east Australia between populations in the MDB and the Eastern Province. Mitochondrial gene flow at these locations appears absent even with the widespread contemporary presence of farm dams, which *C. longicollis* regularly inhabits. Limited cold tolerance may be acting to constrain dispersal of *C. longicollis* in this region. Although *C. longicollis* is active at low temperatures (Kennett *et al.* 2009) and nests at montane sites on the south-east tablelands (Cooma, 793 m above sea level: pers. Obs.), these attributes appear insufficient to allow gene flow over the Great Dividing Range in this region.

Unexpected diversity

Contrary to expectations of panmixia, signals of isolation by distance and significant mitochondrial genetic diversity characterise each haplogroup. Isolation by distance reflects equilibrium between gene flow and genetic drift and is established over long periods with stable populations and limited barriers to dispersal. Neutrality indices also support demographic stability and historically subdivided populations within each haplogroup. We propose that these patterns result from population

contraction and persistence in the MDB and the Eastern Province during recent Pleistocene glacial oscillations.

Haplogroup A diversity

High mitochondrial genetic diversity and signals of population subdivision and demographic stability in Haplogroup A suggest that the eastern population of *C. longicollis* has long persisted in the Eastern Province. Further, highly localised haplotypes point to a pattern of range contractions during Pleistocene aridity and population persistence in multiple freshwater isolates. The complex topography of the Eastern Province could have harboured multiple refugia during glacial cycles. Freshwater taxa including shrimp (*Paratya australiensis*) (Cook *et al.* 2006), hardyhead (*Craterocephalus marjoriae*) (Unmack and Dowling 2010), smelt (*Retropinna semoni*) (Hammer *et al.* 2007), and flathead gudgeon (*Philypnodon macrostomus*) (Thacker *et al.* 2008) show similarly localised haplotypes and isolation by distance. The Hunter drainage, in particular, likely played an important role in harbouring and promoting diversity during Pleistocene population contraction. This drainage has the highest haplotype diversity in *C. longicollis*, and also harbours a divergent lineage of the freshwater catfish (*Tandanus tandanus*) (Jerry 2008).

Haplogroup B diversity

Haplogroup B has a strong association with the MDB and we expected this region above all others to show very limited genetic structure. Although Haplotype 16 has an enormous distribution, extending over 1500 km, signals of isolation by distance and moderate mitochondrial genetic diversity dominate. The two most common haplotypes are separated by a large number of mutational steps from the B group haplotypes (20, 21 and 23) endemic to the Eastern Province. We propose that throughout the LGM, both the MDB and the Eastern Province independently harboured Haplogroup B haplotypes that originated from earlier diversification. Pleistocene refugia in the MDB have been suggested on the basis of localised divergent haplotypes and significant genetic structure in freshwater fish and crustaceans (Austin *et al.* 2003; Nguyen *et al.* 2004; Hughes and Hillyer 2006; Hammer *et al.* 2007; Faulks *et al.* 2008). The upland regions of the Border Rivers, Gwydir, and Namoi drainages, in particular, are strong candidates for Pleistocene refugia in *C. longicollis*. These headwaters contain ancestral haplotypes of the southern purple spotted gudgeon (*Mogurnda adspersa*) (Faulks *et al.* 2008), and the highly restricted and endangered western sawshelled turtle (*Myuchelys bellii*) (Fielder *et al.* 2012). Relictual turtle populations are the product of range contraction from a formerly widespread distribution (Fielder *et al.* 2012), and highlight the headwaters of the Border Rivers, Gwydir, and Namoi drainages as suitable refuge sites for freshwater fauna in the present day, and possibly during the LGM.

Highly localised and divergent haplotypes in the north and north-west Eastern Province suggest that this region also harboured population isolates during the LGM. The Fitzroy–Dawson and the Burnett drainages both present a mosaic of freshwater isolates where haplotypes could have persisted through hostile Pleistocene conditions. A close relationship between the Burnett drainage and the northern MDB characterises carp gudgeon (*Hypseleotris klunzingeri* and *H. galii*) (Thacker *et al.* 2007), lineages of dwarf flathead gudgeon (*Philypnodon macrostomus*) (Thacker *et al.* 2008), and lineages of hardyhead (*Craterocephalus stercusmuscarum fulvus*) (Unmack and Dowling 2010), and expose this area as a potentially important source of diversification before expansion into the MDB.

