Biological Journal of the Linnean Society, 2008, **95**, 175–192. With 6 figures

Phylogeography of *Philypnodon* species (Teleostei: Eleotridae) across south-eastern Australia: testing patterns of connectivity across drainage divides and among coastal rivers

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Received 30 April 2007; accepted for publication 2 October 2007

Phylogeographical studies based on DNA sequences offer insights into intraspecific genetic patterns, elucidating the history and structure of populations and their habitats. We used mitochondrial DNA (cytochrome *b*) to study the phylogeography and population genetics in two sympatric species in the freshwater fish genus *Philypnodon* throughout south-eastern Australia. We sought to determine how populations were related across drainage divides, and whether transfer among adjacent coastal drainages was related to continental shelf width or intradrainage distance. Phylogeographical structure was greater in *Philypnodon macrostomus* Hoese & Reader, 2006 compared with *Philypnodon grandiceps* (Krefft, 1864), with results for *P. macrostomus* showing evidence for the presence of distinct groupings in different areas of south-eastern Australia. There was evidence of drainage-divide crossings in *P. grandiceps* in western Victoria, and in *P. macrostomus* between the Burnett River and the Murray-Darling Basin in south-eastern Queensland. Both species showed low levels of divergence along the narrow continental shelf of New South Wales, but as continental shelf width increased moving north in south-eastern Queensland, population divergence also generally increased. Thus, as the potential riverine connectivity during periods of low sea levels increased, genetic divergences also increased, counter to expectations. Also, population Φ_{st} measures did not correlate as predicted with continental shelf width, nor was a significant relationship detected between Φ_{st} and the distance between populations.

ADDITIONAL KEYWORDS: biogeography – continental shelf – freshwater – gobioidei – sea-level change – sympatric species.

INTRODUCTION

For freshwater organisms, potential distribution ranges are constrained not only by the boundaries of their riverine or lacustrine habitats, but also by the presence of saltwater barriers dividing freshwater outflows. Transfer among rivers may be accomplished over time by changes in flow regimes and drainage connectivity, and/or by transient connections established between waterways during natural events such as floods. Rivers may also become temporarily confluent during periods of sea-level regression, if exposed shelf habitats become inundated with fresh water and river outlets are merged (Unmack, 2001).

South-eastern Australia provides an opportunity to investigate the phylogeographical patterns of

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freshwater organisms that inhabit riverine systems influenced by both sea-level changes and isolation by drainage divides. Numerous relatively short coastal rivers flow east or south from the major drainage divide formed by the Eastern Highlands (Eastern Coast: EC), whereas west-flowing rivers coalesce in the Murray–Darling Basin (MDB) to form the Murray River, the outlet of which is to the west (Fig. 1). There are a number of close relationships between fishes in the area, with approximately 16 species or species pairs occurring on either side of the Eastern Highlands. This pattern suggests there have been recent movements of fishes across this drainage divide (McGlashan & Hughes, 2001; Unmack, 2001). Similarity of the fish fauna between MDB and EC drainages is highest in the north, in the vicinity of the Brisbane River, Queensland, and progressively decreases until around Genoa River on the Victoria–New South Wales border. From that point west, faunal similarity between EC drainages and MDB increases again until the mouth of the Murray River is reached (Unmack, 2001). Today, EC drainages are isolated from one another by seawater; however, fluctuations in sea level have resulted in large areas of

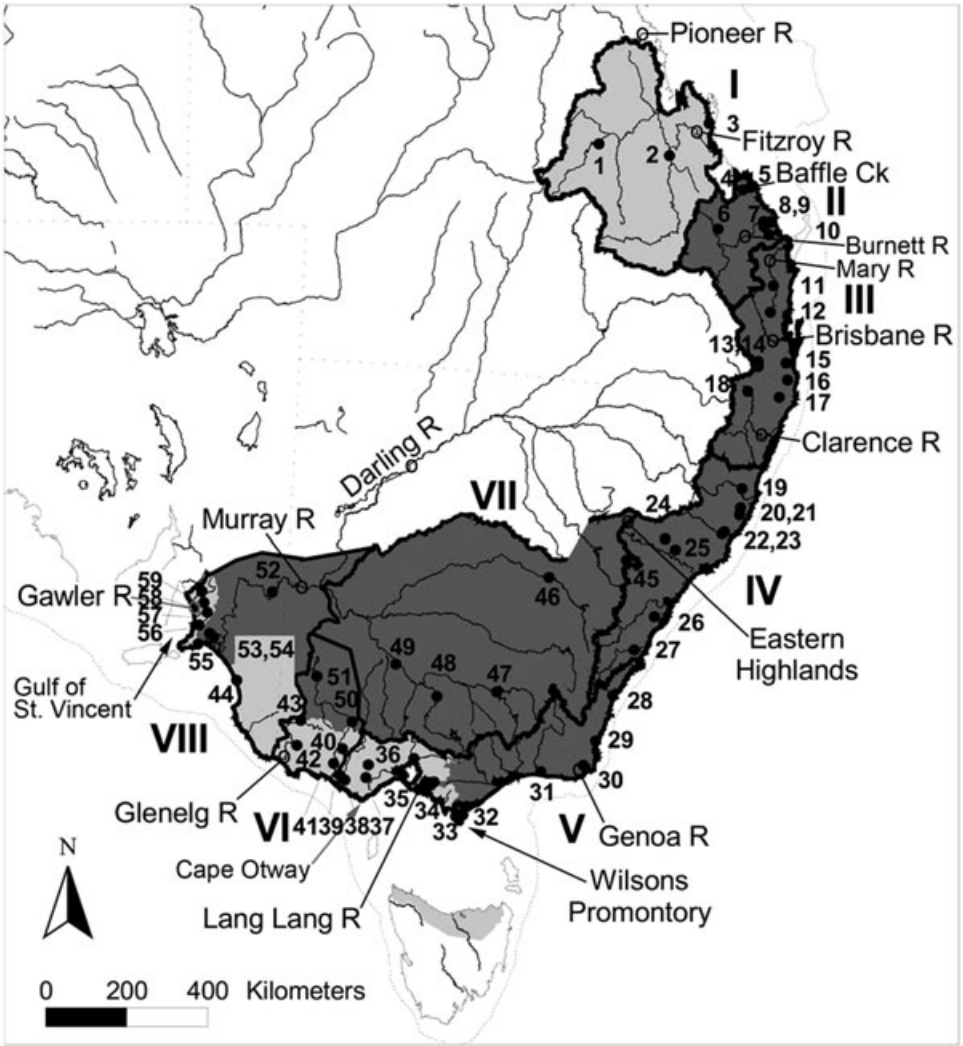

Figure 1. Map of localities sampled in eastern Australia. The dark-shaded areas are those in which both *Philypnodon grandiceps* and *Philypnodon macrostomus* are found; light shading indicates the presence of *P. grandiceps* only. Ranges are subdivided into partitions, indicated by roman numerals, in accordance with drainage basin boundaries. Sampling localities are numbered in accordance with Table 1. The Eastern Highlands, separating Eastern Coast (EC) drainages from those of the Murray–Darling Basin (MDB) is represented by the heavy black line at the eastern edge of the shaded areas; the other major rivers and features are also indicated. The dotted line represents the boundary of the continental shelf.

continental shelf being periodically exposed. These shallow continental shelves may support freshwater or estuarine habitat during sea-level drops, providing opportunities for the dispersal of riverine organisms between coastal drainages.

Nine genera of gudgeons (family Eleotridae) are found in Australian freshwaters, including three that are endemic. Most species are endemic or are only shared with New Guinea (Allen, Midgley & Allen, 2002). The endemic genus *Philypnodon* is primarily found in freshwater and estuarine habitats throughout south-eastern Australia (Fig. 1). The genus includes two recognized species: the flathead gudgeon *Philypnodon grandiceps* (Krefft, 1864) and the dwarf flathead gudgeon *Philypnodon macrostomus* Hoese & Reader, 2006 (Allen *et al*., 2002; Hoese & Reader, 2006). The species are sympatric throughout most of their ranges (Fig. 1). *Philypnodon grandiceps* is the more widespread species, occurring in coastal drainages from the Gawler River (South Australia) continuously to just north of the Fitzroy River (Queensland), as well as a few drainages in northern Tasmania. Additional records from further north in Queensland exist from the Pioneer and Burdekin rivers (Pusey, Kennard & Arthington, 2004), but these may represent misidentifications or translocations, respectively. No specimens were retained from the Pioneer River survey, and no *Philypnodon* have been recorded in subsequent surveys. Records exist from two separate localities in the Burdekin River (Pusey *et al*., 2004), but are thought to have been introduced accidentally as contaminants from sport fishing introductions (B. Pusey, pers. comm.).

