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# East-west genetic differentiation in Musk Ducks (*Biziura lobata*) of Australia suggests late Pleistocene divergence at the Nullarbor Plain.

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**Running head:** *Phylogeography of the Musk Duck*

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## Abstract

Musk Ducks (*Biziura lobata*) are endemic to Australia and occur as two geographically isolated populations separated by the Nullarbor Plain, a vast arid region in southern Australia. We studied genetic variation in Musk Duck populations at coarse (eastern versus western Australia) and fine scales (four sites within eastern Australia). We found significant genetic structure between eastern and western Australia in the mtDNA control region ($\Phi_{ST} = 0.747$), one nuclear intron ($\Phi_{ST} = 0.193$) and eight microsatellite loci ($F_{ST} = 0.035$). In contrast, there was little genetic structure between Kangaroo Island and adjacent mainland regions within eastern Australia. One small population of Musk Ducks in Victoria (Lake Wendouree) differed from both Kangaroo Island and the remainder of mainland eastern Australia, possibly due to genetic drift exacerbated by inbreeding and small population size. The observed low pairwise distance between the eastern and western mtDNA lineages (0.36%) suggests that they diverged near the end of the Pleistocene, a period characterised by frequent shifts between wet and arid conditions in central Australia. Our genetic results corroborate the display call divergence and Mathews' (1914) subspecies classification, and confirm that eastern and western populations of Musk Duck are currently isolated from each other.

Keywords: Arid zone, microsatellite, mitochondrial DNA, nuclear intron, Nullarbor Plain, Waterfowl

## Introduction

The Nullarbor Plain, a vast arid region of porous limestone and calcareous sandstone in southern Australia, represents a major biogeographic barrier for east-west dispersal of temperate Australian plant and animal species (e.g. Keast 1981). The avifauna of southern Australia is characterised by an east-west division at the Nullarbor (Cracraft 1986), with numerous avian species displaying morphological divergence on either side of the Nullarbor sufficient to suggest subspecific differentiation (Schodde & Mason 1999).

Because surface water in the Nullarbor drains away rapidly, this region does not support permanent wetlands. The distribution of Australian waterbirds depends on availability of water in the arid zone (Morgan 1954; Frith 1957, 1959; Roshier *et al.* 2002; Poiani 2006), and thus the Nullabor is predicted to be an important boundary in the distribution of waterbirds. While the importance of the Nullarbor has been examined using genetic data (Toon *et al.* 2007; Neaves *et al.* 2009; Salinas *et al.* 2009), its role as a barrier for dispersal in waterbirds has not been investigated.

Australian waterfowl (Anatidae) such as Grey Teal (*Anas gracilis*), Australasian Shoveler (*Anas rhynchotis*), Pink-eared Duck (*Malacorhynchus membranaceus*) and Freckled Duck (*Stictonetta naevosa*) are highly nomadic and can be found at widespread inland locations after major flooding events (Frith 1967; Briggs 1992). For these species, large scale east-west dispersal seems to occur through the Northern Territory rather than through the Nullarbor (Frith 1962). Other species like Chestnut Teal (*Anas castanea*), Blue-billed Duck (*Oxyura australis*) and Musk Duck (*Biziura lobata*) are less nomadic and have regular movement patterns, moving to inland ephemeral wetlands in winter and spring for breeding, and returning to permanent wetlands closer to the coast in summer and autumn (Frith 1967). For the latter three species, the lack of wetlands on the Nullarbor presumably impedes east-west dispersal, but transient flooding may allow some dispersal to occur through the Nullarbor in wet years.

Musk Ducks are a particularly interesting species among Australian waterfowl, because their distribution does not extend to the Northern Territory. Although fossil records suggest that Musk Ducks were formerly more widely distributed (e.g. Worthy 2002), they currently occur as two geographically isolated populations separated by the Nullarbor (Marchant & Higgins 1990). Thus, their east-west dispersal capacity is predicted to depend critically on the availability of water in the Nullarbor. Musk Ducks also occur on Kangaroo Island and in Tasmania (Barrett *et al.* 2003), which are separated from mainland Australia by Backstairs Passage and Bass Strait, respectively.