Phylogeographic break in the Eastern Province

A mitochondrial phylogeographic break occurs in *C. longicollis* between the Richmond and Burnett drainages. Potential drivers of this break include the McPherson Range and the Conondale Range. The McPherson Range forms the high-elevation (~1500 m above sea level) northern boundary of the Richmond drainage and acted as a significant barrier to gene flow in some species throughout the Miocene and Pliocene (McGuigan *et al.* 1998; Keogh *et al.* 2003; Chapple *et al.* 2011; Smissen *et al.* 2013). The Conondale Range delineates the Mary and Brisbane drainages and is an influential biogeographic barrier in many freshwater species, including a turtle (Page and Hughes 2014; Hodges *et al.* 2014). Future sampling is required in *C. longicollis* to determine the exact location of the phylogeographic break, and whether haplogroups overlap or have hard boundaries in this region.

Phylogeographic break between the Bass Province and the Eastern Province

We did not observe any gene flow between the Eastern Province and the Bass Province. These two freshwater bioregions showed the strongest signal of differentiation despite bordering each other in southern Australia and a continuous distribution of *C. longicollis* throughout. We predicted that *C. longicollis* would be insensitive to this freshwater bioregional boundary as the region is characterised by open lowlands, a habitat type over which gene flow should readily occur. Further, high population connectivity in *C. longicollis* is expected to have dominated this region during the last glacial cycle owing to the presence of the freshwater Lake Bass on the Pleistocene land-bridge between south-east Australia and the island of Tasmania (Blom and Alsop 1988). Unmack *et al.* (2012) suggest that divergence between the Bass Province and the Eastern Province in fish populations may have been maintained during Pleistocene aridity by limited floodplain connectivity surrounding Lake Bass and potentially high salinities in the lake itself. High regional aridity during the LGM coupled with severe localised salinisation in south-east Australia (Bowler *et al.* 2005) may have also limited the distribution of Haplogroups A and B of *C. longicollis*, and population connectivity over this region may have not yet recovered from earlier contractions. Sampling of geographically intermediate populations of *C. longicollis* in this region is necessary to ascertain the exact location and extent of this phylogeographic break.

Haplotype sharing demonstrates contemporary connectivity between bioregions

Chelodina longicollis populations are characterised by seven instances of haplotype sharing between major freshwater biogeographic regions. The geographic extent of haplotype sharing differs markedly between haplogroups with limited

distributions in Haplogroup A, and vast distributions in Haplogroup B. Shared haplotypes can be interpreted variously as evidence of contemporary gene flow, convergence, or the retention of ancestral haplotypes in disconnected populations. We recognise haplotype sharing as an indicator of very recent and potentially ongoing gene flow as all instances are characterised by geographic proximity and contemporary environmental conditions that promote connectivity.

Four cases of mitochondrial haplotype sharing between freshwater bioregions occur across lowland drainage divides. These characterise Haplogroup B and occur in the north-west and south-west MDB. Population connectivity in *C. longicollis* is expected at these locations owing to indistinguishable drainage divides and broad low plains that ensure hydrological connection during wet periods. Affinity between bioregions bordering the north-west MDB is demonstrated in many other freshwater taxa. Connectivity between the Condamine and Fitzroy–Dawson drainages is evident in golden perch (*Macquaria ambigua*) (Musyl and Keenan 1992; Faulks *et al.* 2010) and in populations of Midgley's carp gudgeon (*Hypseleotris* sp.) (Thacker *et al.* 2007). Faunal connections among the Burdekin, Warrego, and Cooper Creek drainages characterise eight species of freshwater fish (Unmack 2001; Thacker *et al.* 2007). Population connectivity in the south-west between the MDB and the Bass Province is demonstrated in sister lineages of the river blackfish (*Gadopsis marmoratus*) (Miller *et al.* 2004), Australian smelt (*Retropinna semoni*) (Hammer *et al.* 2007), subspecies of the freshwater crayfish (*Cherax destructor*) (Nguyen *et al.* 2004), and in populations of flathead gudgeon (*Philypnodon grandiceps*) (Thacker *et al.* 2008).