In coastal drainages, *P. macrostomus* occurs from Wilsons Promontory (Victoria) north to Baffle Creek (Queensland), with a single recently discovered outlier population in the Lang Lang River, Victoria (T. Raadik, pers. comm.). Both species tend to be relatively common wherever they are found. In southern coastal rivers both *Philypnodon* species tend to be restricted to the lower reaches of rivers. In more northerly drainages, they are are found further upstream, and from the Brisbane River north they may occur in the upper reaches of drainages. *Philypnodon* species are also often found in the upper reaches of estuaries, although typically only in lower salinities. Within the MDB, *P. grandiceps* and *P. macrostomus* are widespread in the southern portion (Murray River and its tributaries), but have only been historically recorded from the upper Macquarie River within the northern Darling River tributaries; their presence there implies that they may have been more widespread in the northern portion of the MDB during different climatic periods. Recent additional records of *P. grandiceps* and *P. macrostomus* were made in 2001, and afterwards, from the Condamine River, an upper Darling River tributary (QM 33154; M. Hutchison, pers. comm.), although it remains unclear as to whether these populations were introduced or are remnant populations.

The biology of both species has been little studied, although a summary of existing information was provided by Pusey *et al*. (2004), and the following is based on their account. *Philypnodon* species occur in a diverse range of low-velocity habitats ranging from small streams to large rivers and upper estuaries, as well as being common in floodplain habitats and large natural lakes. Both species are commonly associated with some type of cover, such as aquatic macrophytes or woody debris, especially *P. macrostomus*. They appear to have broad environmental tolerances, with salinity tolerance in *P. grandiceps* varying between 24 and 40 ppt. Their breeding biology appears to be typical for eleotrids, with the males defending a territory and guarding the eggs until hatching. Both species probably reach maturity by the end of their first year; their total longevity is unknown. Maximum size for *P. grandiceps* is 120 mm, and for *P. macrostomus* it is 50 mm. *Philypnodon grandiceps* has been recorded in mass migrations, especially between estuarine and freshwater reaches across weirs and other barriers to movement, although these movements are not associated with any specific aspect of their life history or the size class of individuals. Information on movement is lacking for *P. macrostomus*, although it was likely to be present in these same migrations but misidentified as juvenile *P. grandiceps*.

In this study, we use phylogeographical and population genetic analyses to investigate several aspects of historical dispersal in both *Philypnodon* species. We seek to determine the pattern of movement between adjacent coastal drainages, as well as across the Eastern Highlands. There are six species or species pairs that are widespread and occur in a large proportion of drainages on both sides of the Eastern Highlands [in order of number of drainages they are: *Retropinna semoni* (Weber, 1895), *P. grandiceps*, *P. macrostomus* (these three species occur in most drainages), *Galaxias olidus* Günther, 1866 (occurs in most drainages south of the Clarence River), the species pair *Hypseleotris galii* (Ogilby, 1898) and Murray-Darling carp gudgeon (*Hypseleotris* sp.. 3) (these two species occur in coastal drainages from the Georges River north), and *Tandanus tandanus* (Mitchell, 1838) (occurs in drainages as far south as the Hunter River)], and none of these species have been genetically examined in detail except *H. galii* and *Hypseleotris* sp.. 3 (Thacker *et al*., 2007), and *R. semoni* (Hammer *et al*., 2007). All other species either only occur across drainage divides in southern drainages in Victoria or occur mostly north of Clar-

ence River in northern New South Wales and Queensland (Unmack, 2001). Although neither *Philypnodon* species occurs in headwater regions of many streams in the central portion of their coastal range (they must be present near drainage divides in order to cross them), they may have occurred further upstream under different climatic conditions, and they do occur in headwater regions north of Brisbane River and east of Mt Emu Creek (western Victoria). In addition, because the *Philypnodon* species are largely sympatric, our study allows for comparative phylogeographical comparisons between two closely related species with similar habitat requirements and life-history characteristics. We hypothesize that patterns in *P. grandiceps* will be congruent with those in *P. macrostomus*.

To test the hypothesis that a wider continental shelf leads to more mixing among drainages because of confluences established in times of lowered sea levels, we use population genetic analyses (pairwise population divergence (Φ_{st}) among geographical partitions based on drainage basin boundaries) in conjunction with our phylogeographical trees. We also determine whether *Philypnodon* species exhibit a linear pattern of divergences around the coast based on drainage proximity, which would indicate exchange among populations consistent with an isolation-by-distance model. Our expectation is that areas adjacent to the wider continental shelf will show more mixing among drainages, and that because of the variation in shelf width around south-eastern Australia, a strict isolation-by-distance pattern will not be observed. Isolation by distance may be observed at smaller scales, however, with variance in strength corresponding to shelf width, such that a narrower shelf may contribute to a stronger pattern.

MATERIAL AND METHODS

Frozen or ethanol-preserved samples of *Philypnodon* species for DNA analysis were collected from 59 localities across south-eastern Australia: 49 for *P. grandiceps* and 22 for *P. macrostomus* (Fig. 1; Table 1). Representative genetic material was deposited in the Evolutionary Biology Unit of the South Australian Museum, and formalin-fixed representatives were deposited in the Australian, Victorian, and South Australian museums. These samples may be identified based on their station code (Table 1).

Muscle tissue from each specimen was used for total genomic DNA extraction, performed with the DNeasy Tissue Kit (Qiagen). Amplification of the cytochrome *b* (cyt*b*) gene was achieved in two portions, using primer pairs HYPSLA (5′-GTGGCTTGA AAAACCACCGTT-3′) to HYPSHD (5′-GGGTTGTTG GAGCCAGTTTCGT-3′) for the 5′ end, and hypsl510 (5′-agataatgcaaccctmacccg-3′) or HYPSL500 (5′-CTTYTCMMTAGATAATGCAACCC-3′) to ph15938 (5′-CGGCGTCCGGTTTACAAGAC-3′) for the 3′ end. PCR was performed using Platinum Taq DNA polymerase (Invitrogen) or Gibco Taq polymerase (Life Technologies), with a profile of 94 °C for 3 min, followed by 40 cycles of 94 °C for 15-s denaturation, 50–53 °C for 45-s annealing, and 70 °C for 30-s extension, with a final hold at 70 °C for 7 min. PCR products were electrophoresed on a low-melting-point agarose gel, visualized and photographed, and were then excised and purified with the QIAquick gel extraction kit (Qiagen). Using the same primers (1 μM rather than 10 μM), PCR fragments were cycle sequenced using the Big Dye terminator/Taq FS ready reaction kit version 3.1, purified by passing the reactions through 750-μL Sephadex columns (2.0 g in 32.0 mL ddH_2O), and were then visualized on an ABI 377XL automated sequencer (Applied Biosystems). The heavy and light strands were sequenced separately. The resultant chromatograms for the heavy and light strands were reconciled using Sequencher 4.1.2 (GeneCodes Corp.) to check base calling, translated to an amino acid sequence using the 'mammalian mtDNA' code, concatenated for each taxon, and were finally aligned by eye. There were no ambiguities or gaps in the alignment; all the gaps present in the final matrix were to the result of missing data, and were treated as such (coded as '?' rather than as a new character state) in the analysis. Aligned nucleotide sequences were exported as NEXUS files from Sequencher. In addition to newly sequenced taxa, 12 additional sequences were obtained from GenBank. In accordance with the basal gobioid phylogeny of Thacker & Hardman (2005), cyt*b* sequences from the taxa *Microphilypnus ternetzi* Myers, 1927 (AY722253), *Leptophilypnus fluviatilis* Meek & Hildebrand, 1916 (AY722197-9), *Leptophilypnus panamensis* (Meek & Hildebrand, 1916) (AY722195-6), *Odontobutis potamophila* (Günther, 1861) (AY722225, AY722247), and *Percottus glenni* Dybowski, 1877 (AY 722208, AY 722217, AY 722243–4) were included. *Odontobutis potamophila* and *P. glenni* were designated as outgroups and were used to root the phylogeny.