Historically, Musk Ducks were thought to be flightless (Ramsey 1867; Campbell 1901), but now are known to fly long distances to colonise ephemeral wetlands after inland rain (Frith 1967; Brooker et al. 1979; Marchant & Higgins 1990). Band recoveries demonstrate that Musk Ducks disperse locally (Anonymous 1988a; Guay 2007). Musk Ducks have been observed on ephemeral wetlands on the Nullarbor after major flooding, but never in large numbers (Brooker *et al.* 1979; Burbidge *et al.* 1987). They also occur on, and forage in, marine habitats (Wood 1960; McCracken 1999) and have been observed in small flocks on the coast of the Nullarbor (Martindale 1980; Congreve & Congreve 1982, 1985; Barrett et al. 2003). These anecdotal sightings suggest that Musk Ducks may move between eastern and western Australia, albeit in low numbers, either inland through connecting ephemeral wetlands or possibly along the coast. Nevertheless, it remains unclear whether there is significant dispersal across the Nullarbor.

Mathews (1914, 1927) described the eastern populations of Musk Ducks as a separate subspecies (*B. l. menziesi*) distinct from the nominate western populations, although others (Phillips 1926; Hartert 1931; Delacour 1959; Parker *et al.* 1985) considered the species to be monotypic. Display postures and vocalisations of Musk Ducks have been described in detail for populations in eastern and western Australia (Serventy 1946; Stranger 1961; Johnsgard 1966; Lowe 1966). Although some or all postures are shared between eastern and western populations (Fullagar & Carbonell 1986), vocalisations differ markedly (Robinson & Robinson 1970; McCracken et al. 2002). These differences led Robinson and Robinson (1970) and McCracken *et al.* (2002) to conclude that the two populations probably have been isolated for an extended period.

While numbers of Musk Duck seem stable in Western Australia (Saunders & Ingram 1995) and Tasmania (Bryant & Jackson 1999; S. Blackhall, unpublished data), they have decreased in mainland eastern Australia (e.g. Parker *et al.* 1985; Davey 1989; Paton *et al.* 1994). Musk Ducks are currently listed as vulnerable in Victoria (Victorian Department of Sustainability and Environment 2007) and rare in South Australia (Robinson et al. 2000), but are yet to be listed in New South Wales. Little information on population size is available, but the combined eastern and western population is estimated to be 20,000 to 50,000 individuals (Wetlands International 2006). Major threats to the population include habitat loss due to drainage for agriculture and possible competition for food with introduced European carp (*Cyprinus carpio*) in the Murray Darling Basin (McCracken 2005). Decreased habitat availability over the last decade, due to a long lasting drought in southeastern Australia, probably has contributed to recent population declines. Because numbers of Musk Ducks are not declining nationally, conservation or recovery efforts have not been initiated. Conservation efforts should not be limited to species or subspecies, but rather target populations that are ecologically and/or genetically distinct (Moritz 1994; Crandall *et al.* 2000). Understanding patterns of divergence and gene flow between eastern and western Australia is therefore essential for the proper management of the species.

We investigated genetic variation in Musk Ducks in the mitochondrial DNA (mtDNA) control region, two nuclear introns, and eight microsatellite loci, to estimate levels of population connectivity. We analysed genetic structure at a coarse scale (eastern versus western Australia) and at fine scales (four populations within eastern Australia). We predicted that genetic structure between eastern and western Australia would be consistent with display divergence, but that the dispersive ability of Musk Ducks would limit structure within eastern Australia.

## Methods

### *Sample collection*

We collected blood and/or feather samples from 89 Musk Ducks captured or collected from eastern and western Australia between 1995 and 2005 and obtained an additional 71 samples from museum tissue collections and from historical museum specimens dating as far back as the late 1800s (Appendix 1). We grouped samples geographically into five populations (Figure 1): Western Australia (WA; *n* = 16), Kangaroo Island (KI; *n* = 47), Tasmania (TAS; *n* = 8), mainland eastern Australia (SE; *n* = 55) and Lake Wendouree (LW; *n* = 34).

### *Sampling and study sites*

Most of the live captures took place on two wetlands: Murray Lagoon, Cape Gantheaume Conservation Park, Kangaroo Island, South Australia (35°54'S, 137°24'E; 1995-1997; n = 46) and Lake Wendouree in Ballarat, central Victoria (33°33'S, 143°49'E; 2003-2004; n = 34).

Murray Lagoon is a 750-1,000ha natural wetland on Kangaroo Island which greatly increased in size after flooding in 1995 (McCracken *et al.* 2000). Musk Ducks breed on Murray Lagoon (Baxter 1989; McCracken *et al.* 2000). The population fluctuates seasonally, and between 1995 and 1997 numbers peaked in early to mid-October (McCracken 1999). We captured Musk Ducks on Murray Lagoon using night-lighting, baited traps, and walk-in-nest-traps (McCracken *et al.* 2003).