Three incidences of haplotype sharing occur across complex and relatively high-elevation landscapes (average elevation ~850 m above sea level). These characterise Haplogroup A and occur at the eastern boundary of the MDB and the Eastern Province. We propose that haplotype sharing represents very recent population expansion from the east to the west. This directionality is inferred as the shared haplotypes all belong to Haplogroup A, which has a strong affiliation with the Eastern Province and signatures of expansion from *in situ* refugia.

An ephemeral upland wetland complex encompassing the Clarence and Macleay drainages in the Eastern Province and the Border Rivers and Gwydir drainages in the MDB (Bell *et al.* 2008) may facilitate sharing of Haplotypes 2 and 4 over the Great Dividing Range. These wetlands formed during late Pleistocene glacial cycles (Haworth *et al.* 1999) and may explain the limited extent of these haplotypes in the MDB as *C. longicollis* populations in the Eastern Province were afforded the opportunity to expand eastward over the Great Dividing Range only very recently. Sharing of Haplotype 8 between the Eastern Province and the upland MDB is likely assisted by the Cassilis Gap at the headwaters of the Hunter drainage. The Cassilis Gap is a broad open valley and a well known biogeographic barrier to upland-forest-adapted fauna (Moussalli *et al.* 2005; Colgan *et al.* 2009; Chapple *et al.* 2011; Rix and Harvey 2012). The same landscape features that inhibit north–south dispersal in terrestrial species assist east–west dispersal in *C. longicollis* and a range of freshwater fish (Unmack 2001; Jerry 2008). A similar pattern characterises the Burdekin Gap in the Eastern Province. There, an arid corridor contributes to vicariance in terrestrial faunal lineages yet freshwater turtle species are relatively insensitive to the north–south 'gap' (Todd *et al.* 2014).

Recent mitochondrial gene flow in *C. longicollis* from the Eastern Province into the MDB may be also assisted by permanent water provided by farm dams. The upper reaches of drainages in the Eastern Province became major agricultural areas after European settlement in the 1800s, and saw the proliferation of privately owned dams. *C. longicollis* is abundant in these artificial permanent water bodies and it is possible that these new habitats assist contemporary populations to extend from the Eastern Province into the upland MDB. Furthermore, the recency of this habitat availability is consistent with the limited geographic extent of Haplogroup A in the MDB.

Conclusion

The longstanding biogeographic impediment of the Great Dividing Range, plus Pleistocene climate change, has significant influence on the recent evolutionary history of Australian freshwater taxa, and a far greater effect on *C. longicollis* than predicted. In contrast to expectations of insensitivity to barriers, we find east–west phylogeographic partitioning dating to the Miocene, and caused by the Great Dividing Range, which, on global standards, is of relatively low elevation. In contrast to predictions of panmixia we instead find signals of isolation by distance and diversity within each haplogroup shaped by diversification within, and limited connectivity among, multiple Pleistocene refugia.

Mitochondrial phylogeography of *C. longicollis* demonstrates that different evolutionary processes dominate at different times to create complex patterns of divergence and connectivity. Landscape history has driven ancient patterns of mitochondrial divergence and diversity, and overwhelmed life-history traits that could connect populations. Contemporary processes, however, have reinstated the influence of life history, with some populations dominated by dispersal and gene flow, leading to sympatry of haplogroups. As such, the eastern and western distributions of *C. longicollis* may be moving from divergence towards homogenisation as the convergent effects of gene flow between bioregions has a greater effect than the divergent effects of genetic drift between them.

Supplementary material

Additional supplementary material may be found in the online version of this article.

Acknowledgements

We thank colleagues who have contributed tissues to the University of Canberra Wildlife Tissue Collection (GenBank UC <Aus>) and we are grateful to many people who provided specimens and assisted in the field, in particular: Melanie Twidale, Olivier Baggiano, and Garry Peterson. This study was supported by an Australian Research Council Linkage Grant LP0560985 awarded to AG, SD and Mark Hutchinson with supplementary funding from the Murray–Darling Basin Authority, the University of Adelaide and the University of Canberra.

