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Environmental change as a driver of diversification in temporary aquatic habitats: does the genetic structure of extant fairy shrimp populations reflect historic aridification?

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SUMMARY

1. Over the past 65 my, the Australian continent experienced a pronounced shift from predominantly wet, tropical, conditions to a much drier climate. Little is known, however, about the effect of this important continent-wide event on freshwater organisms and ecosystems. Fairy shrimps (Crustacea; Anostraca) are ancient and specialist inhabitants of temporary and saline aquatic habitats that typically prevail under semiarid conditions. Therefore, they present suitable evolutionary models to study scenarios of historic environmental change and the impact of a drying climate on aquatic ecosystems in particular.

2. Focussing on both macro- and micro-evolution in the fairy shrimp genus *Branchinella* and using mitochondrial DNA data (16S and COI), we evaluated whether patterns of contemporary genetic variation reflect historic climate change.

3. There is a close match between episodes of Cenozoic climate change and macro-evolutionary diversification in Australian fairy shrimps, presumably mediated by a progressive increase in the abundance and diversity of temporary aquatic habitats on the continent. Micro-evolutionary patterns reflect both range expansion and recent contraction, linked to extreme drying events during the Pleistocene glacial periods.

4. This study effectively illustrates the potential long-term effects of environmental change on the diversity and the evolutionary trajectories of the fauna of temporary waters. Moreover, it demonstrates the importance of adaptation to new environments and non-adaptive processes, such as divergence in isolation, for explaining extant diversity patterns in this particular environment.

Keywords: Anostraca, Australia, climate change, habitat shift, molecular phylogeny

Introduction

Throughout the earth's history, the global climate has been characterised by significant variations, usually occurring as oscillations (Zachos *et al.*, 2001; Ruddiman, 2008), which affect the distribution of species and genetic lineages (Byrne *et al.*, 2008; Mayhew, Jenkins & Benton, 2008; Finarelli & Badgley, 2010). Accordingly, both the fossil record and the phylogeny, phylogeography and genetic population structure of contemporary genera and species often reflect episodes of historic climate change (Larmuseau *et al.*, 2009a,b; Vanhove *et al.*, 2012). Well-characterised examples of this include the hot conditions in the middle Cretaceous, the early Eocene 'greenhouse earth', the Oligocene 'icehouse earth' and the Quaternary ice ages (Hewitt, 2000, 2004; Wilson & Norris, 2001; MacFadden, 2006). Although periods of environmental change are frequently linked to species

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extinctions, they may also provide opportunities by creating new niches. These can be filled either as a result of dispersal and range expansion of pre-adapted species or lineages, or through local adaptation and adaptive radiation (Crowley & North, 1988; Gillespie, 2004; Mayhew *et al.*, 2008).

The Australian biogeographical region is a prime example of an area that has experienced pronounced environmental change in its relatively recent geological history (Hill, 1994; Martin, 2006; Byrne *et al.*, 2008). Between 65 and 45 mya (million years ago), Australia detached from Antarctica and began to drift northwards. At that time, the continent was almost entirely covered by rainforest, and conditions were warm with an average annual temperature of around 20 °C and wet with an average annual precipitation (AAP) between 1200 and 2400 mm. Today, about two-thirds of the land surface area is covered with shrub- and woodland vegetation and is classified as either arid or semiarid (AAP < 500 mm) (Hopper & Gioia, 2004; Sniderman *et al.*, 2007) with only a few wet areas remaining in Tasmania, in parts of the continental east coast and the north.

The transition from tropical to arid conditions can be roughly divided into three main episodes. The first involved the establishment of arid conditions (AAP < 500 mm) throughout most of north-west Australia from the Palaeocene (65.5–55.8 mya) until the mid-Miocene (65.5–15.0 mya) and with seasonal and semiarid conditions expanding into central Australia and later, near the end of the Oligocene (25.0–23.0 mya), to large parts of Western and eastern Australia (Quilty & Telfer, 1994; Martin, 2006). In a second phase (15.0–5.3 mya), arid conditions developed in most of the areas that currently make up the arid zone (Byrne *et al.*, 2008). Finally, from the late Pliocene-Pleistocene onwards (3.0 mya-present), cyclical glacial events resulted in major oscillations in precipitation regime. With a probable overall decrease in precipitation throughout the continent of around 40%, notably around 0.5–0.4 mya, this third episode can be considered a period of extreme aridification (Fujioka *et al.*, 2005; Martin, 2006).

Although the impact of these episodes of environmental change on the Australian continent has been explored to various extent in a number of plants, terrestrial invertebrates and vertebrates (Byrne *et al.*, 2008), very little is known about how these changes affected aquatic ecosystems. Transitions to drier conditions and less predictable rainfall patterns can result in changes in the abundance and nature of inland waters (Wellborn, Skelly & Werner, 1996). Aridification may, for instance, lead to salinisation of fresh waters (Alley, 1998) and a general shift from permanent to episodic aquatic habitats (Street & Grove, 1979; Williams, 2006). As a result, distinct aquatic communities develop, dominated by species that can bridge dry periods or saline conditions (Williams, 2006). For instance, large branchiopod crustaceans (fairy shrimp: Anostraca, tadpole shrimp: Notostraca and clam shrimp: Spinicaudata, Laevicaudata and Cyclestherida) produce dormant eggs resistant to desiccation (Dumont & Negrea, 2002; Brendonck & De Meester, 2003; Brendonck *et al.*, 2008).

After the radiation of early jawed fishes (Mesozoic marine revolution), which resulted in highly effective predators, large branchiopods became restricted to fishless waters (Kerfoot & Lynch, 1987). Temporary or saline habitats provided refuges for these predation-sensitive organisms (Pennak, 1953). Therefore, from the second half of the Mesozoic onwards, large branchiopod diversity is thought to be strongly linked to the prevalence of temporary and saline aquatic systems, which are especially abundant in arid and semiarid regions (Eriksen & Belk, 1999; Brendonck *et al.*, 2008). The Australian fairy shrimp fauna is relatively well studied and highly diverse (Timms, 2012). About 15% of all described fairy shrimps are endemic to Australia. Australia, and especially Western Australia and the Paroo Area, is therefore recognised as a biodiversity hot spot for fairy shrimps (Timms & Richter, 2002; Timms & Sanders, 2002). Of these Australian species, about 60% (35 species) belong to the ecologically and morphologically diverse Thamnocephalid genus *Branchinella* (Timms, 2012). A recent phylogenetic and taxonomic review of the Australian *Branchinella* revealed substantial levels of cryptic diversity (Pinceel *et al.*, 2013), although the link between diversification and speciation events and Australia's history of environmental change remains unexplored. Currently, *Branchinella* species occupy almost the entire range of temporary aquatic habitats found on the Australian continent. As specialist inhabitants of these systems, fairy shrimps present a suitable evolutionary model to study the relation between historic environmental change (e.g. transition of permanent to temporary aquatic habitats) and both macro- and micro-evolutionary responses such as diversification, genetic bottlenecks and range expansions and contractions.

Here, we investigated whether current continent-wide genetic variation of *Branchinella* in Australia reflects historical episodes of climate and environmental change. We considered two mitochondrial (16S rDNA and COI) genes to detect signals of historic climate change, both at macro-evolutionary (within genus) and at micro-evolutionary (within species) scales.