Lake Wendouree is an artificial wetland maintained by the Ballarat city council for recreational purposes. The lake was traditionally topped up in summer, but water restrictions imposed by a recent drought meant that this practice was abandoned in 2003. In 2005, the lake dried up entirely for the first time in 50 years. Musk Ducks bred regularly and, prior to 2005, were sedentary on Lake Wendouree (Thomas & Wheeler 1983; Anonymous 2000; Guay & Mulder 2007). We captured Musk Ducks on Lake Wendouree by hand or using a hand net after they were enticed to the shore using bread morsels (Guay & Mulder 2007).

*Mitochondrial DNA and intron sequencing*

We extracted DNA from blood samples using the salting out method (Bruford et al. 1992) and from feathers, tissue and toe-pad samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA). Only DNA isolated from feathers, muscle tissue or toe-pad samples was used for amplification of mtDNA, and DNA isolated from blood or tissue was used to amplify nuclear introns and microsatellites. We amplified the 5'end of the mitochondrial genome control region (positions 82-773 in the chicken genome), intron 4 of ornithine decarboxylase (ODC1) and intron 7 of beta-fibrinogen (FGB). Primers included L81 (TATTTGGTTATGCATATTCGTGCAT; M. D. Sorenson unpublished), H493 (Sorenson & Fleischer 1996) and H774 (Sorenson *et al.* 1999) for control region, ODC1-5F and ODC1-6R (McCracken *et al.* 2009) and FGB-7F (CTCAGAAGACTGGAGCTCATTTG; M. D. Sorenson unpublished) and FGB-7R (CCRCCRTCTTCTTTNGARCACTG; M. D. Sorenson unpublished). We performed polymerase chain reactions (PCR) on a Corbett Research PC-960C thermocycler using standard recipes. Betaine (1.0M) was added to PCR reactions of samples from study skin feathers and toe-pads (Johnson & Dunn 2006). We performed PCR amplification as follows: one cycle of 7min at 94°C followed by 45 cycles of 94°C for 20s, 56°C for 20s and 72°C for 60s, and one cycle of 72°C for 7min. PCR products were separated by agarose gel electrophoresis, gel purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA), and sequenced either commercially (Macrogen, Seoul, Korea) or with the BigDye Terminator kits on ABI 3100 Genetic Analyzers (Applied Biosystems, Foster City, CA). We sequenced both strands of the mtDNA, but nuclear introns were only sequenced in one direction unless the individual was found to be heterozygous for an insertion/deletion (indel), in which case both strands were sequenced to resolve the indel (e.g. Peters *et al.* 2007).

We sequenced 652bp from the 5' end of the mtDNA control region of birds collected for this study and 373bp from most recent samples obtained from museum study skins. All but one of the variable sites were located within the first 244 bp from the 5' end of the sequence. The only variable site outside the first 244bp was a transition at position 250; this transition was unique to two specimens from Tailem Bend, South Australia (SAMA B25005 and SAMA B23004) that also shared a unique haplotype within the first 244bp. Thus, we designed an internal primer (H364; GCATGAGTAATGGGAGGTAGC) to amplify the first 244bp of the control region from older specimens for which amplification using both L81/H774 and L81/H493 had failed. Finally, we sequenced 363bp from ODC1 and 450bp from FGB. Sequences from complementary mtDNA strands were reconciled and double peaks in intron sequences were coded with IUPAC degeneracy codes and treated as polymorphisms using SEQUENCHER 3.1 (Gene Codes, Ann Arbor, USA).

### *Microsatellite genotyping*

Individuals were genotyped at eight Musk Duck-specific microsatellite loci (*Blm2, Blm3, Blm4*, *Blm5*, *Blm7*, *Blm9*, *Blm11* and *Blm12*; Guay & Mulder 2005). In all eight cases, one primer of each pair was attached with an M13 tail (CACGACGTTGTAAAACGAC) to allow for universal dye labelling (Boutin-Ganache et al. 2001). PCR reactions were performed on a Corbett Research PC-960C thermocycler as described by Guay and Mulder (2005). Fragments were separated on a CEQ 8000 automatic DNA sequencer (Beckman Coulter) and fragment size was estimated using the CEQ 8000 Genetic Analysis System software (Beckman Coulter, 2004; version 8.0.52). Genotyping was repeated for 10% of the individuals and in all cases confirmed that allelic designations were accurate.