References

Austin, C., Nguyen, T. T., Meewan, M., and Jerry, D. R. (2003). The taxonomy and evolution of the "*Cherax destructor*" complex

(Decapoda: Parastacidae) re-examined using mitochondrial 16S sequences. *Australian Journal of Zoology* **51**, 99–110. doi:10.1071/ZO02054

Banarescu, P. 1990. 'Zoogeography of Fresh Waters. Volumes 1–3.' (AULAVerlag: Wiesbaden.)

Bell, D. M., Hunter, J. T., and Haworth, R. J. (2008). Montane lakes (lagoons) of the New England Tablelands Bioregion. *Cunninghamia* **10**, 475–492.

Blom, W. M., and Alsop, D. B. (1988). Carbonate mud sedimentation on a temperate shelf: Bass Basin, southeastern Australia. *Sedimentary Geology* **60**, 269–280. doi:10.1016/0037-0738(88)90124-8

Bower, D. S., and Hodges, K. (2014). *Chelodina expansa* Gray 1857 – broad-shelled turtle, giant snake-necked turtle. *Chelonian research monographs* **5**, 71.1–81.1.

Bowler, J. M., Kotsonis, A., and Lawrence, C. (2005). Environmental evolution of the mallee region, western Murray Basin. *Proceedings of the Royal Society of Victoria* **118**, 161–210.

Burridge, C. P., Craw, D., and Waters, J. M. (2007). An empirical test of freshwater vicariance via river capture. *Molecular Ecology* **16**, 1883–1895. doi:10.1111/J.1365-294X.2006.03196.X

Cann, J. (1998). 'Australian Freshwater Turtles.' (Beaumont Publishing: Singapore.)

Chapple, D. G., Chapple, S. N. J., and Thompson, M. B. (2011). Biogeographic barriers in south-eastern Australia drive phylogeographic divergence in the garden skink, *Lampropholis guichenoti*. *Journal of Biogeography* **38**, 1761–1775. doi:10.1111/J.1365-2699.2011.02531.X

Chessman, B. (1984*a*). Food of the snake-necked turtle, *Chelodina longicollis* (Shaw) (Testudines: Chelidae) in the Murray Valley, Victoria and New South Wales. *Australian Wildlife Research* **11**, 573–578. doi:10.1071/WR9840573

Chessman, B. (1984*b*). Evaporative water loss from three south-eastern Australian species of freshwater turtle. *Australian Journal of Zoology* **32**, 649–655. doi:10.1071/ZO9840649

Chessman, B. (1988). Habitat preferences of fresh-water turtles in the Murray Valley, Victoria and New South Wales. *Wildlife Research* **15**, 485–491. doi:10.1071/WR9880485

Colgan, D. J., O'Meally, D., and Sadlier, R. A. (2009). Phylogeographic patterns in reptiles on the New England tablelands at the south-western boundary of the McPherson Macleay overlap. *Australian Journal of Zoology* **57**, 317–328. doi:10.1071/ZO08088

Cook, B. D., Baker, A. M., Page, T. J., Grant, S., Fawcett, J. H., Hurwood, D. A., and Hughes, J. M. (2006). Biogeographic history of an Australian freshwater shrimp, *Paratya australiensis* (Atyidae): the role life history transition in phylogeographic diversification. *Molecular Ecology* **15**, 1083–1093. doi:10.1111/J.1365-294X.2006.02852.X

Costanzo, J. P., Iverson, J. B., Wright, M. F., and Lee, R. E. (1995). Cold hardiness and overwintering strategies of hatchlings in an assemblage of northern turtles. *Ecology* **76**, 1772–1785. doi:10.2307/1940709

Craw, D., Burridge, C. P., Upton, P., Rowe, D. L., and Waters, J. M. (2008). Evolution of biological dispersal corridors through a tectonically active mountain range in New Zealand. *Journal of Biogeography* **35**, 1790–1802. doi:10.1111/J.1365-2699.2008.01936.X