To test the hypothesis that patterns of aridification coincide with periods of diversification in *Branchinella*, we used tMRCA analyses (time to Most Recent Common Ancestor) and clock-constrained phylogenetic trees to reconstruct the temporal pattern of diversification in *Branchinella* (macro-evolutionary scale) and performed constraint analyses to assess the importance of adaptive (habitat- and trophic shifts) and non-adaptive processes (habitat availability, isolation) fuelling diversification in this genus. Aridification may also change the variety and lifespan of waterbodies, providing opportunities for species to invade new habitat types. To test this, we use ecological data, molecular rates of evolution and constraint analyses to detect and date niche shifts of fairy shrimps from freshwater systems to typical arid aquatic systems, such as salt pans and rock pools. Additionally, since a species-level resolution is too coarse to detect the impact of relatively recent events, we attempt to reconstruct micro-evolutionary patterns within the widespread and morphologically diverse *Branchinella longirostris*, a species endemic to ephemeral rock pool habitats in south-western Australia. Since rock pools, which are typically shallow and short-lived, are among the first aquatic habitats affected by climate change (Hulsmans *et al*., 2008), the species is particularly suitable to assess the impact of relatively recent (Pliocene-Pleistocene) events on genetic diversity and demography. Here, we hypothesised that, although ongoing aridification has probably resulted in range expansion, more recent shifts to extreme conditions with unpredictable rainfall and short inundations would be reflected in a genetic bottleneck.

Methods

Model organisms and study system

Australian *Branchinella* exhibit substantial variation both in phenotypic traits such as body size (8–60 mm), eye size and egg size (113–565 μm), and in the habitat types occupied (Geddes, 1981; Timms, 2004, 2009; Timms & Lindsay, 2011). *Branchinella* is known from all sorts of small (<2 ha), shallow (<1 m) temporary aquatic systems ranging from ephemeral puddles and rock pools to larger vernal ponds, salt pans, claypans and temporary creeks across broad ranges of temperature and salinity (0–62 g L^{-1}) that may hold water from 1 up to 10 months (Timms, 2009; Pinceel *et al*., 2013).

Since the autecology of most *Branchinella* species is reasonably well documented (Timms, 2012), they can be reliably categorised by their habitat preference and diet. Two larger (typically 50–60 mm) species, *B. australiensis* and *B. occidentalis*, are predators (Timms, 2004), consuming smaller zooplankton, while the remaining species are opportunistic filter feeders. Additionally, most *Branchinella* species occur exclusively in certain habitat types. In our analyses, we considered a species a specialist of saline or turbid habitats when it occurs exclusively in habitats characterised by a salinity of >3 g L^{-1} (Hammer, 1986) or a Secchi depth of <2 cm, respectively. A species is considered a rock pool specialist when it does not occur in any other habitat type.

Currently, barren granite outcrops (inselbergs) that hold temporary rock pool habitats are typical and relatively abundant landforms in the drier regions of Australia (Withers, 2000). Although the granite itself is 2500 my old, the inselberg domes were only exposed through erosion around 50–100 mya (Bourne & Twidale, 2000). Granite outcrops in tropical and moist areas typically lack temporary pools, since they are covered by topsoil and lush vegetation (Porembski & Barthlott, 2000). This was probably also the case for the majority of inselbergs in the Australian subtropics until about 15.0–5.3 mya. Exposed to a drier climate, vegetation is typically lost, topsoil is removed due to erosion and weathered depressions are exposed that can develop into temporary rock pools (Twidale & Sved, 1978; Twidale, 2007) of various depths (3 to at least 50 cm), which may be colonised by specialised fauna such as fairy shrimps (Pinder *et al*., 2000; Timms, 2006; Vanschoenwinkel *et al*., 2011). Due to their small water volume and the fact that precipitation is the only water source, while evaporation is the main source of water loss, temporary rock pools are particularly responsive to climate-driven (rainfall, temperature) changes in hydroperiod, as shown using realistic simulation models (Hulsmans *et al*., 2008; Vanschoenwinkel *et al*., 2009). As a result, the genetic structure of species endemic to these systems is probably more likely to reflect recent climate events than the genetic structure of other species inhabiting more stable habitats. One of the dominant competitors in this habitat is the fairy shrimp *B. longirostris*, which is endemic to Western Australia (Timms, 2002) and which was the subject of a study by Zofkova & Timms (2009) who uncovered a conflict in morphological and genetic patterns within the species.

Sample information

All *Branchinella* specimens were collected by the authors over the period 1985–2009 using standard sampling

equipment (dip or kick nets). The *B. longirostris* specimens were collected in 2009. The 16S *Branchinella* data set comprising 34 evolutionary lineages (see Table 1) has also served as the basis for a molecular taxonomic revision of the genus in Australia (Pinceel *et al.*, 2013). Locality data on the newly sequenced *B. longirostris* inselberg (meta) populations along with GenBank accession numbers (total: 236 specimens) are in Table 2). We assembled a genetic data set of up to 18 *B. longirostris* specimens from a total of 32 inselbergs. Of these 32 inselbergs, 12 inselbergs along the dominant north–south axis of the species' range were represented by at least 10 and up to 18 specimens from 10 different pools on each inselberg (total: 192 specimens; Table 2) yielding regional *B. longirostris* samples. This data set encompasses the entire range of the species and includes most major inselbergs in the area.

DNA isolation, molecular markers, polymerase chain reaction and sequencing

Branchinella specimens were preserved in absolute ethanol. Specimens were dissected to obtain phyllopod tissue for DNA extraction. Genomic DNA was extracted from tissue using the NucleoSpin® extraction kit for individual samples (Macherey-Nagel, Düren, Germany). Details on the assembly of the 16S *Branchinella* data set are presented by Pinceel *et al.* (2013). The mitochondrial COI DNA region of *B. longirostris* was amplified using the forward (5′ GGT CAA CAA ATC ATA AAG ATA TTG G 3′) and reverse (5′ TAA ACT TCA GGG TGA CCA AAA AAT CA 3′) primers used by Folmer *et al.* (1994).

The PCR volume of 25 μL contained 2 μL of template DNA, 2 mM MgCl2, 1 mM 10× reaction buffer, 0.2 mM dNTPs, 0.4 μM of each primer and 1.1U TAQ polymerase.

Table 1 Overview and specifications of the sequenced *Branchinella* specimens and sequences drawn from GenBank (modified from Pinceel *et al.*, 2013)

	Species	Site	GenBank accession numbers
1	*B. affinis* Linder, 1941	Paroo area, NSW & Pandie Pandie, SA	AF308942.1/AF527568.1
2	*B. arborea* Geddes, 1981	Paroo area, NSW	AF308945.1
3	*B. australiensis* Richters, 1876	Paroo area, NSW & Claremont, Qld	AF527556-7.1
4	*B. basispina* Geddes, 1981	Balladonia Rock, WA	JN812774
5	*B. buchananensis* Geddes, 1981	Bloodwood station, NSW	JN812775
6	*B. buchananensis*	Paroo-Gidgee Lake, Bloodwood station, NSW	AF308943.1, AF527562.1
7	*B. budjiti* Timms, 2001	Paroo area, NSW & Warburton Crossing, SA	AF527563-67.1
8	*B. campbelli* Timms, 2001	Paroo area, NSW	AF527576.1
9	*B. clandestina* Timms, 2005	Paroo river, Currawinya National Park, Qld	JN812776
10	*B. compacta* Linder, 1941	Avon Lake, Monaro, NSW	JN812772
11	*B. complexidigitata* Timms, 2002	Lake Logue, WA	JN812777
12	*B. frondosa* Henry, 1924	Rockwell, Qld	AF308941.1
13	*B. halsei* Timms, 2002	Lake Cronin, WA	JN812778
14	*B. hattahensis* Geddes, 1981	Kaponyee Lake, Currawinya National Park, Qld	AF308944.1
15	*B. kadjikadji* Timms, 2002	Kadji Kadji Station, WA	AF527569.1
16	*B. lamellata* Timms & Geddes, 2003	Clifton Hills Station, SA	JN812783
17	*B. longirostris* Wolf, 1911	King Rocks, WA	AF527575.1
18	*B. lyrifera* Linder, 1941	Bloodwood Station, NSW	JN812779
19	*B. lyrifera*	Kaponyee Lake, Qld & Bobbiemongie, SA	AF527559-61.1/AF308940.1
20	*B. mcraeae* Timms, 2005	Onslow, WA	JN812784
21	*B. nichollsi* Linder, 1941	Kalgoorlie, WA	JN812771
22	*B. occidentalis* Dakin, 1914	Paroo area, NSW & Nilpinna, SA	AF308947.1/AF527558.1
23	*B. papillata* Timms, 2008	Kau Nature Reserve, WA	JN812782
24	*B. pinderi* Timms, 2008	Onslow, WA	JN812785
25	*B. pinnata* Geddes, 1981	Paroo area, NSW & Rockwell, Qld	AF308946.1/AF527570.1
26	*B. proboscida* Henry, 1924	Paroo area, NSW	AF527574.1
27	*B. simplex* Linder, 1941	Cue, WA	JN812780
28	*B. tyleri* Timms & Geddes, 2003	Victoria River, NT	JN812781
29	*B. wellardi* Milner, 1929	Bloodwood Station, NSW	JN812788
30	*B. wellardi*	Paroo area, NSW	AF527571.1
31	*B.* sp. *nov. U*	Moora, WA	JN812787
32	*B.* sp. *nov. S*	Claremont, Qld	AF527572.1
33	*B.* sp. *nov. M*	Moora, WA	JN812773
34	*B.* sp. *nov. Y*	Yarromere Station, Qld	JN812786