Phylogenetic analyses were performed using MrBayes, version 3.1.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Bayesian analyses were conducted by first determining the appropriate model for base and substitution frequencies with the likelihood-ration test (LRT) and Akaike information criteria (AIC), as implemented in MrModeltest 2.0 (Nylander, 2004). Default Dirichlet priors were specified using the command prset statefreqpr = dirichlet(1,1,1,1). The MrBayes 3.1.1 search was run for 2 300 000 generations with two

Table 1. Sampling localities and sample sizes for *Philypnodon* species

Partition, population number and locality	Station code	*P. grandiceps*	*P. macrostomus*
I. Fitzroy River, Queensland (EC)			
1 Fairbairn Dam, Emerald, QLD	PU01-53	1	–
2 Dawson R, Moura, QLD	PU01-55	2	–
3 Maryvale Ck, Maryvale Station, QLD	PU02-49	2	–
II. North Queensland (EC)			
4 Baffle Ck, Miriam Vale, QLD	PU02-50	0	2
5 Oyster Ck, Agnes Waters, QLD	PU02-42	1	0
6 Burnett R, Ceratodus, QLD	PU02-51	0	3
7 Burnett R, Mingo Crossing, QLD	PU99-55	2	0
8 Elliott R, Elliott, QLD	PU02-38	0	2
9 Gregory R, Goodwood, QLD	PU02-37	0	3
10 Isis R, Childers, QLD	PU97-48	1	0
III. Southern Queensland and Northern New South Wales (EC)			
11 Amamoor Ck, Amamoor, QLD	PU02-33	2	2
12 Delaneys Ck, D'Aguilar, QLD	PU97-71	0	2
13 Moogerah Lake, Moogerah, QLD	PU02-27	3	0
14 Maroon Dam, Maroon, QLD	PU02-28	2	0
15 Coomera R, Flying Fox, QLD	PU02-24	1	0
16 Oxley R, Eungella, NSW	PU02-22	2	2
17 Leycester Ck, Leycester, NSW	PU02-17	2	3
18 Clarence R, Tabulam, NSW	PU99-43	1	0
IV. Midcoast New South Wales (EC)			
19 Hickeys Ck, Millbank, NSW	PU02-13	2	3
20 Bril Bril Ck, Rowland Plains, NSW	PU02-11	1	0
21 Hastings R, Wauchope, NSW	PU99-38	2	2
22 Dingo Ck, Wingham, NSW	PU02-10	1	0
23 Cedar Party Ck, Wingham, NSW	PU02-09	3	0
24 Hunter R, Muswellbrook, NSW	PU02-07	1	0
25 Goorongoola Ck, Mt. Pleasant, NSW	PU02-08	3	0
26 Georges R, Liverpool, NSW	IW94-52	1	4
27 Kangaroo R, Kangaroo Valley, NSW	PU02-58	3	0
V. Southern New South Wales and Coastal Victoria (EC)			
28 Mogo Ck, Mogo, NSW	PU02-60	2	0
29 Millingandi Ck, Millingandi, NSW	PU99-83	4	5
30 Maramingo Ck, Genoa, VIC	PU99-84	0	2
31 Snowy R Lagoon, Orbost, VIC	PU99-85	5	5
32 Miranda Ck, Wilsons Prom., VIC	PU02-69	0	2
33 Darby R, Wilsons Promontory, VIC	PU02-73	2	–
34 Lang Lang R, Lang Lang, VIC	n/a	0	2(as *P.* sp.)
35 Steele Ck, Melbourne, VIC	F-FISHADD4	2	–
36 Woady Yaloak R, Cressy, VIC	PU00-28	1	–
37 Lake Colac, Colac, VIC	PU02-89	1	–
38 Curdies R, Curdie, VIC	PU00-24	2	–
39 Mt. Emu Ck, Panmure, VIC	PU02-112	1	–
VI. Western Victoria (EC)			
40 Lake Bolac, Lake Bolac, VIC	PU02-116	2	–
41 Hopkins R, Mortlake, VIC	PU02-115	2	–
42 Palmer Ck, Merino, VIC	PU02-119	1	–
43 Glenelg R, Burke Bridge, VIC	PU00-15	2	–
50 Mount Cole Ck, Warrak, VIC	PU00-06	4	–
51 Wimmera R, Jeparit, VIC	PU01-60	2	–

Table 1. *Continued*

Partition, population number and locality	Station code	*P. grandiceps*	*P. macrostomus*
VII. Murray-Darling East (MDB)			
45 Cudgegong R, Rylstone, NSW	TR02–497B	3	0
46 Lake Forbes, Forbes, NSW	PU99-36	1	0
47 Murray R, Albury, NSW	PU02-55	1	0
48 Reedy Swamp, Shepparton, VIC	IW94-38	2	0
49 Black Swamp, Cohuna, VIC	IW94-33	1	2
VIII. Murray-Darling West (MDB) and Coastal South Australia			
44 Cortina Lakes, Cortina, SA	F-FISH90	2	–
52 Murray River, Berri, SA	F-FISHLAB	1	3
53 Bremer R, Lake Alexandrina, SA	IW94-23	5	0
54 Angas R, Strathalbyn, SA	PU94-29	0	2
55 Hindmarsh R, Victor Harbour, SA	F-FISHY2	2	0
56 Onkaparinga R, Clarendon, SA	F-FISH94	2	2
57 Torrens R, Cuddle Creek, SA	F-FISHY2	0	3
58 Gawler R, Gawler, SA	F-FISHADD4	4	–
59 Light R, Hamley Bridge, SA	F-FISHY2	3	–

Partitions are indicated on the map in Figure 1. Station codes can be used to track references to genetic material deposited in the South Australian Museum, and morphological samples deposited in the Australian, South Australian, and Victorian museums. For analysis of population structure (Table 2), some partitions were grouped. A dash (–) indicates *P. macrostomus* is absent from that drainage; zero indicates the species is recorded from that drainage, but was not sampled for this study.

replicates, each with four simultaneous chains. This length of search ensured that the runs converged and that stationarity was achieved, as indicated by the standard deviation of split frequencies and by examination of a graph of posterior probability vs. replicates. Trees were sampled every 1000 generations, and the first 300 trees (300 000 generations) were discarded as burn-in. The Bayesian estimates of posterior probabilities were included to indicate support for clades. We also performed parsimony analysis of a restricted data set (to reduce computation time and memory demands). Based on the results of the Bayesian analysis, representative sequences for each clade were selected from the recovered groups and analysed under the parsimony criterion with PAUP* version 4.0b8 (Swofford, 2003). Sequences used in the parsimony analysis included *P. grandiceps* from Burnett River, Queensland (QLD; 7), Coomera River QLD (15), Snowy River Lagoon, Victoria (VIC; 31), Cudegong River, New South Wales (NSW; 45), and Wimmera River VIC (51); *P. macrostomus* from Millingandi Creek NSW (29), Hickeys Creek NSW (19), Torrens River, South Australia (SA; 57), and two individuals from Miranda Creek VIC (32); the two *Philypnodon* sp. from Lang Lang River VIC (34), and the six outgroup sequences from *O. potamophila* and *P. glenni*. One thousand replications of a heuristic search were run, using tree bisection–reconnection (TBR) branch swapping, with the data designated as equally weighted. Because this study examines intraspecific as well as interspecific relationships, a neighbour-joining dendrogram was also constructed, based on pairwise distances of sequences, using PAUP*. Support for nodes in the dendrogram was assessed using 1000 replications of jackknife resampling, also in PAUP*.

To examine population-level patterns within these species, so as to further examine the influence of continental shelf width on population structure, and to determine if population structuring is related to genetic distance, the DNA sequence data were analysed with distance-based population methods. Populations within each species were analysed in accordance with the partitions in Table 1, except that some smaller samples were pooled to increase sample size (partitions II and III for *P. grandiceps*, and VII and VIII for *P. macrostomus*; Table 2). Analyses of population structure, including haplotype diversity (h), genetic diversity (π), and pairwise population Φ_{st} were performed with Arlequin version 2.001 (Schneider, Roessli & Excoffier, 2000). The significance of pairwise Φ_{st}–statistics was corrected for multiple comparisons using a Bonferroni correction (Rice, 1989; Buonaccorsi, McDowall & Graves, 2001). Arlequin was also used to test conformation with a sudden expansion model, by generating expected values for the mismatch distribution (distribution of pairwise divergences between individuals). To determine

Table 2. Φ_{st} values for population comparisons for *Philypnodon* species

Φ_{st} among populations of *Philypnodon grandiceps*. All values are significant after Bonferroni correction ($\alpha = 0.007$), except those italicized.						
	1	2	3	4	5	6	7
1 Fitzroy River (I)	0						
2 QLD & North NSW (II + III)	*0.06251*	0					
3 Midcoast NSW (IV)	0.25956	0.08285	0				
4 South NSW & Coastal VIC (V)	0.64220	0.53814	0.67894	0			
5 Western VIC (VI)	0.68583	0.37724	0.37898	0.83196	0		
6 Murray-Darling East (VII)	0.47993	0.19009	*0.13865*	0.77487	0.29110	0	
7 Murray-Darling West (VIII)	0.59682	0.33174	0.29248	0.79696	*0.01360*	*0.07174*	0
Φ_{st} among populations of *Philypnodon macrostomus*. All values are significant after Bonferroni correction ($\alpha = 0.01$), except the italicized value.						
	1	2	3	4	5		
1 North QLD (II)	0						
2 South QLD & North NSW (III)	0.30916	0					
3 Midcoast NSW (IV)	0.53470	0.20979	0				
4 South NSW & Coastal VIC (V)	0.54529	0.23326	*0.12098*	0			
5 Murray-Darling (VII + VIII)	0.27383	0.58374	0.77537	0.73270			

whether or not population structuring is related to distance between populations, a Mantel test for correlation between Φ_{st} and geographical distance was performed with Arlequin, using 1000 permutations of the matrices. Haplotype networks were constructed with Arlequin, and were rendered with Adobe Illustrator CS (Adobe Systems). Geographic distances between each population were calculated using ArcView version 3.3 (Environmental Systems Research Institute), and the distance and azimuth matrix extension, version 2.1 (Jenness, 2005). All values were calculated as straight-line distances because there is no ideal way to calculate intrariverine distances for rivers that flow to the ocean and never physically connect. Many variables may influence the effective distance when connectivity is established; we use straight-line distances merely as a best estimate.