Drummond, A. J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214. doi:10.1186/1471-2148-7-214

Excoffier, L., and Lischer, H. (2010). Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–567. doi:10.1111/J.1755-0998.2010.02847.X

Faulks, L. K., Gilligan, D. M., and Beheregaray, L. B. (2008). Phylogeography of a threatened freshwater fish (*Mogurnda adspersa*) in eastern Australia: conservation implications. *Marine and Freshwater Research* **59**, 89–96. doi:10.1071/MF07167

Faulks, L. K., Gilligan, D. M., and Beheregaray, L. B. (2010). Clarifying an ambiguous evolutionary history: range-wide phylogeography of an Australian freshwater fish, the golden perch (*Macquaria ambigua*). *Journal of Biogeography* **37**, 1329–1340. doi:10.1111/J.1365-2699.2010.02304.X

Fielder, D., Vernes, K., Alacs, E., and Georges, A. (2012). Mitochondrial variation among Australian freshwater turtles (genus *Myuchelys*), with special reference to the endangered *M. bellii*. *Endangered Species Research* **17**, 63–71. doi:10.3354/ESR00417

Fu, Y. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **47**, 915–925.

Georges, A., and Thompson, M. B. (2010). Diversity of Australasian freshwater turtles, with an annotated synonymy and keys to species. *Zootaxa* **2496**, 1–37.

Georges, A., Zhang, X., Unmack, P., Le, M., and McCord, W. (2014). Contemporary genetic structure of an endemic freshwater turtle reflects Miocene orogenesis of New Guinea. *Biological Journal of the Linnean Society. Linnean Society of London* **111**, 192–208. doi:10.1111/BIJ.12176

Goode, J. 1967. 'Freshwater Tortoises of Australia and New Guinea.' (Lansdown Press: Melbourne.)

Hammer, M. P., Adams, M., Unmack, P. J., and Walker, K. F. (2007). A rethink on *Retropinna*: conservation implications of new taxa and significant genetic sub-structure in Australian smelts (Pisces: Retropinnidae). *Marine and Freshwater Research* **58**, 327–341. doi:10.1071/MF05258

Haworth, R. J., Gale, S., Short, S. A., and Heijnis, H. (1999). Land use and lake sedimentation on the New England Tablelands of New South Wales, Australia. *The Australian Geographer* **30**, 51–73. doi:10.1080/00049189993765

Hodges, K., Georges, A., and Donnellan, S. (2014). Phylogeography of the Australian freshwater turtle *Chelodina expansa* reveals complex relationships among inland and coastal bioregions. *Biological Journal of the Linnean Society. Linnean Society of London* **111**, 789–805. doi:10.1111/BIJ.12221

Hughes, J. M., and Hillyer, M. (2006). Mitochondrial DNA and allozymes reveal high dispersal abilities and historical movement across drainage boundaries in two species of freshwater fishes from inland rivers in Queensland, Australia. *Journal of Fish Biology* **68**, 270–291. doi:10.1111/J.0022-1112.2006.01073.X

Hughes, J. M., Schmidt, D. J., and Finn, D. S. (2009). Genes in streams: using DNA to understand the movement of freshwater fauna and their riverine habitat. *Bioscience* **59**, 573–583. doi:10.1525/BIO.2009.59.7.8

Hughes, J. M., Huey, J. A., and Schmidt, D. J. (2013). Is realised connectivity among populations of aquatic fauna predictable from potential connectivity? *Freshwater Biology* **58**, 951–966. doi:10.1111/FWB.12099

Irwin, D. E. (2002). Phylogeographic breaks without geographic barriers to gene flow. *Evolution* **56**, 2383–2394. doi:10.1111/J.0014-3820.2002.TB00164.X

Jerry, D. R. (2008). Phylogeography of the freshwater catfish *Tandanus tandanus* (Plotosidae): a model species to understand evolution of the eastern Australian freshwater fish fauna. *Marine and Freshwater Research* **59**, 351–360. doi:10.1071/MF07187

Jerry, D. R., and Cairns, S. C. (1998). Morphological variation in the catadromous Australian bass, from several geographically distinct riverine drainages. *Journal of Fish Biology* **52**, 829–843. doi:10.1111/J.1095-8649.1998.TB00823.X

Kennett, R., and Georges, A. (1990). Habitat utilization and its relationship to growth and reproduction of the eastern long-necked turtle, *Chelodina longicollis* (Testudinata: Chelidae), from Australia. *Herpetologica* **46**, 22–33.