Table 2 Overview of the sampled *Branchinella longirostris* metapopulations along with locality data. The coordinates (latitude, S and longitude, E), location of the Nearest Weather Station (NSW), the mean Average Annual Precipitation (AAP) and the mean average annual minimum and maximum temperatures [T(min-max)] are specified for each sampling site. A unique ID for every inselberg is defined between brackets. Sampling size (*N*) and the number of identified unique haplotypes (*R*) are specified for each metapopulation as well. Climate data are based upon monthly measurements from 1907 to 2009 and were obtained from the Bureau of Meteorology of the Australian Government; www.bom.gov.au

Site name (site ID)	Longitude				Latitude				NWS	AAP	T(min–max)	N	R
Walloo Hill (WO)	S	27°	14′	49″	E	117°	25′	44″	Cue	231.4	14.7–28.4	18	2
Walga Rock (WA)	S	27°	24′	16″	E	117°	27′	46″	Cue	231.4	14.7–28.4	18	4
Ballan Rock (BA)	S	28°	10′	51″	E	117°	25′	32″	Mt Magnet	238.7	14.2–28.6	2	1
Bullamanya Rocks (PA)	S	29°	10′	18″	E	117°	39′	44″	Paynes Find	279.3	12.8–27.7	15	3
Wardagga Rock (WD)	S	29°	23′	17″	E	117°	30′	21″	Paynes Find	279.3	12.8–27.7	18	7
Wanarra Rock (WN)	S	29°	31′	–	E	116°	48′	–	Paynes Find	279.3	12.8–27.7	2	2
Old Rainy Rocks (OL)	S	29°	44′	–	E	119°	37′	–	Menzies	249.8	12.6–26.3	2	1
Hospital Rocks (HO)	S	29°	50′	–	E	120°	7′	–	Menzies	249.8	12.6–26.3	2	1
Old Remplap Rocks (RE)	S	30°	2′	4″	E	117°	37′	53″	Paynes Find	279.3	12.8–27.7	16	3
Xantippe Rocks (XA)	S	30°	17′	–	E	116°	58′	–	Paynes Find	279.3	12.8–27.7	2	1
Elachbutting Rock (EL)	S	30°	35′	34″	E	118°	36′	39″	Merredin	326.4	11.3–24.9	16	3
Yanneymooning Rock (YA)	S	30°	42′	55″	E	118°	33′	20″	Merredin	326.4	11.3–24.9	16	3
Baladjie Rock (BE)	S	30°	57′	12″	E	118°	52′	52″	Merredin	326.4	11.3–24.9	18	5
Yarragin Rock (YN)	S	31°	2′	–	E	117°	57′	–	Merredin	326.4	11.3–24.9	1	1
Weowannie Rocks (WW)	S	31°	8′	–	E	119°	45′	–	Southern Cross	294.5	11.3–24.9	2	1
Moorine Rock (MO)	S	31°	13′	–	E	118°	59′	–	Southern Cross	294.5	10.7–25.5	2	1
Sandford Rock (SA)	S	31°	14′	–	E	118°	46′	–	Southern Cross	294.5	10.7–25.5	2	1
Yorkrakine Rock (YO)	S	31°	25′	–	E	117°	30′	–	Merredin	326.4	11.3–24.9	2	1
Strawberry Rock (ST)	S	31°	27′	17″	E	119°	16′	59″	Southern Cross	294.5	10.7–25.5	16	2
Jilbadgie Rock (JI)	S	31°	29′	–	E	119°	12′	–	Southern Cross	294.5	10.7–25.5	18	6
Frog Rock (FR)	S	31°	30′	–	E	119°	14′	–	Southern Cross	294.5	10.7–25.5	2	2
Borayukkin Rock (BO)	S	31°	55′	26″	E	118°	46′	48″	Narembeen	335	10.5–25.0	3	1
McDermid Rocks (MD)	S	32°	2′	–	E	120°	43′	–	Norseman	289	10.6–24.6	2	1
Mt Walker (WR)	S	32°	4′	0.3″	E	118°	45′	14″	Hyden	343.6	9.8–24.8	13	2
Newman Rocks (NE)	S	32°	7′	–	E	123°	10′	–	Balladonia	262	9.8–24.7	2	1
Anderson Rocks (AN)	S	32°	10′	16″	E	118°	51′	–	Hyden	343.6	9.8–24.8	5	3
Bushfire Rocks (BU)	S	32°	26′	–	E	119°	20′	–	Hyden	343.6	9.8–24.8	2	1
Wave Rock (WE)	S	32°	26′	45″	E	118°	54′	11″	Hyden	343.6	9.8–24.8	3	1
McPherson Rocks (MC)	S	32°	27′	–	E	121°	40′	–	Norseman	289	10.6–24.6	2	1
Graham Rock (GR)	S	32°	27′	34″	E	119°	3′	8″	Hyden	343.6	9.8–24.8	10	1
Lilian Stokes Rocks (LI)	S	33°	4′	–	E	120°	6′	–	Newdgate	369.3	8.7–23.2	2	1
Mt Madden (MA)	S	33°	14′	20″	E	119°	50′	45″	Newdgate	369.3	8.7–23.2	2	1

The cycle settings were modified from Adamowicz, Hebert & Marinone (2004) with an initial denaturation of 3 min at 94 °C, followed by five liberal amplification cycles (denaturation for 1 min at 94 °C, annealing for 1.5 min at 45 °C and elongation for 1.5 min at 72 °C) and 35 more rigid cycles (denaturation for 1 min at 94 °C, annealing for 1.5 min at 50 °C and elongation for 1.5 min at 72 °C), followed by a final elongation of 6 min at 72 °C. Reaction contaminants were removed from the samples using the NucleoFast® 96 PCR Clean-Up kit (Macherey-Nagel). Samples were sequenced with the help of the Big Dye Terminator 3.1 kit (Applied Biosystems, Gent, Belgium), following a 1/8 dilution of the Big Dye Terminator sequencing protocol, using the same primers. Finally, the products were run on an ABI PRISM 3130 Avant Genetic Analyser automated sequencer (Applied Biosystems). Consensus sequences were constructed and checked for quality in SeqScape v.2.5 (Applied Biosystems).

Phylogenetic and phylogeographical reconstructions

Two hundred and thirty-six newly obtained *B. longirostris* sequences, representing 37 unique haplotypes (GenBank accession codes: KC335159–KC335195) from 12 locations (inselbergs), were aligned (ClustalW multiple alignment) (Higgins *et al.*, 1994) and trimmed to a length of 535 bp in BioEdit Sequence Alignment Editor v.7.0.0 (Hall, 1999). Twenty-eight *B. longirostris* COI sequences from Zofkova & Timms (2009), all representing unique haplotypes from 19 additional locations, were drawn from GenBank and aligned to the newly obtained DNA

fragments (Table 2 gives additional details and GenBank accession codes).