RESULTS

Sequences and analyses

A total of 155 *Philypnodon* individuals were sequenced, including 99 *P. grandiceps* and 56 *P. macrostomus*. These, along with 12 individuals of five basal gobioid outgroup species, were included in the phylogenetic analysis, making a total of 167 individuals. The overall matrix was composed of 1179 base pairs, of which 512 were phylogenetically informative, including the complete cyt*b* gene, as well as the first 39 base pairs of the threonine transfer RNA (sequences are available in GenBank under accession numbers DQ463807–DQ463961). Fewer informative characters were present in within-species comparisons: 101 for *P. grandiceps* and 77 for *P. macrostomus*. MrModeltest indicated that the GTR + I + G model was most appropriate for these data, based on both the LRT and AIC. Results from the Bayesian, neighbour-joining, and parsimony analyses were generally consistent with one another; the most notable difference among hypotheses was in the placement of *P. macrostomus* individuals from the coastal Victoria localities Snowy River (31) and Miranda Creek (32). In the Bayesian analysis, these individuals are placed in a basal polytomy with the other *P. macrostomus*; the neighbour-joining and parsimony analyses place them as a sister group to individuals from the EC drainages. The Bayesian results are presented in Figure 2, and the neighbour-joining results are presented in Figure 3: both are discussed below. For the Bayesian hypothesis, posterior probability values are indicated for major nodes; values range from 98 to 100%. The jackknife values for major nodes in the neighbour-joining hypothesis are 96–100%, but most nodes within species are supported at less than 60%.

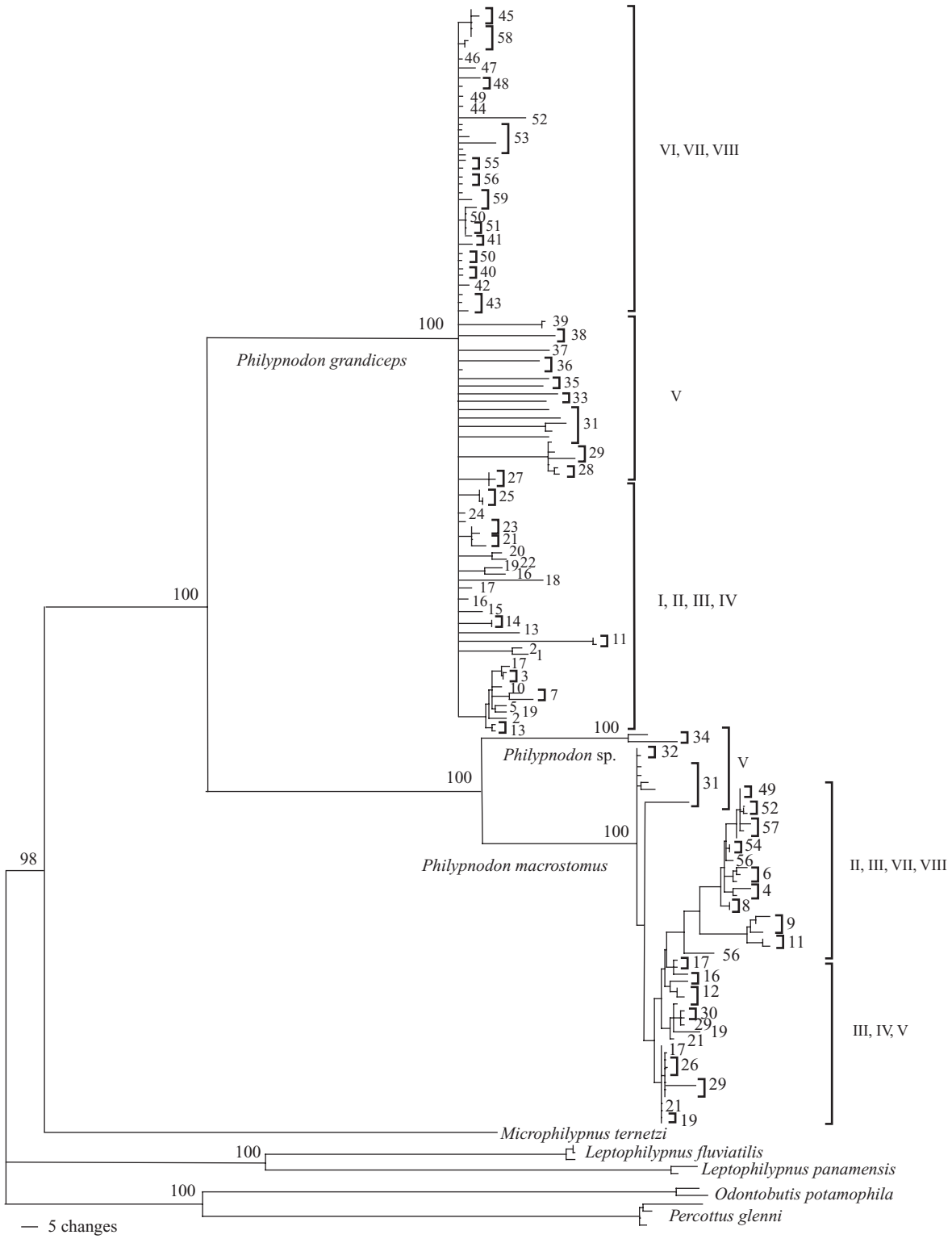

Figure 2. Bayesian estimate of the phylogeny for *Philypnodon* individuals, based on 1179 base pairs of sequence data, including the complete cytochrome *b* gene and partial threonine transfer RNA. Numbers on nodes are the posterior probability values of clades (multiplied by 100). Species are identified on the branch subtending their clades; numbers on the terminals represent collection localities, and roman numerals indicate geographical partitions, in accordance with Figure 1 and Table 1.

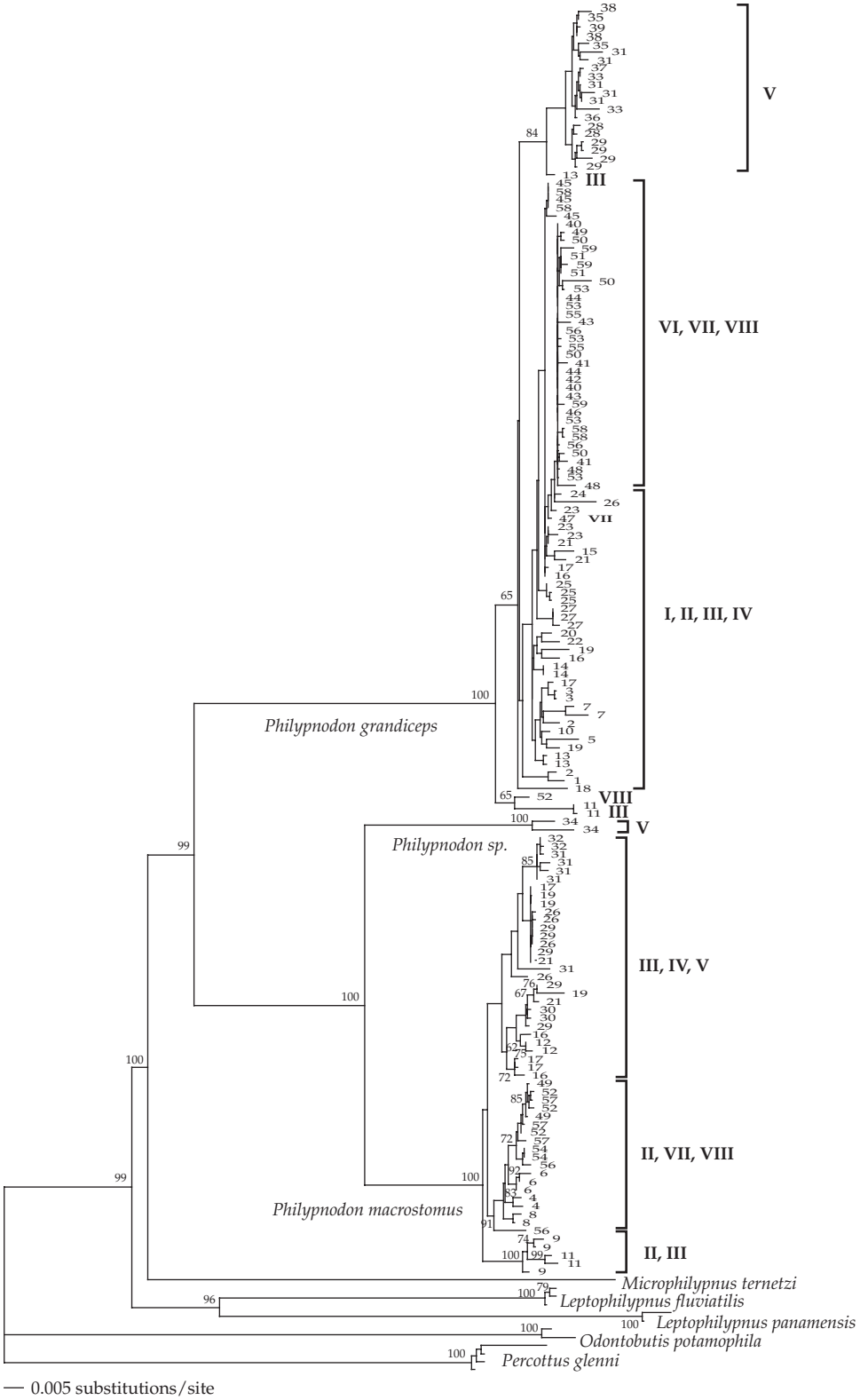

Figure 3. Neighbour-joining dendrogram of *Philypnodon* individuals, based on the same DNA sequence data used for Figure 2. The numbers on the terminals represent collection localities; the roman numerals indicate geographical partitions, in accordance with Figure 1 and Table 1.