Kennett, R., Roe, J., Hodges, K., and Georges, A. (2009). *Chelodina longicollis* (Shaw 1784) – eastern long-necked turtle, common long-necked

turtle, common snake-necked turtle. *Chelonian Research Monographs* **5**, 31.1–31.8.

Keogh, J. S., Scott, I., Fitzgerald, M., and Shine, R. (2003). Molecular phylogeny of the Australian venomous snake genus *Hoplocephalus* (Serpentes, Elapidae) and conservation genetics of the threatened *H. stephensii*. *Conservation Genetics* **4**, 57–65. doi:10.1023/A:1021823423944

Librado, P., and Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452. doi:10.1093/BIOINFORMATICS/BTP187

Masci, K. D., Ponniah, M., and Hughes, J. M. (2008). Patterns of connectivity between the Lake Eyre and Gulf drainages, Australia: a phylogeographic approach. *Marine and Freshwater Research* **59**, 751–760. doi:10.1071/MF07178

McGlashan, D. J., and Hughes, J. M. (2001). Genetic evidence for historical continuity between populations of the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae) east and west of the Great Dividing Range. *Journal of Fish Biology* **59**, 55–67. doi:10.1111/J.1095-8649.2001.TB01378.X

McGuigan, K., McDonald, K., Parris, K., and Moritz, C. (1998). Mitochondrial DNA diversity and historical biogeography of a wet forest-restricted frog (*Litoria pearsoniana*) from mid-east Australia. *Molecular Ecology* **7**, 175–186. doi:10.1046/J.1365-294X.1998.00329.X

Miller, A. D., Waggy, G., Ryan, S., and Austin, C. M. (2004). Mitochondrial 12S rRNA sequences support the existence of a third species of freshwater blackfish (Gadopsidae) from south-eastern Australia. *Memoirs of the Museum of Victoria* **61**, 121–127.

Moussalli, A., Hugall, A. F., and Moritz, C. (2005). A mitochondrial phylogeny of the rainforest skink genus *Saproscincus*, Wells and Wellington (1984). *Molecular Phylogenetics and Evolution* **34**, 190–202. doi:10.1016/J.YMPEV.2004.08.022

Murphy, N. P., and Austin, C. M. (2004). Phylogeography of the widespread Australian freshwater prawn, *Macrobrachium australiense* (Decapoda, Palaemonidae). *Journal of Biogeography* **31**, 1065–1072. doi:10.1111/J.1365-2699.2004.01105.X

Musyl, M. K., and Keenan, C. P. (1992). Population genetics and zoogeography of Australian freshwater golden perch, *Macquaria ambigua* (Richardson 1845) (Teleostei: Percichthyidae), and electrophoretic identification of a new species from the Lake Eyre basin. *Marine and Freshwater Research* **43**, 1585–1601. doi:10.1071/MF9921585

Musyl, M. K., and Keenan, C. P. (1996). Evidence for cryptic speciation in Australian freshwater eel-tailed catfish, *Tandanus tandanus* (Teleostei: Plotosidae). *Copeia* **1996**, 526–534. doi:10.2307/1447516

Nguyen, T. T. T., Austin, C. M., Meewan, M. M., Schultz, M. B., and Jerry, D. R. (2004). Phylogeography of the freshwater crayfish *Cherax destructor* Clark (Parastacidae) in inland Australia: historical fragmentation and recent range expansion. *Biological Journal of the Linnean Society. Linnean Society of London* **83**, 539–550. doi:10.1111/J.1095-8312.2004.00410.X

Nock, C. J., Elphinstone, M. S., Rowland, S. J., and Baverstock, P. R. (2010). Phylogenetics and revised taxonomy of the Australian freshwater cod genus, *Maccullochella* (Percichthyidae). *Marine and Freshwater Research* **61**, 980–991. doi:10.1071/MF09145

Obbard, M. E., and Brooks, R. J. (1981*a*). Fate of overwintered clutches of the common snapping turtle (*Chelydra serpentina*) in Algonquin Park, Ontario. *Canadian Field Naturalist* **95**, 350–352.