The use of COI to reconstruct the evolutionary history of *Branchinella* at the generic level was also evaluated. Mutation rates on this gene were too high, however, to render any robust reconstructions for the evolutionary relations between these distantly related species. Finally, an 18S gene fragment of approximately one kilobase was obtained for a subset of 20 taxa, but mutation rates were too low (uncorrected *P*-distance: 0–1.6%) to render robust phylogenetic reconstructions; hence, these sequences were excluded from further analyses.

The anostracan *Branchinella affinis* was selected as an outgroup and aligned to the *B. longirostris* COI data as it was used as outgroup in a previous genetic study on *B. longirostris* (Zofkova & Timms, 2009). The alignment quality was checked by eye and in trimAl (Capella-Gutierrez, Silla-Martinez & Gabaldon, 2009). jModeltest 0.1.1 (Posada, 2008) selected the TPM2uf + I + G (I = 0.3930, G = 0.5100) model with nucleotide frequencies A = 0.26139, C = 0.21111, G = 0.17414 and T = 0.35336 and rate matrix (1.9808 22.7268 1.9808 1.0000 22.7268 1.0000) as best fitting model of evolution for the COI *B. longirostris* data set. Model-averaged phylogenetic analyses in the same software indicated that all 88 models tested rendered identical trees. Substitution saturation was tested using DAMBE 5.2.13 (Xia *et al.*, 2003; Xia & Lemey, 2009). The index of substitution saturation (Iss) was found to be significantly smaller than its critical value (Iss c), indicating little saturation. Genetic Kimura two-parameter (K2P) distances (Kimura, 1980) were computed using MEGA v. 4.1 (Tamura *et al.*, 2007). Phylogenetic analyses for the *Branchinella* 16S data set were carried out as specified in the study by Pinceel *et al.* (2013). maximum parsimony (MP) analyses were conducted in PAUP* 4.0b10 (Swofford, 2000) using the PaupUp graphical interface (Calendini & Martin, 2005). Maximum likelihood (ML) analyses were run in PhyML (Guindon *et al.*, 2005) and Quartet puzzling (QP) maximum likelihood analyses in TREE-PUZZLE (Schmidt *et al.*, 2002), each time according to the model and parameters as selected by jModeltest. Neighbour-joining (NJ) analyses were performed in MEGA v. 4.1 using the following settings: Maximum composite likelihood, defined G = 0.5100 and 1000 bootstrap replicates. For BI analyses, MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) was used. MrBayes ran for 3×10^6 generations (lset number of substitution types = 6, rates = invgamma, number of rate categories for the gamma distribution = 4) with a defined outgroup (*B. affinis*) and a sampling frequency of 100 generations. To include only trees in which the convergence of the Markov chain had been reached, we defined a burn-in of 25%. The remaining trees were used to construct a 50% majority consensus tree. For the *B. longirostris* COI data set, only ML and BI were used since the combination of both methods produced a robust tree topology and it is possible to run both searches according to a uniform molecular clock.

Constraint analyses were performed in PAUP* on the 16S *Branchinella* data set to evaluate whether the observed niche shifts in the genus *Branchinella*, which include habitat shifts from fresh water to saline systems and dietary shifts from filter feeding to predation, occurred only once in a common ancestor or a number of times independently throughout the evolutionary history of the genus. Both Kishino–Hasegawa (Kishino & Hasegawa, 1989) and Shimodaira–Hasegawa (Shimodaira & Hasegawa, 1999) tests for the ML tree and Kishino–Hasegawa and Templeton (Wilcoxon signed-ranks) and winning site tests for the MP tree were conducted to test whether enforcing a single niche shift (grouping species that live in saline systems, grouping all predatory species) resulted in a significantly more likely or more parsimonious tree.

NETWORK v. 4.6.0.0 (Bandelt, Forster & Röhl, 1999) was used to construct a statistical parsimony network of the *B. longirostris* haplotypes. The network topology was also evaluated using TCS v. 1.3 (Clement, Posada & Crandall, 2000).

Molecular clock and evolutionary time-frame

Gene genealogies can be used to reconstruct historic patterns of diversification by applying molecular clocks. For such methods to render truly robust results, the use of internal fossil calibration points or well-dated historic geological events has been recommended (Ho & Phillips, 2009). Fairy shrimps are poorly represented in the fossil record, however, and there are no fossils available of *Branchinella* or any closely related genera which would allow for accurate calibration. Moreover, in freshwater zooplankton, the possibility of intercontinental long-distance dispersal mediated by avian vectors prevents the use of geological events, such as continental break-up, to calibrate molecular clocks (Vanschoenwinkel *et al.*, 2012). It is therefore only possible to obtain estimates of the timing of evolutionary events by applying a broad range of mutation rates, calibrated for related organisms. In arthropods, COI was shown to mutate at 1.2% per million years in beetles (Caccone & Sbordoni, 2001), which is the slowest

evolutionary rate identified. Intermediate mutation rates of around 1.4% per million years were found in snapping shrimps (Knowlton & Weigt, 1998), while pairwise divergence rates of 1.66–2.33% per million years were found in crabs (Schubart, Diesel & Hedges, 1998). Although Papadopoulou, Anastasiou & Vogler (2010) found indications for even higher COI mutation rates (3.54% per million years) in insects, we chose to use the 2.33% per million years identified by Schubart *et al.* (1998) as a realistic upper bound since their clock was calibrated for crabs, which are also crustaceans and hence more closely related to our study group. For 16SrDNA, a pairwise divergence rate of 0.41% per million years was calibrated based on 13 vertebrate and invertebrate animal species (Lynch & Jarrell, 1993). Rates of 0.53% per million years (Stillman & Reeb, 2001), 0.65–0.88% per million years (Schubart *et al.*, 1998) and 0.96% per million years (Sturmbauer, Levinton & Christy, 1996) were found for crabs and rates of 0.46–0.67% per million years for barnacles (Wares, 2001). Taking into account the molecular divergence rates discussed above as well as rates of evolution adopted in previous large branchiopod studies (Zofkova & Timms, 2009; Vanschoenwinkel *et al.*, 2011, 2012), we use 1.2–2.33% and 0.41–0.96% per million years as broad and realistic ranges of pairwise divergence rates for COI and 16S, respectively.

A likelihood ratio test (Felsenstein, 1988) was performed in TREE-PUZZLE to determine whether the 16S and COI data sets evolved in a clocklike manner. To date evolutionary splits, both between and within species, tMRCA of various monophyletic clades was estimated using Bayesian inference as implemented in BEAST v. 1.6.1 (Drummond & Rambaut, 2007) according to the evolutionary model identified using jModeltest. The rate variation between sites was modelled using a gamma distribution with eight rate categories. Divergence times with credibility intervals were computed under the assumption of constant population size, with expansion growth and a strict molecular clock. Different rates of molecular evolution were specified (0.00205 and 0.0048 mutations/site/my for 16S and 0.006 and 0.01165 mutations/site/my for COI which correspond to pairwise sequence divergence rates of, respectively, 0.41 and 0.96% for 16S and 1.2 and 2.33% for COI). All estimated dates were approximated by sampling parameters at an interval of 500 generations over 10^7 Markov chain Monte Carlo steps, after discarding 10^6 burn-in steps. The convergence of the sampled parameters was subsequently verified using TRACER v. 1.5 (Drummond & Rambaut, 2007).

Demographic analyses

To test whether the Tertiary–Quaternary drying sequence in Australia's history initiated diversification and demographic and geographical expansion in *B. longirostris*, the occurrence of range contractions or expansions was verified and dated by analysing the frequency distribution of pairwise differences among haplotypes (mismatch distribution) (Harpending, 1994) in ARLEQUIN (Excoffier, Laval & Schneider, 2005). Furthermore, the demographic history of the species was examined by constructing a coalescent Bayesian Skyline Plot (BSP) model (Drummond *et al.*, 2005) implemented using BEAST v. 1.6.1 and visualised using TRACER v. 1.5. The BSP analyses were run for $3x10^7$ generations with a defined burn-in of 3×10^6 generations. Analyses were repeated with 5, 10 and 20 grouped intervals under both a strict and relaxed molecular clock.