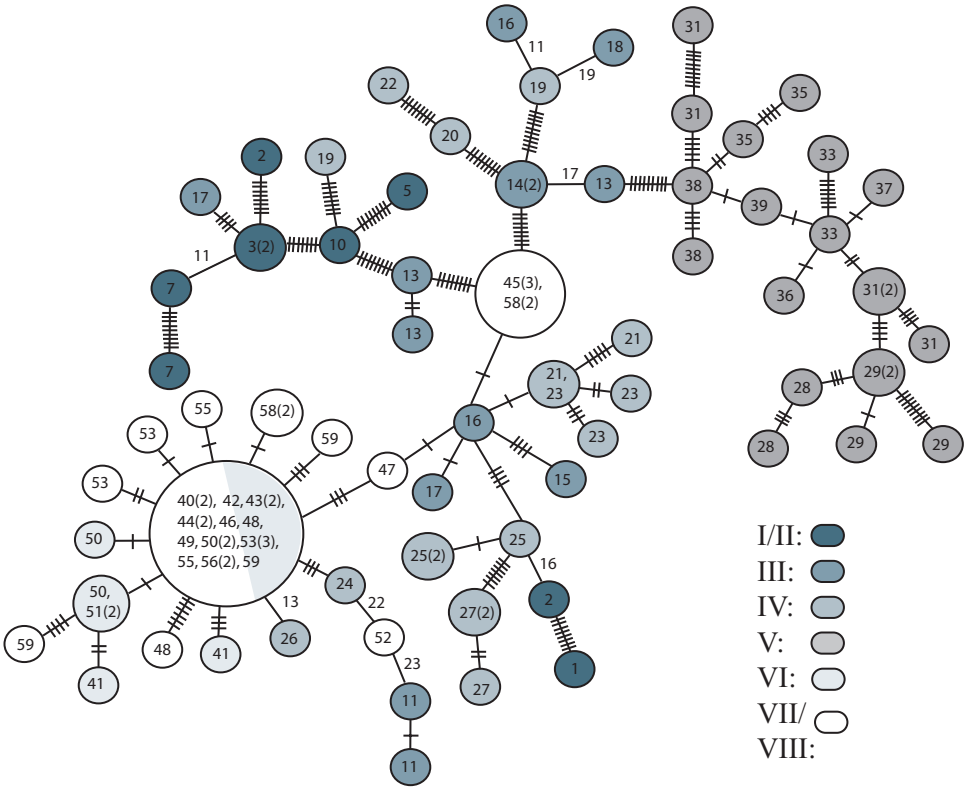

Figure 4. Haplotype network for *Philypnodon grandiceps*. Haplotypes are represented by circles, with numerals denoting sample localities, in accordance with Table 1. If more than one individual from one locality shares the haplotype, the number of individuals is indicated after the locality code in parentheses. Tick marks indicate the number of changes between haplotypes; lines are not drawn to scale, and greater than ten changes are indicated by numerals. Shading indicates the geographical partitions.

Thus, although more structure is present in the neighbour-joining hypothesis, it should be recognized that much of this structure is weakly supported. This lack of resolution is likely to have resulted from the recency of the population divergences, yielding few informative characters in the cyt*b* sequences. Additionally, only a few individuals were sampled from each drainage, revealing broad-scale patterns, but this sampling is likely to be insufficient for extremely fine-scale interpretations. Haplotype networks are also presented for *P. grandiceps* (Fig. 4) and *P. macrostomus* (Fig. 5), with partition groupings indicated.

Phylogeography of *Philypnodon grandiceps*

The Bayesian and neighbour-joining hypotheses show consistent results for relationships of populations within *P. grandiceps*, with less resolution in the Bayesian hypothesis. In the Bayesian hypothesis (Fig. 2), most individuals are placed in a basal polytomy, with a few smaller groups within it. Populations from southern New South Wales/coastal Victoria, including individuals from Mogo Creek NSW (28), Millingandi Creek NSW (29), Snowy River VIC (31), Darby River VIC (33), Steele Creek VIC (35), Woady Yaloak River VIC (36), Lake Colac VIC (37), Curdies River VIC (38), and Mt Emu Creek VIC (39), exhibit notably longer branch lengths than the majority of the other populations. These drainages are clustered on the south-eastern coast of Australia (partition V of Table 1), and all drain to the ocean. The Mogo Creek NSW (28) and Millingandi Creek NSW (29) individuals (the most northerly populations in partition V) are grouped together.

Other samples in *P. grandiceps* are placed in a polytomy, with little additional structure being evident. Individuals from Cudgegong River NSW (45) are grouped with those from Gawler River SA (58). Another group, including individuals from Dawson River QLD (2), Maryvale Creek QLD (3), Oyster Creek QLD (5), Burnett River (Mingo Crossing) QLD (7), Isis River QLD (10), Moogerah Lake QLD (13), Leycester Creek NSW (17), and Hickeys Creek NSW (19), is distinguished, although some individuals from Dawson River QLD (2), Leycester Creek NSW (17), and Hickeys Creek NSW (19) are also present in the

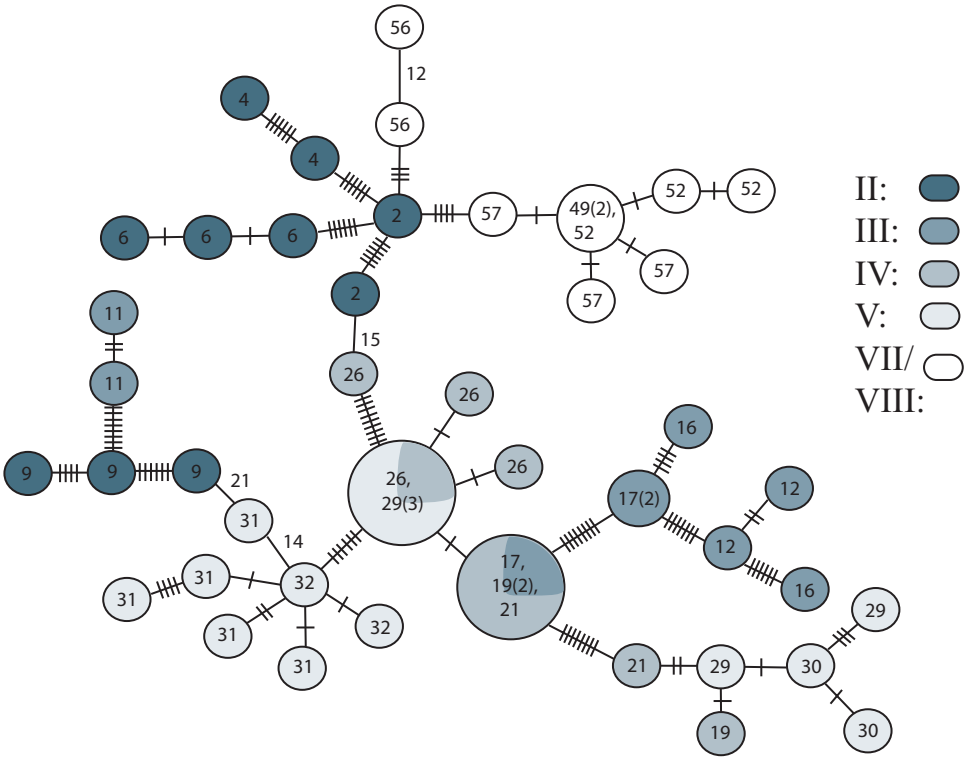

Figure 5. Haplotype network for *Philypnodon macrostomus*. Numbering and conventions follow those for *Philypnodon grandiceps* in Figure 4.

overall polytomy. The remainder of *P. grandiceps* individuals show unresolved relationships, with individuals from the same locality grouping together in some cases.