Obbard, M. E., and Brooks, R. J. (1981*b*). A radio telemetry and mark recapture study of activity in the common snapping turtle *Chelydra serpentina*. *Copeia* **1981**, 630–637. doi:10.2307/1444568

Page, T. J., and Hughes, J. M. (2014). Contrasting insights provided by single and multispecies data in a regional comparative phylogeographic study. *Biological Journal of the Linnean Society. Linnean Society of London* **111**, 554–569. doi:10.1111/BIJ.12231

Parmenter, C. J. (1976). The natural history of the Australian freshwater turtle *Chelodina longicollis* Shaw (Testudinata, Chelidae). Ph.D. Thesis. University of New England, Biddeford, ME.

Peakall, R., and Smouse, P. E. (2006). GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288–295. doi:10.1111/J.1471-8286.2005.01155.X

Posada, D., and Crandall, K. A. (2001). Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution* **16**, 37–45. doi:10.1016/S0169-5347(00)02026-7

Rabosky, D. L., Donnellan, S. C., Talaba, A. L., and Lovette, I. J. (2007). Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proceedings. Biological Sciences* **274**, 2915. doi:10.1098/RSPB.2007.0924

Rambaut, A., and Drummond, A. J. (2007). Tracer. Available at http://beast.bio.ed.ac.uk/tracer [Verified 14 February 2015].

Ramos-Onsins, S., and Rozas, J. (2002). Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution* **19**, 2092–2100. doi:10.1093/OXFORDJOURNALS.MOLBEV.A004034

Rix, M. G., and Harvey, M. S. (2012). Phylogeny and historical biogeography of ancient assassin spiders (Araneae: Archaeidae) in the Australian mesic zone: evidence for Miocene speciation within Tertiary refugia. *Molecular Phylogenetics and Evolution* **62**, 375–396. doi:10.1016/J.YMPEV.2011.10.009

Roe, J. H. (2008). *Chelodina longicollis* (eastern long-necked turtle): drinking behaviour. *Herpetological Review* **39**, 212–213.

Roe, J., and Georges, A. (2007). Heterogeneous wetland complexes, buffer zones, and travel corridors: landscape management for freshwater reptiles. *Biological Conservation* **135**, 67–76. doi:10.1016/J.BIOCON.2006.09.019

Roe, J. H., and Georges, A. (2008). Terrestrial activity, movements and spatial ecology of an Australian freshwater turtle, *Chelodina longicollis*, in a temporally dynamic wetland system. *Austral Ecology* **33**, 1045–1056. doi:10.1111/J.1442-9993.2008.01877.X

Roe, J. H., Georges, A., and Green, B. (2008). Energy and water flux during terrestrial estivation and overland movement in a freshwater turtle. *Physiological and Biochemical Zoology* **81**, 570–583. doi:10.1086/589840

Rogers, L. J. (1966). The nitrogen excretion of *Chelodina longicollis* under conditions of hydration and dehydration. *Comparative Biochemistry and Physiology* **18**, 249–260. doi:10.1016/0010-406X(66)90184-8

Rowland, S. J. (1993). *Maccullochella ikei*, an endangered species of freshwater cod (Pisces: Percicthyidae) from the Clarence River system, NSW and *M. peeli mariensis*, a new subspecies from the Mary River system, Qld. *Records of the Australian Museum* **45**, 121–145. doi:10.3853/J.0067-1975.45.1993.132

Ruzzante, D. E., Walde, S. J., Macchi, P., Alonso, M., and Barriga, J. (2011). Phylogeography and phenotypic diversification in the Patagonian fish *Percichthys trucha*: the roles of Quaternary glacial cycles and natural selection. *Biological Journal of the Linnean Society. Linnean Society of London* **103**, 514–529. doi:10.1111/J.1095-8312.2011.01682.X