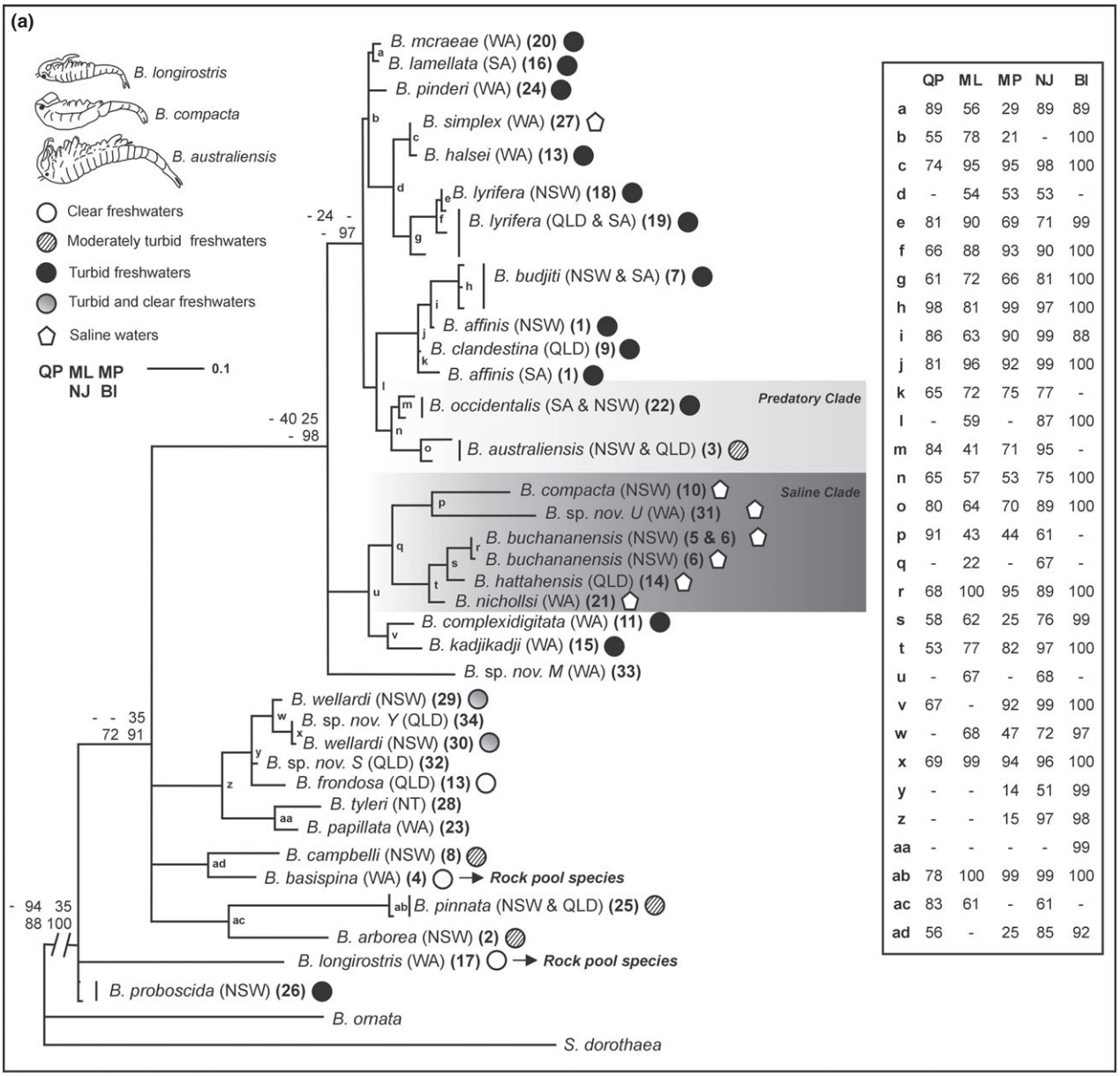
Results

Sequence variation

The final 16S rDNA *Branchinella* and COI *B. longirostris* data sets comprised 47 and 236 sequences, respectively, and represented 47 and 66 unique haplotypes of 267 and 535 bp long with a total of 104 (39%) and 168 (31%) polymorphic sites of which 82 (31%) and 105 (20%) were parsimony-informative. Among the 31 Australian *Branchinella* species in our data set, the number of nucleotide differences ranged from one, between *B. wellardi and Branchinella* specimens from Yarromere station in Queensland (Qld) (=minimum K2P distance of 0.4%), to 47 between *B. longirostris* from King Rocks (WA) and *B.* sp. *nov. U* from Moora (WA) (=maximum K2P distance of 26.6%). Considering all species, the overall average genetic divergence was 11.4%. Within *B. longirostris*, the number of nucleotide differences between the populations from 32 inselbergs ranged from one, between a population from Frog Rock and Jilbadgie Rock (JI) (=minimum K2P distance of 0.2%), to 42 between a Sandford Rock (SA) and Bushfire Rocks (BU) haplotype (=maximum K2P distance of 9.9%).

Macro-evolutionary patterns

In general, the different methods of phylogenetic inference (ML, MP, NJ, QP and BI) retrieved the same tree topology in *Branchinella.* Reconstructions were most robust at shallow and intermediate levels of divergence (Pinceel *et al.*, 2013; Fig. 1a).



	QP	ML	MP	NJ	BI
a	89	56	29	89	89
b	55	78	21	-	100
c	74	95	95	98	100
d	-	54	53	53	-
e	81	90	69	71	99
f	66	88	93	90	100
g	61	72	66	81	100
h	98	81	99	97	100
i	86	63	90	99	88
j	81	96	92	99	100
k	65	72	75	77	-
l	-	59	-	87	100
m	84	41	71	95	-
n	65	57	53	75	100
o	80	64	70	89	100
p	91	43	44	61	-
q	-	22	-	67	-
r	68	100	95	89	100
s	58	62	25	76	99
t	53	77	82	97	100
u	-	67	-	68	-
v	67	-	92	99	100
w	-	68	47	72	97
x	69	99	94	96	100
y	-	-	14	51	99
z	-	-	15	97	98
aa	-	-	-	-	99
ab	78	100	99	99	100
ac	83	61	-	61	-
ad	56	-	25	85	92