The neighbour-joining hypothesis (Fig. 3) exhibits more resolution than the Bayesian hypothesis (because of the constraints of the method), but many internodes are very shallow. The hypothesis places the partition-V individuals as a distinct grouping, with one partition-III individual from Moogerah Lake QLD (13) included as the sister to the remainder. Most individuals from the remaining partitions form a group: with partition-I, -II, -III and -IV individuals placed as a grade basal to the partition-VI, -VII and -VIII individuals. There are a few instances of overlap: one partition-VII individual [from Murray River, Albury NSW (47)] is placed among those of partition IV, and three individuals from partitions-VIII and III [from Murray River, Berri SA (52) and Amamoor Creek, Amamoor QLD (11), respectively] are placed outside the remainder of the sampled *P. grandiceps*. The placement of these individuals apart from the majority of those in their geographical partitions is consistent with incomplete lineage sorting or retained ancestral polymorphism.

The haplotype network presented in Figure 4 reinforces the patterns seen in the phylogenetic trees. The most distinct grouping of haplotypes is that consisting of individuals from partition V [closest to Moogerah Lake QLD (13), as in the phylogenies], with no overlap between these haplotypes and the others. Other partitions are distributed in between two and a few clusters throughout the network, with some haplotypes shared between partitions VI, VII, and VIII. As indicated in the neighbour-joining hypothesis, individuals from Cudegong River NSW (45) and Gawler River SA (58) share haplotypes, and individuals from Murray River, Berri SA (52), and Amamoor Creek (11) form a distinct grouping.

PHYLOGEOGRAPHY OF *PHILYPNODON MACROSTOMUS* AND *PHILYPNODON* SP.

The Bayesian hypothesis indicates that *P. macrostomus* and *Philypnodon* sp. form the sister clade to *P. grandiceps*, supported with 100% posterior probability. Two individuals from *Philypnodon* sp. were included: both from Lang Lang River VIC (34). Individuals of *P. macrostomus* from nearby Snowy River VIC (31) and Miranda Creek VIC (32) form a basal polytomy, with the exception of one Snowy River individual, placed as sister to the remainder of *P. macrostomus*. All other *P. macrostomus* individuals are grouped together. Within the major clade of

P. macrostomus, populations are structured into several smaller groups. Populations from Delaneys Creek QLD (12), Oxley River NSW (16), Leycester Creek NSW (17), Hickeys Creek NSW (19), Hastings River NSW (21), Georges River NSW (26), Millingandi Creek NSW (29), and Maramingo Creek VIC (30) form a clade with some internal structure. The crown group within *P. macrostomus* includes the east-coast localities Baffle Creek QLD (4), Burnett River (Ceratodus) QLD (6), Elliot River QLD (8), Gregory River QLD (9), and Amamoor Creek QLD (11), as well as the MDB sites Black Swamp VIC (49), Murray River SA (52), Angas River SA (54), Onkaparinga River SA (56), and Torrens River SA (57). This group consists of individuals from the westernmost extent of the range of *P. macrostomus* in the MDB and the northeasternmost extent in the coastal streams (partitions II, III, VII, and partition VIII; *P. macrostomus* is absent from partitions I and VI).

For *P. macrostomus*, the neighbour-joining and parsimony analyses differ from the Bayesian analysis with respect to placement of some populations. Specifically, the partition-V populations Snowy River VIC (31) and Miranda Creek VIC (32) are not placed as sister to the remainder; rather, they fall inside a cluster containing other partition-V individuals, as well as those from partitions III and IV. Sister to that group is one consisting of individuals from partitions II, VII, and VIII, and finally the most basal grouping consists of populations from Gregory River QLD (9) and Amamoor Creek QLD (11). In the Bayesian hypothesis, Gregory River and Amamoor Creek individuals are grouped with other members of partitions II, III, IV, and V. In both the Bayesian and the neighbour-joining hypotheses, Gregory River and Amamoor Creek individuals are placed separately from more northerly partition-II populations [Baffle Creek, Burnett River (Ceratodus) and Elliot River] and more southerly partition-III populations (Delaneys Creek, Oxley River, and Leycester Creek), revealing a more complex, finer-scale set of population divergences than is shown in the geographical partitions in Figure 1.

Neither the Bayesian nor the neighbour-joining hypotheses show an exact parallel in *P. macrostomus* with the evidence in *P. grandiceps* for a distinct group of individuals from the southern New South Wales/coastal Victoria areas (partition V). However, the westernmost of those localities (Snowy River VIC and Miranda Creek VIC; 31 and 32) correspond to the most basal *P. macrostomus* individuals, which are well-separated from the remainder of the individuals in the Bayesian hypothesis, and are a distinct, derived group in the neighbour-joining hypothesis. Both hypotheses also indicate that within *P. macrostomus*, populations in partitions II, VII, VIII, and northern partition III, a disjunct set of populations from northern Queensland and MDB, are different from the remainder.

The haplotype network (Fig. 5) paralells these conclusions. There is overlap among populations from partitions III, IV, and V, with common haplotypes shared between partitions V and VI, and between partitions VI and V. Individuals from partitions II, VII, VIII, and the northern population from partiton III form two groupings distinct from the remainder and from each other. One group consists of individuals from Gregory River QLD (9) and Amamoor Creek QLD (11), which are linked to a partition-V population [Miranda Creek VIC (31)] but with many changes in haplotypes. The second includes the remaining partition-II sites, joined with those from VII and VIII, and linked to a partition-IV population [Georges River NSW (26)]. but again only distantly.

Population structure in *Philypnodon* species

The genetic diversity measures h and π were similar for all partitions; values for h were the maximum possible (1.0), and for π were very low (0.042–0.0186 for *P. grandiceps*, and 0.0049–0.0158 for *P. macrostomus*). Simulated and observed patterns for the mismatch distribution are presented in Figure 6. The results for population Φ_{st} measures are given in Table 2. With the exception of a few neighbouring partitions, all values are significant after Bonferroni correction, indicating a high degree of structure among population partitions. Mantel tests indicted that neither species exhibited a significant correlation ($r^2 = 0.0603$, $P = 0.166$ for *P. grandiceps*, and $r^2 = 0.0835$, $P = 0.185$ for *P. macrostomus*) between population Φ_{st} and geographical distance.

DISCUSSION

Phylogenetic relationships among *Philypnodon* species and outgroups

The phylogenetic hypotheses presented in Figures 2 and 3 support the monophyly of *Philypnodon*, as well as of the included species *P. grandiceps*. *Philypnodon macrostomus* populations also form a clade, with individuals from the Lang Lang River (34; the western extent of the range for *P. macrostomus*) widely separated from the remainder. These individuals may represent an undescribed species; the clade is here labelled *Philypnodon* sp., and is sister to *P. macrostomus*. Hoese & Reader (2006) also noted in their description of *P. macrostomus* that additional undescribed species of *Philypnodon* may exist, and that the systematics of this genus is in need of revision. Support values for species-level groupings and above are high (96–100%). The place-