Šlechtová, V., Bohlen, J., Freyhof, J., Persat, H., and Delmastro, G. B. (2004). The Alps as barrier to dispersal in cold-adapted freshwater fishes? Phylogeographic history and taxonomic status of the bullhead in the Adriatic freshwater drainage. *Molecular Phylogenetics and Evolution* **33**, 225–239. doi:10.1016/J.YMPEV.2004.05.005

Smissen, P. J., Melville, J., Sumner, J., and Jessop, T. S. (2013). Mountain barriers and river conduits: phylogeographical structure in a large, mobile lizard (Varanidae: *Varanus varius*) from eastern Australia. *Journal of Biogeography* **40**, 1729–1740. doi:10.1111/JBI.12128

Swofford, D. L. (2002). 'PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10.' (Sinauer Associates: Sunderland, MA.)

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–595.

Thacker, C. E., Unmack, P. J., Matsui, L., and Rifenbark, N. (2007). Comparative phylogeography of five sympatric *Hypseleotris* species (Teleostei: Eleotridae) in south-eastern Australia reveals a complex pattern of drainage basin exchanges with little congruence across species. *Journal of Biogeography* **34**, 1518–1533. doi:10.1111/J.1365-2699.2007.01711.X

Thacker, C. E., Unmack, P. J., Matsui, L., Duong, P., and Huang, E. (2008). Phylogeography of *Philypnodon* species (Teleostei: Eleotridae) across south-eastern Australia: testing patterns of connectivity across drainage divides and among coastal rivers. *Biological Journal of the Linnean Society. Linnean Society of London* **95**, 175–192. doi:10.1111/J.1095-8312.2008.01000.X

Thompson, J., Gibson, T., Plewniak, F., Jeanmougin, F., and Higgins, D. (1997). The CLUSTAL_X windows interface. Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4882. doi:10.1093/NAR/25.24.4876

Todd, E. V., Blair, D., Farley, S., Farrington, L., FitzSimmons, N. N., Georges, A., Limpus, C. J., and Jerry, D. R. (2013). Contemporary genetic structure reflects historical drainage isolation in an Australian snapping turtle, *Elseya albagula*. *Zoological Journal of the Linnean Society* **169**, 200–214. doi:10.1111/ZOJ.12049

Todd, E. V., Blair, D., and Jerry, D. R. (2014). Influence of drainage divides versus arid corridors on genetic structure and demography of a widespread freshwater turtle, *Emydura macquarii krefftii*, from Australia. *Ecology and Evolution* **4**, 606–622. doi:10.1002/ECE3.968

Unmack, P. J. (2001). Biogeography of Australian freshwater fishes. *Journal of Biogeography* **28**, 1053–1089. doi:10.1046/J.1365-2699.2001.00615.X

Unmack, P. J., and Dowling, T. E. (2010). Biogeography of the genus *Craterocephalus* (Teleostei: Atherinidae) in Australia. *Molecular Phylogenetics and Evolution* **55**, 968–984. doi:10.1016/J.YMPEV.2010.02.019

Unmack, P. J., Bagley, J. C., Adams, M., Hammer, M. P., and Johnson, J. B. (2012). Molecular phylogeny and phylogeography of the Australian freshwater fish genus *Galaxiella*, with an emphasis on dwarf galaxias (*G. pusilla*). *PLoS ONE* **7**, e38433. doi:10.1371/JOURNAL.PONE.0038433

Walker, D. E., and Avise, J. C. (1998). Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. *Annual Review of Ecology and Systematics* **29**, 23–58. doi:10.1146/ANNUREV.ECOLSYS.29.1.23

Wellman, P. (1979). On the cainozoic uplift of the southeastern Australian highland. *Journal of the Geological Society of Australia* **26**, 1–9. doi:10.1080/00167617908729061

Zamudio, K. R., and Greene, H. W. (1997). Phylogeography of the bushmaster (*Lachesis tnuta*: Viperidae): implications for neotropical biogeography, systematics, and conservation. *Biological Journal of the Linnean Society. Linnean Society of London* **62**, 421–442. doi:10.1111/J.1095-8312.1997.TB01634.X