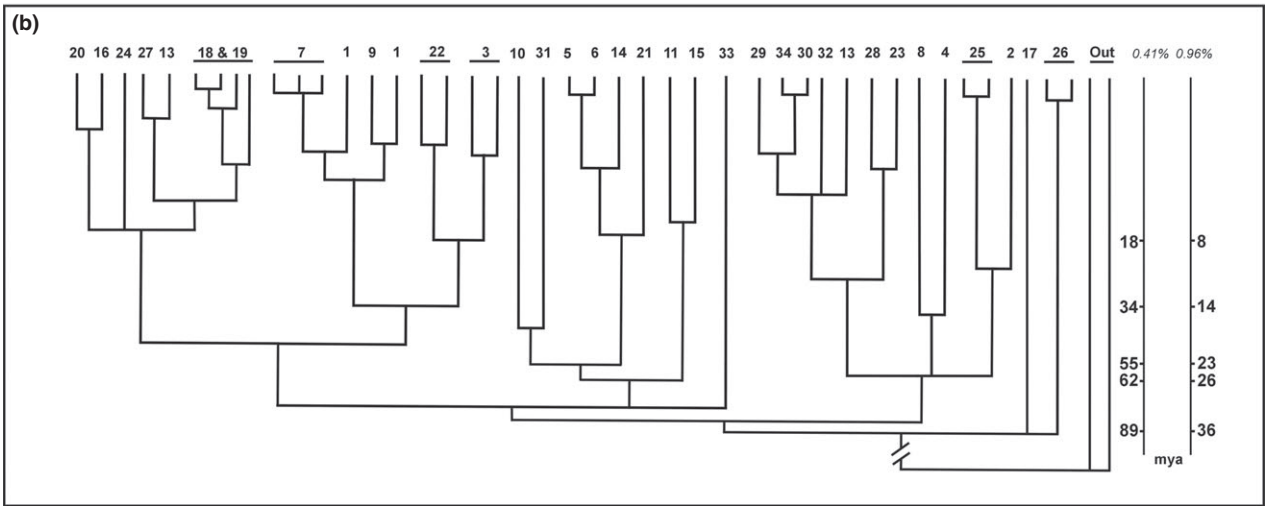


Fig. 1 (a) 16S consensus phylogram of the Australian *Branchinella* with *Carinophallus ornata* and *Streptocephalus dorothae* as outgroup. Bootstrap values are indicated for statistically supported groupings (cut-off value of 50) by quartet puzzling (QP), maximum likelihood (ML), maximum parsimony (MP), neighbour-joining (NJ) and Bayesian inference (BI) analyses. If groupings were supported by at least one analysis with a value of >50, lower support values were also shown. The habitat preferences of the species are specified. Two groupings are highlighted: a predatory *Branchinella* clade and a clade uniting *Branchinella* species inhabiting saline waters (Modified from Pinceel *et al.*, 2013). (b) BI tree constrained by a uniform pairwise divergence rate. Taxon numbers are provided and correspond to those specified in Table 1.

The *Branchinella* consensus tree was characterised by a series of habitat- and trophic shifts during the evolutionary history of the genus. While most *Branchinella* species survive in turbid claypans, non-turbid wetlands or various types of temporary pools, two distantly related lineages (*B. basispina* and *B. longirostris*) occur in ephemeral rock pools. Although 75% of the *Branchinella* species are found strictly in freshwater habitats, three lineages are mostly restricted to saline habitats: *B. simplex* and two well-supported clades comprising five salt-tolerant species which cluster together into one (poorly supported) monophyletic clade (Saline habitat clade, Fig. 1a). Additionally, the two predatory branchinellids (the very large *B. occidentalis* and the slightly smaller *B. australiensis*) emerged as sister species within a monophyletic clade (predator clade). Constraint analysis (Kishino-Hasegawa, non-parametric Templeton- and winning-sites tests) confirms that grouping the different members into the saline clade and the predator clade, respectively, leads to significantly ($P < 0.0001$) more parsimonious or more likely trees. No convergence between the turbid water specialists and the tree topology was found as the turbid water specialists did not cluster in monophyletic groups.

The simpler clocklike tree for the *Branchinella* 16S data (excluding outgroups) could not be rejected on a significance level of 5% (log L with and without clock, respectively: −3451.66 and −5094.66). Therefore, a BI tree, constrained by a uniform pairwise divergence rate of 0.41–0.96% per million years, was constructed (Fig. 1b). Based on tMRCA analyses in BEAST, the most recent common ancestor of all extant *Branchinella* taxa lived around 88.7–36.4 mya. The saline clade emerged between 62.05–54.88 and 26.33–23.31 mya, whereas the predatory clade evolved much more recently between 33.89–18.31 and 13.96–7.81 mya.

Micro-evolutionary patterns

The distribution range of *B. longirostris* in Western Australia, including the localities of surveyed inselbergs, is provided in Fig. 2a. Use of two different methods of phylogenetic inference (ML, BI) resulted in the emergence of highly similar tree topologies (Fig. 2b). Both tree and network analyses (Fig. 2b,c,d) confirmed the presence of two distinct clades in *B. longirostris*: a diverse, widely distributed, clade A (Fig. 2c) and a less diverse, southerly distributed, clade B (Fig. 2d). Two haplotypes (BO and Newman Rocks) were not included in either clade but instead emerged as sisters to clade A. Contrary to clade B, clade A is characterised by a star-shaped statistical parsimony haplotype network. All haplotypes were endemic to the inselbergs on which they were collected. Within each clade, nucleotide differences ranged, respectively, from 1–26 (clade A) and 1–27 (clade B), with an overall average K2P distance of 2.8 and 3.8% and a maximum of 5.6%, found between SA and JI, and 6.0%, between AN and WR haplotypes. Clades A and B were separated by an overall average K2P distance of 7.0%, a minimum of 4.8% between PA and Lilian Stokes Rocks and a maximum distance of 9.9% between SA and BU haplotypes.

Analyses of tMRCA, performed using BEAST according to pairwise divergence rates of, respectively, 1.2 and 2.33% per million years, dated the split between clades A and B in the range of 7.54–3.85 mya and the onset of diversification of clade A to between around 5.04–3.67 mya and 2.58–1.88 mya. The most recent common ancestor of the clade B haplotypes lived between 7.54–5.00 and 3.85–2.55 mya.

Since only a small number of extant lineages belong to clade B, the clade B data set was too limited to obtain meaningful results from BSP and mismatch analyses. The mismatch distribution of the *B. longirostris* clade A haplotypes was unimodal. The mismatch distribution fitted the predicted distribution under a model of both spatial and demographic expansion (Fig. 3a; Table 3). The clade A demographic expansion could be dated to approximately 2.13–1.10 mya and the spatial expansion to 1.97–1.01 mya (Fig. 2b). Bayesian Skyline Plot analysis was used to date major historic shifts in population size within the radiated clade A. The plot shows a period of expansion that started around 2.08–1.07 mya and a major period of population size contraction beginning between 417 and 215 kya, and progressing up until today, according to the 2.33 and 1.2% clocks (Fig. 3b). The results were robust as different coalescent models in the BEAST analysis resulted in similar estimates.