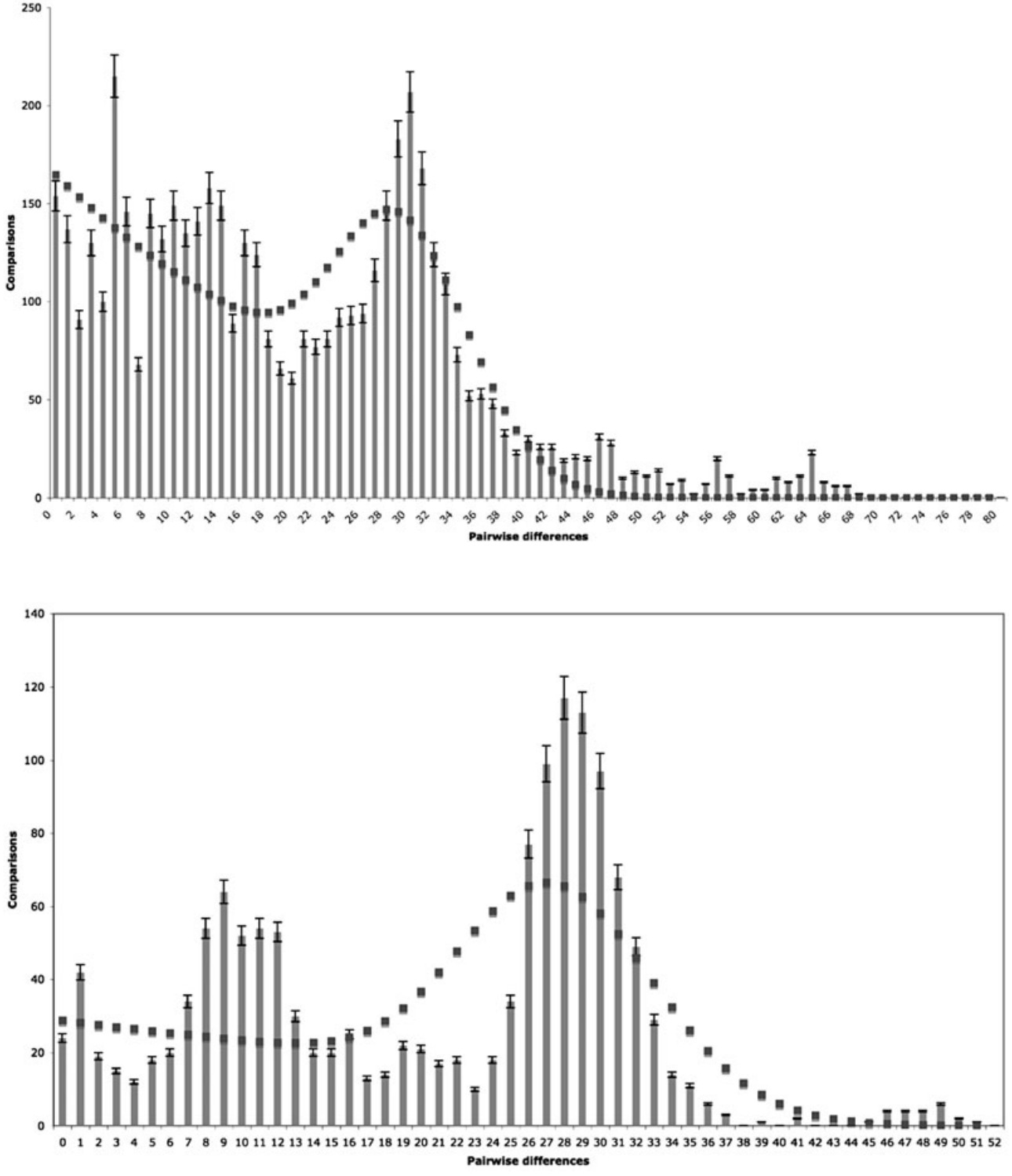

Figure 6. Mismatch distribution graphs for *Philypnodon grandiceps* (above) and *Philypnodon macrostomus* (below). Bars indicate the observed frequencies of pairwise differences (with 5% error bars); plotted lines indicate the patterns predicted by a sudden population expansion model.

ment of *Philypnodon* relative to outgroup taxa *L. fluviatilis*, *L. panamensis*, *M. ternetzi*, *O. potamophila*, and *P. glenni* is concordant with the relationships shown in Thacker & Hardman (2005), with the addition of *P. macrostomus* and *Philypnodon* sp. as sisters to *P. grandiceps*.

Phylogeographical comparison between species across the Eastern Highlands

The mitochondrial DNA-based phylogeny shows some structure among populations of both *P. grandiceps* and *P. macrostomus*, but much of it is weakly supported, consistent with historical gene flow among populations. Related haplotypes within *P. grandiceps* are found on either side of the Eastern Highlands in western Victoria and South Australia, although it is unclear whether populations mixed via coastal connections (between drainages adjacent to the Murray River mouth), or across the Eastern Highlands. Either is possible, as the Eastern Highlands are very subdued in this region, with little significant separation between north- and south-flowing drainages. Unfortunately, *P. macrostomus* is absent from these coastal drainages (but is present in MDB), thus limiting comparisons within *Philypnodon*. However, a similar biogeographical pattern is found in the fishes *R. semoni* (Hammer *et al*., 2007) and *Gadopsis marmoratus* Richardson, 1848 (Miller *et al*., 2004), with populations intermixed or closely related across the Eastern Highlands in western Victoria and South Australia. Evidence for a sister relationship between populations of the crayfish *Cherax destructor* Clark, 1936 from western Victoria and MDB also exists (Nguyen *et al*., 2004; Nguyen & Austin, 2005). However, patterns within the crayfish genus *Euastacus*, which have a similar distribution, were not congruent (Shull *et al*., 2005); crayfish from Glenelg River group with those from further east (Yarra River), and both are sister to MDB populations.

Despite the long distance of the drainage divide separating MDB and EC populations, the only region that has clear evidence for divide crossing is between the Burnett River and MDB within *P. macrostomus*. There is a close relationship between three northern drainages, Baffle Creek QLD (4), Burnett River QLD (6), and Elliott River QLD (8), and MDB, which is evident in both the phylogenies and the haplotype network. Of these three EC drainages, only the Burnett River shares a drainage divide with MDB, and is thus the most likely area in which the crossing occurred. Surprisingly, despite sympatry, there is no evidence of a similar relationship within *P. grandiceps*. Some other fishes show a similar pattern with recent movement across this region of the Eastern Highlands: *Hypseleotris klunzingeri* (Ogilby, 1898) and *Hypseleotris* sp.. 3/*H. galii* (Thacker *et al*., 2007). However, several species also lack this pattern, despite high levels of sympatry (both locally and broadly) with species that have crossed this divide: *Hypseleotris* sp.. 5 (Midgleys carp gudgeon) (Thacker *et al*., 2007) and *R. semoni* (Hammer *et al*., 2007). It seems likely that *P. grandiceps* has also crossed the Eastern Highlands earlier in their evolutionary history, given that some EC populations appear to be sister to MDB. However, it is unclear, given the lack of resolution, where such crossings may have occurred; it was likely to be somewhere in the northern half of New South Wales (Fig. 3).

One other region is separated by local drainage divides not associated with the Eastern Highlands. Populations inhabiting rivers draining into the Gulf of St Vincent SA (pops 55–59), are separated from populations in the MDB by the Mount Lofty Ranges. In both *Philypnodon* species, Gulf of St Vincent populations are closely related to those from MDB, with both species sharing identical or very similar haplotypes with other MDB individuals. Whether movement occurred between these basins over drainage divides or via coastal connections is ambiguous. Connections during lowered sea levels are quite likely (Unmack, 2001); however, patterns are further complicated by water transfers via pumping from the MDB into Gulf of St Vincent drainages. For instance, *P. grandiceps* has a long record of collection from this region, but *P. macrostomus* along with several other MDB species have only more recently been recorded, suggesting recent introductions may have occurred (Hammer & Walker, 2004).

Phylogeographical comparison between species among coastal drainages: the influence of continental shelf width

The continental shelf of Australia adjacent to the range of *Philypnodon* species varies in width, which in turn influences the degree to which present-day rivers may coalesce during periods of lower sea levels, and thus allow populations to mix (Unmack, 2001). Continental shelf width is broad from the Gulf of St Vincent (250 km, measured to 200 m below sea level) to slightly east of the mouth of the Murray River (120 km), it then narrows to between 30 and 60 km until near Cape Otway. East of this point, all land between southern Victoria and northern Tasmania is exposed during low sea levels, with drainages all coalescing into one system. The eastern edge of this drainage system has its drainage divide near Wilsons Promontory during low sea levels, potentially isolating populations between coastal Victorian drainages east and west of Wilsons Promontory. East of Wilsons Promontory, the continental shelf quickly becomes

especially narrow (20–40 km) along most of coastal NSW, and gradually broadens (50–100 km) from Brisbane River north to Burnett River, and becomes very broad by the Fitzroy River (greater than 200 km). Little coalescence between major rivers appears to occur during low sea levels in regions of especially narrow (less than 50 km) continental shelf width (Unmack, 2001).

Both species demonstrate some slight differentiation in drainages around the coast of south-eastern Australia. Within *P. grandiceps* the largest phylogeographical discontinuity occurs at the boundaries of partition V: between Kangaroo River NSW (27) and Mogo Creek NSW (28), as well as between Mt Emu Creek VIC (39) and Lake Bolac/Hopkins River VIC (40, 41) (all three are part of the same drainage system, albeit separated by Hopkins Falls). This discontinuity is broadly congruent with a significant change in continental shelf width between western Victoria and the former terminus of 'Lake Bass', a large drainage that existed between southern Victoria and northern Tasmania during times of lowered sea levels (Blom & Alsop, 1988; Unmack, 2001). No other clear genetic divergences exist within *P. grandiceps*, but one broad trend is that most northern coastal individuals from Moogerah Lake QLD (13) north to Dawson River QLD (2) weakly group together along with two haplotypes from southern populations, one individual each from Leycester Creek NSW (17) and Hickeys Creek NSW (19); this grouping is also reflected in the haplotype network. Three northern populations are outside of this group and are somewhat distinctive relative to other populations. These consist of two haplotypes from Fairbairn Dam QLD and Dawson River QLD (1, 2, Fitzroy River), and Amamoor Creek QLD (11, Mary River), each of which are genetically more distinct than the surrounding populations, and each of which cluster apart from the remaining haplotypes, separated by several changes. The remaining populations from Kangaroo River NSW (27) north to Coomera River QLD (15) do not form any obvious coherent grouping.

Within *P. macrostomus* and *Philypnodon* sp. the largest coastal genetic separation occurs between Lang Lang River VIC (34) and Miranda Creek VIC (32). At present no geographically intermediate populations are known. This separation corresponds to Wilsons Promontory, which forms the fish faunal boundary between Eastern and Bass provinces (Unmack, 2001). There appears to be little significant congruence with these coastal patterns in *Philypnodon* and other aquatic species. One region between the Brisbane and Mary rivers (QLD) has been identified as being a barrier in several fishes: *H. klunzingeri*, *Hypseleotris* sp. 5, *Nannoperca oxleyana* Whitley, 1940, *Pseudomugil signifer* (Kner, 1866), and *Rhadinocentrus ornatus* Regan, 1914 (Hughes *et al*., 1999; Page, Sharma & Hughes, 2004; Wong, Keogh & McGlashan, 2004; Thacker *et al*., 2007). However, many of these species differ in the degree of genetic divergence, and in some cases populations from geographically intermediate areas have not been examined. Thus, they can only be considered congruent in a broad tentative sense.