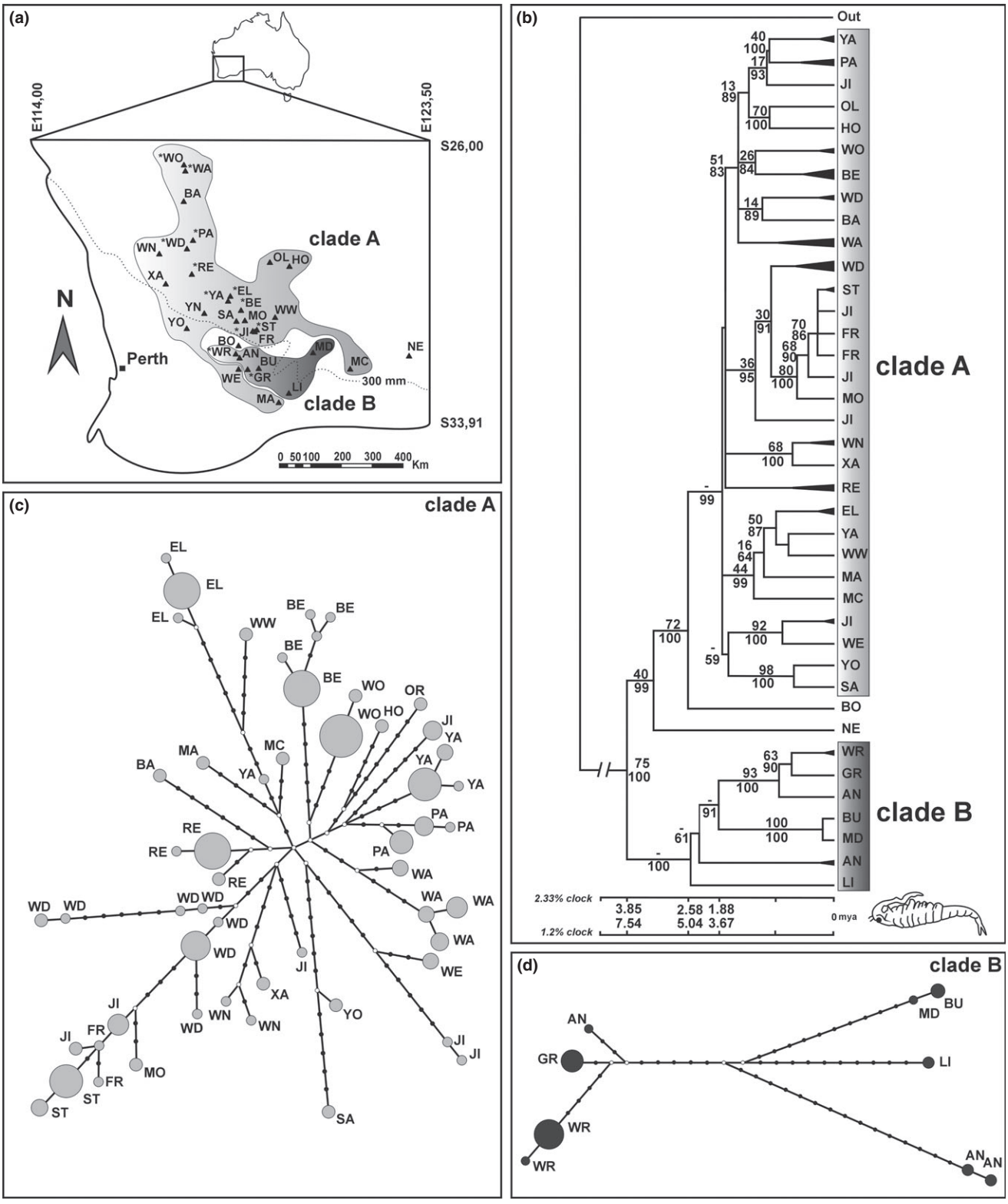


Fig. 2 (a) Geographical overview of the sampled inselbergs, (locations for which a regional sample was obtained are marked with an '*'). (b) Clock-constrained COI consensus phylogram of *Branchinella longirostris* samples from 32 locations across its distribution area. The sister species *Branchinella affinis* was defined as an outgroup. Bootstrap values are indicated for statistically supported groupings for maximum likelihood (above branch) and Bayesian inference (below branch). Monophyletic clades containing haplotypes from single metapopulations were collapsed. (c–d) Statistical parsimony networks of clade A and B, respectively. Black dots indicate hypothetical, unsampled, haplotypes and circle sizes are proportionate to haplotype frequencies.

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Table 3 Results of the mismatch analyses for the *Branchinella longirostris* COI sequences

	Demographic mismatch distribution					Spatial mismatch distribution				
	τ	t_c (1.2%)	t_c (2.33%)	P(rag)	P(SDD)	τ	t_c (1.2%)	t_c (2.33%)	P(rag)	P(SDD)
Clade A	13.664	2.128	1.096	0.790	0.757	12.631	1.967	1.013	0.778	0.532

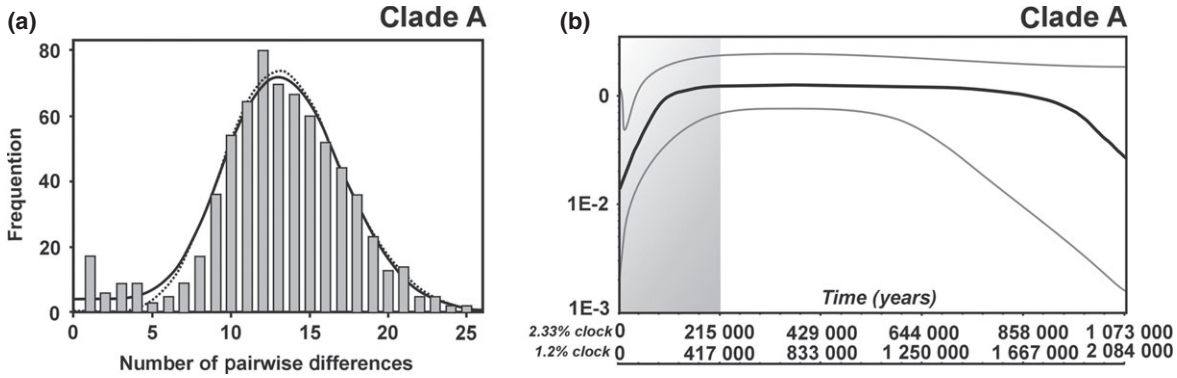

Fig. 3 (a) Mismatch distribution of COI haplotypes within the *Branchinella longirostris* clade A under a model of demographic (dashed line) and geographical expansion (full line). (b) Extended Bayesian Skyline Plot showing changes in population size for the *B. longirostris* A clade through time. These historical demographic trends of the COI lineages are interpreted according to the 1.2 and 2.33% molecular clocks. The episode of negative expansion is marked grey. The black line represents the median estimate; upper and lower limits (95% HPD) are drawn in grey.

Discussion

A reconstruction of the evolutionary history of *Branchinella* yielded patterns of diversification consistent with the gradual spreading of (semi)arid conditions from the Palaeocene onward into the Miocene. However, most of the extant clades seem to have evolved during a more intense period of aridification from the Mid-Miocene until the Pliocene.

Taking into account the ecological information available for extant lineages, including habitat- and dietary preference, we showed that the present diversity of Australian fairy shrimps is most likely to have resulted from a series of dietary and habitat shifts, as well as from dispersal limitation and genetic drift, illustrating the importance of both adaptive and non-adaptive processes. Traces of the most recent episode of severe aridification (late Pliocene onwards) were detected at the intraspecific level. Initial aridification seems to have driven radiation and demographic as well as spatial expansion, whereas the extreme climate change events during the alternation of Pleistocene glacial and interglacial periods resulted in demographic contraction.

Macro-evolutionary patterns

According to a range of known mutation rates of mtDNA in invertebrates, the most recent common ancestor of all present-day Australian *Branchinella* species presumably lived around 88.7-36.4 mya. This is consistent with the oldest known fairy shrimp fossil that resembles *Branchinella* in terms of brood sac morphology, found in the late Mesozoic of Victoria (Jell & Duncan, 1986). The predominantly moist and tropical Australian climate during that period probably confined fairy shrimps to areas with dryer micro-climates, where low precipitation and high evapotranspiration ensured periodic drying of lentic habitats. Around 65.5–55.8 mya, the majority of Australia was still covered by tropical vegetation with environmental conditions comparable to those of current equatorial rainforests (Hill, 1994). Under these circumstances, most inland aquatic habitats held fresh water, were permanently inundated and contained fish. They were therefore unsuitable for large crustaceans, such as fairy shrimps, which cannot tolerate fish predation (Kerfoot & Lynch, 1987). The paucity of fish-less aquatic habitats is considered the main reason for the absence of fairy shrimps in the wet tropics (Brendonck *et al.*, 2008). Areas with semiarid conditions or seasonal droughts first started to develop in the beginning of the Cenozoic in central and north-west Australia (Martin, 2006). This presumably resulted in a transition from permanent to temporary aquatic habitats suitable for colonisation by large branchiopods. The origin of the three major evolutionary clades within *Branchinella* should be situated within that first period of gradual

aridification. Besides changes in permanence, drought can also modify aquatic habitats by increasing the concentrations of dissolved salts (Williams, 2006). Our analyses suggest that the appearance of the first saline-tolerant *Branchinella* (Fig. 1b) also took place during the first period of aridification with the common ancestor of this clade appearing between 62.1–54.9 and 26.3–23.3 mya. This indicates that saline habitats were already present by this time and that fairy shrimps managed to colonise them as a result of a successful habitat shift from fresh waters to inland saline waters. The limited character data set, however, did not allow us to assess more rigorously the history of character states and the process of character evolution (e.g. using Mesquite software; Maddison & Maddison, 2011). The finding that the halophilic Australian fairy shrimp genus *Parartemia* Daday, 1910 appears to have diversified around the same time (Remigio, Hebert & Savage, 2001) can be considered additional support for this scenario.

Although several *Branchinella* clades diverged in the early Cenozoic, most of the current lineages in the genus originated during the second episode of aridification in the Mio-Pliocene (15.5–5.3 mya). The origin of the predatory clade is, for example, situated between 33.89–18.31 and 13.96–7.81 mya. The emergence of this clade, which groups two species that effectively prey on zooplankton and other fairy shrimps using extended phyllopods (Timms, 2004), represents a significant innovation. Currently these species are restricted to large, nutrient rich, temporary pools where fairy shrimps can grow very large and turbidity obscures them from visual predators such as water birds (Woodward & Kiesecker, 1994; Dumont & Negrea, 2002). Analogous to similar habitats in the African savannah (Nhiwatiwa *et al.*, 2011), it is possible that these large and turbid lowland habitats suitable for the evolution of predatory taxa only became abundant after the eventual disappearance of tropical forest.

Micro-evolutionary patterns

We have shown that early and middle Cenozoic climate change was associated with speciation and diversification in Australian *Branchinella*. Additionally, intraspecific analyses of the genetic structure of the ephemeral rock pool specialist *B. longirostris* revealed a correlative link between climate events that took place since the late Pliocene and patterns of genetic differentiation. Phylogeographical reconstructions, network and mismatch analyses and historical demographic reconstructions suggest three distinct periods within the evolutionary history of *B. longirostris*.

First of all, a major diversification occurred about 7.5–3.9 mya, from the late Miocene until the mid Pliocene (Fig. 2b). Although both basal clades (A and B) are genetically quite dissimilar, with a minimum pairwise K2P distance of 4.8% and a maximum divergence of 9.9%, no consistent morphological differences were identified in a preceding morphological study on specimens from both clades (Zofkova & Timms, 2009). Populations of clade B, however, are geographically structured, occupying a range nested within the southern part of the species range and not co-occurring on inselbergs with members of clade A (Fig. 2a).

Secondly, the star-shaped network topology of clade A (Fig. 2c) and unimodal mismatch distribution of pairwise haplotype differences (Fig. 3a) support correspondence of late Tertiary aridification (3.7–1.9 mya) with a range- and a demographic expansion in this *B. longirostris* clade. The BSP analysis did not support a significant demographic expansion although a trend is visible (Fig. 3b). No such radiation was observed within clade B (Fig. 2d). Based on evidence of increased aridification in other parts of Australia (Fujioka *et al.*, 2005), it is probable that large numbers of south-west Australian inselbergs lost their lush vegetation cover at that time and rock pool basins were exposed (Twidale & Bourne, 2001; Twidale, 2007), creating opportunities for *B. longirostris* to expand its range. It is unclear why an expansion was observed in only one of the two major *B. longirostris* clades. Potential explanations for this discrepancy that remain to be tested include a shorter reproduction time or a tolerance to higher temperature in clade A, allowing establishment in more short-lived temporary rock pool systems in the warmer and drier northern and inland areas of Western Australia. Potential differences in dispersal capacity between the members of both clades are unlikely since the morphology of the dispersing propagules is identical (Timms & Lindsay, 2011).

Finally, after initial expansion, BSP analyses indicate a demographic contraction during the late Pleistocene (417–215 ka) within *B. longirostris*. This hypothesis appears to be consistent with the reconstructed haplotype network, as well as the fact that terminal haplotype radiations were scarcer than would be expected under stable or expanding demographies. The observed demographic contraction is presumably related to the Pleistocene glaciations. Australia experienced severe climatic oscillations with periods of extreme aridity during the last 400 ka (Martin, 2006). Hydrological models (Hulsmans

et al., 2008) support the notion that, during such periods with reduced precipitation and higher temperature, only the deepest rock pools with the longest hydroperiods would be able to support fairy shrimps. Genetic bottlenecks and extinction of populations in short-lived pools associated with these extreme climatic fluctuations therefore provide a suitable explanation for the observed demographic contraction.

The lack of shared haplotypes suggesting low levels of recent gene flow could be the result of different processes. The isolation of inselbergs, typically separated by several (tens of) kilometres, indicates that passive dispersal of resting eggs by animal vectors (Figuerola, Green & Michot, 2005) is more likely than other vectors, such as wind. Australian wood ducks (*Chenonetta jubata*) could be effective dispersal vectors as they occasionally visit rocky outcrops to feed on the semi aquatic quillwort *Isoetes* (T. Pinceel pers. obs). Additionally, it is likely that mammals such as kangaroos and wallabies that visit rock pools for drinking purposes will occasionally transport resting eggs (Vanschoenwinkel *et al*., 2008). Finally, the combination of numerical priority effects in combination with the possibility of local adaptation (monopolisation hypothesis; De Meester *et al*., 2002) can reduce establishment success explaining the lack of secondary contact between populations on different inselbergs.

Adaptive versus non-adaptive processes

Adaptive radiation is now generally defined as the differentiation of a single ancestor into an array of species inhabiting a variety of environments and differing in adaptive traits (Schluter, 2000). Following this definition, the evolutionary history of the genus *Branchinella* on the Australian continent appears to be at least partly adaptive with strong indications of specialisation.

We found clear indications for niche specialisation in *Branchinella*, sometimes evolving independently in distantly related lineages. *Branchinella* has invaded saline habitats while two lineages, *B. longirostris* and *B. basispina*, have colonised extreme rock pool habitats independently. Besides niche diversity provided by substantial variation in habitat characteristics such as salinity, turbidity and hydroperiod, fairy shrimps occupy different feeding niches, with the emergence of large predatory lineages, and exploit different temporal niches. The latter is, for instance, reflected in succession of fairy shrimp species in long-lived habitats (Timms, 2002) or in differential emergence from dormant propagule banks under various conditions (Donald, 1983; Warner & Chesson, 1985).

On the other hand, we also found indications that non-adaptive processes, such as long-distance dispersal and genetic drift which promote genetic divergence in isolation (Excoffier & Ray, 2008; Vanschoenwinkel *et al*., 2011, 2012), may have played a significant role. First of all, low gene flow among populations, which promotes genetic differentiation and speciation due to genetic drift, can explain the extreme geographical variation in the ornamentation of the male frontal appendage in isolated inselberg populations of the widespread *B. longirostris* (Zofkova & Timms, 2009). This structure, which is involved in copulation, is assumed to be non-adaptive as previous research has shown that its morphology does not carry any phylogenetic signal. As these populations do not share a single haplotype between inselbergs, they do not seem to be connected by recent gene flow (Fig. 2c, d). Consequently, a combination of founder effects and sexual selection has been proposed to explain the observed variation (Zofkova & Timms, 2009). Secondly, the sheer size of Australia, preventing efficient intracontinental dispersal, ensures that similar niches in distant areas can be occupied by different species promoting diversity at regional scales. This is illustrated by *B. compacta* and *B.* sp. *nov. U* which present morphologically similar species that inhabit comparable salt lakes in Eastern and Western Australia, respectively (Pinceel *et al*., 2013).

Overall, our large-scale phylogenetic analysis of the fairy shrimp *Branchinella* and range-wide phylogeographical analysis of the rock pool specialist *B. longirostris* reveal important new insights on the impact of Cenozoic climate change on this specialised aquatic fauna. Additionally, the study illustrates how large branchiopod crustaceans can be used as models to evaluate the impact of environmental change on evolutionary processes in general and on those in aquatic ecosystems in particular. Our results indicate that drying can lead to drastic changes in aquatic ecosystems, with a transition from tropical communities to species assemblages typical for arid environments. In terms of freshwater ecosystems, this transition is most likely to have comprised a shift from permanent waters to temporary and saline systems. Specifically for Australia, the isolation of the continent ensured that the new niches that appeared could only be filled in by local species, a condition that promotes radiation as observed in *Branchinella* and for which indications are also found in other endemic groups of freshwater zooplankton bound to temporary aquatic systems, such as the fairy shrimp genus *Parartemia* (Remigio *et al*., 2001) and the cladoceran genus *Daphnia* (*Daphniopsis* Sars, 1903) (Colbourne, Wilson &

Hebert, 2006). Although our results suggest that large branchiopods were able to benefit from the historic aridification in terms of species diversity, indications of recent range contraction were also observed in this study. It is therefore likely that the ongoing trends towards even drier conditions and less predictable rainfall as a result of climate change may promote (secondary) salinisation of freshwater habitats (Pinceel *et al.*, 2013) and future reduction of hydroperiod, resulting in the loss of suitable fairy shrimp habitat, range contractions and extinctions.

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