When compared with continental shelf width patterns, some interesting patterns emerge. There is a broadly congruent genetic discontinuity in *P. grandiceps* in south-western Victoria, where the continental shelf rapidly broadens extensively. A divergence also exists in *P. macrostomus* and *Philypnodon* sp. in the vicinity of Wilsons Promontory (VIC), also corresponding to a rapid change in continental shelf width, possibly associated with the drainage divide that forms here during low sea levels. Only one notable genetic divergence exists between Wilsons Promontory (VIC) and the Brisbane River (QLD) in *P. grandiceps*. Despite this region having the narrowest continental shelf in the study area, it shows the highest degree of genetic similarity between populations, implying moderately recent movement between at least some of these drainages. North of the Brisbane River, divergences in both species generally increase, with genetically distinct populations occurring from Mary River (Amamoor Creek QLD, 11), as well as some distinct populations between Burnett and Fitzroy River populations. Thus, the pattern appears to be the opposite of what would be expected (more mixing should occur in areas with a broader continental shelf). These northern populations are at the edge of the ranges for both species, and it is possible that other factors such as climate play a role in influencing movement patterns. A similar situation exists within *P. signifer* (Wong *et al*., 2004) and *R. semoni* (Hammer *et al*., 2007). In *P. signifer* there is almost no structure across its range from southern NSW until a discontinuity near Mary River QLD. In *R. semoni*, a single clade occurs in a broad region from Wilsons Promontory north to the vicinity of the NSW–QLD border. North of these clades, in both species, populations show a higher degree of structure, implying a decrease in population connectivity. Thus, in the regions with a narrow continental shelf there is evidence for limited population structure, but once the continental shelf becomes wider, population structure becomes more apparent.

Wong *et al*. (2004) and Hammer *et al*. (2007) hypothesized that there are likely to be differences in the ecology of populations in both *P. signifer* and *R. semoni* in different parts of their ranges. Like *Philypnodon*, both of these species may also be found in upper estuaries, and may have some ability to move between basins during flood plumes that could

occasionally allow mixing between rivers (Williams, 1970; Wolanski & Jones, 1981; Grimes & Kingsford, 1996). Alternatively, these species may differ in their ecology across their range, or may be composed of ecologically divergent cryptic species. Some populations (or cryptic species) may have evolved higher salinity tolerance, and/or a means to disperse as larvae that are capable of very occasionally being able to travel via the ocean between coastal rivers, thereby increasing the likelihood that individuals may move between drainages in the absence of any coalescence between rivers caused by narrow continental shelf width. The other possibility would be via movement across drainage divides between coastal rivers, but this would imply that fishes are moving across many drainages in a relatively short period of time. Additionally, these species (except possibly *R. semoni*) generally do not occur far enough upstream to be able to take advantage of such a method of dispersal.

Overall phylogenetic concordance

Despite similarities in ecology and largely sympatric distributions of *Philypnodon* species, there were no examples of concordance in locations where drainage-divide crossings were inferred. In the Burnett River, only *P. macrostomus* populations appear to have crossed the Eastern Highlands. *Philypnodon grandiceps* may have crossed in western Victoria, but this movement may have alternatively been between the mouth of the Murray River and adjacent drainages. The same situation occurs between Gulf of St Vincent drainages and MDB. The only aspect of coastal congruence between *P. grandiceps* and *P. macrostomus* is the distinctiveness of the Mary River populations (Amamoor Creek QLD; 11). The potential drainage-divide crossings identified are congruent with those found in some other fishes and crayfishes, but only a few aquatic species have been examined in this region. There also appeared to be little congruence in the genetic discontinuities among coastal populations of *Philypnodon* as compared with other aquatic taxa.

Population genetics analyses and isolation by distance

Both *P. grandiceps* and *P. macrostomus* exhibited low genetic and high haplotype diversity for cyt*b*. These measures may indicate that the populations have undergone relatively recent, sudden expansions, possibly associated with colonization into new habitats. However, the mismatch distributions for the populations do not match the predictions of the sudden expansion model very well. When all individuals of a species are combined, and the mismatch distributions examined, the fit still does not match the sudden expansion model (Fig. 4), although the shapes of the distributions are similar.

The population Φ_{st} values presented in Table 2 indicate that most populations are significantly distinct. If continental shelf width is influencing population mixing, we would expect that the Φ_{st} comparisons among populations where the shelf is narrowest (III vs. IV and IV vs. V) would be the highest. The Φ_{st} measures for comparisons of partitions adjacent to a broad shelf (I vs. II and II vs. III) should be the lowest, and those with a moderate shelf width (V vs. VI and VI vs. VIII) should have intermediate values. This hypothesis is only partially supported: in *P. grandiceps*, the Φ_{st} between partition I and II/III is low and nonsignificant (0.06251), as predicted, and there is also a nonsignificant Φ_{st} detected in the intermediate shelf width comparison of VI and VIII (0.01360). However, a second comparison with intermediate shelf width (V vs. VI) is quite high (0.83196). One of the narrow shelf-width comparisons (IV vs. V) shows a high Φ_{st} (0.67894), but the other does not (II/III vs. IV, 0.08285). For *P. macrostomus*, the hypothesis is soundly rebuffed: the comparison predicted to have the lowest Φ_{st} (II vs. III) is significant (0.30916), and those predicted to be the highest are in fact lower (III vs. IV, 0.20979; IV vs. V, 0.12098).

Instead of a simple relationship between shelf width and mixing, the the Φ_{st} values reinforce the patterns seen in the phylogeographical analyses: partition V is the most distinct. For *P. grandiceps*, the highest Φ_{st} values (ranging from 0.53814 to 0.83196) are found in comparisons between partition V and the other areas, with the exception of comparisons between partition I and VI or VIII. For *P. macrostomus*, the most distinct populations, as revealed by Φ_{st}, are those in the MDB (partitions VII and VIII), except as compared with the north Queensland partition II. Mantel tests indicated that neither species exhibited a significant correlation between Φ_{st} and geographical distance, thereby refuting the isolation-by-distance model.

CONCLUSIONS

This study adds to the small but growing body of evidence that suggests most crossings of the Eastern Highlands in south-eastern Australia have either occurred in the vicinity of Burnett River (Thacker *et al*., 2007), or in western Victoria (Miller *et al*., 2004; Nguyen & Austin, 2005; Hammer *et al*., 2007), with species subsequently expanding their coastal range by moving between coastal drainages. In the species studied in south-eastern coastal drainages there appears to be some congruence in where genetic discontinuities occur, with most differences being in the

drainages between the Brisbane and Fitzroy rivers (Hughes *et al*., 1999; Page *et al*., 2004; Wong *et al*., 2004; Hammer *et al*., 2007; Thacker *et al*., 2007), with much of coastal NSW being somewhat genetically uniform. However, there appears to be little evidence for phylogeographical patterns that are congruent in time and space, as genetic divergences across these drainages are often quite different (Thacker *et al*., 2007). This lack of congruence is highlighted in sympatric closely related species, such as those in the genera *Hypseleotris* and *Philypnodon*, with quite similar ecologies and distributions, yet often having different phylogeographical patterns (Thacker *et al*., 2007). The lack of genetic differentiation in coastal NSW drainages suggests higher connectivity between populations in this area (Jerry & Baverstock, 1998; Wong *et al*., 2004; Hammer *et al*., 2007; Thacker *et al*., 2007). This observation runs counter to the prediction that population connectivity should be higher in areas where the continental shelf is widest, facilitating transfer between drainages when the sea level drops. One alternate explanation is that some aspect of their life history or ecology allows occasional movement between rivers (Jerry & Baverstock, 1998; Unmack, 2001; Wong *et al*., 2004). This would manifest in the phylogeographical and population genetic signatures as stochastic patterns of drainage connectivity, which are likely to differ among sympatric species, as demonstrated here for the two species of *Philypnodon*.

ACKNOWLEDGEMENTS

The authors thank the many people who provided tissue samples and assistance with fieldwork in Australia, especially M. Adams, M. Baltzly, M. Hammer, G. Knowles, T. Raadik, &and R. Remington, &and the various state fisheries agencies who provided collecting permits. CET thanks the Australian Museum, Sydney (AMS), for a Collection Fellowship enabling the study of collections of *Philypnodon* species, and AMS staff J. Leis, M. McGrouther, K. Parkinson, and T. Trinski for their assistance. Comments from two reviewers greatly improved the manuscript. This study was supported by a grant from the National Science Foundation (NSF DEB 0108416), and by grants from the W. M. Keck and R. M. Parsons Foundations in support of the program in Molecular Systematics at the Natural History Museum of Los Angeles County.

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