*Statistical Analysis – sequence data*

Two specimens showed a single transition polymorphism in their mtDNA, suggesting heteroplasmy (QVM:1963/2/28 and LSUMZ 34777 from Tasmania and Kangaroo Island respectively). Both haplotypes for these two specimens were considered separately in the analysis. Gametic phase of introns was resolved using PHASE 2.1 (Stephens *et al.* 2001). PHASE uses a Bayesian algorithm to infer haplotypes from diploid genotypic data with recombination and the decay of LD with distance. Each data set was analyzed using the default values (100 main iterations, 1 thinning interval, 100 burn-in) followed by 1,000 main iterations and 1,000 burn-in (-X10 option) for the final iteration. Analysis was performed three times, and all pair probabilities were 1.00 for both loci.

We used FSTAT 2.9.3 (Goudet 1995) to test both introns for deviation from the Hardy-Weinberg equilibrium and for evidence of linkage disequilibrium between loci. We calculated haplotype (h) and nucleotide diversity ($\pi$) using ARLEQUIN 3.01 (Excoffier et al. 2005). We calculated unrooted networks using the software NETWORK 4.2.0.1 (Fluxus Technology).

*Statistical analysis – microsatellites*

Number of alleles (A) and observed ($H_O$) and expected ($H_E$) heterozygosity were calculated using GENALEX 6 (Peakall & Smouse 2006). We also calculated allelic richness (Rs; El Mousadik & Petit 1996) and the inbreeding coefficient ($F_{IS}$) using FSTAT 2.9.3. We tested each locus in each population for deviation from the Hardy-Weinberg equilibrium and tested each pair of loci in each population for linkage disequilibrium using GENEPOP 1.2 (Raymond & Rousset 1995). We performed Hardy's (2003) test using SPAGEDI 1.2g (Hardy & Vekemans 2002) to evaluate which of the two measures of population differentiation ($F_{ST}$ or $R_{ST}$) was more appropriate to use with our dataset. As part of Hardy's test, allele size permutation is performed to calculate simulated $R_{ST}$ ($pR_{ST}$). If observed $R_{ST}$ is larger than $pR_{ST}$, mutation plays an important role in population differentiation and $R_{ST}$ should be used (Slatkin 1995). Otherwise drift is the main driving force of population and $F_{ST}$ should be used (Hardy et al. 2003). $R_{ST}$ and $pR_{ST}$ were not significantly different in our data set ($P > 0.10$). We therefore used $F_{ST}$ for the analysis of population differentiation.

### *Genetic structure analysis*

We analysed genetic structure at two levels: 1) coarse scale (east versus west), and 2) fine scale (within eastern Australia). For the coarse scale analysis, samples from eastern Australia were compared to samples from Western Australia. To avoid potential biases from over-representation of samples from either Lake Wendouree or Kangaroo Island within the eastern Australia sample, we randomly selected eight samples from each of these two populations, as this was the maximum number of sequences from any other population in eastern Australia. For the fine-scale analysis, we compared samples from Kangaroo Island to samples from Tasmania and to samples from mainland eastern Australia (including eight randomly selected samples from Lake Wendouree). Finally we compared samples from Lake Wendouree to samples from Kangaroo Island and the remainder of mainland eastern Australia.

Samples from Tasmania were only used for analysis of mtDNA because of low sample size due to poor PCR amplification success of nuclear markers from old museum specimens.

The HKY model (Hasegawa et al. 1985) was identified as the best-fit model of nucleotide substitution for both mtDNA and nuclear introns in our dataset using MODELTEST 3.7 (Posada & Crandall 1998). We thus calculated pairwise $\Phi_{ST}$ values for mtDNA and introns using the closely related K80 (Kimura 1980) nucleotide substitution model in ARLEQUIN 3.01, as the HKY model is not available in this software. For both mtDNA and the introns, we also calculated $F_{ST}$ based on the haplotype frequencies using ARLEQUIN 3.01. We estimated pairwise $F_{ST}$ for the eight microsatellite loci combined using FSTAT 2.9.3. Significance values for all pairwise $\Phi_{ST}$ and $F_{ST}$ calculations were adjusted using sequential Bonferroni correction to avoid type I error (Holm 1979). Sample sizes varied between markers and populations. Uneven sample size can bias $F_{ST}$ leading to Type I error (Scribner *et al.* 2001). To test for biases in cases where sample size differed between populations and we found significant $F_{ST}$ or $\Phi_{ST}$, we repeated the analysis using a random subsample of the largest population of equal sample size to that of the other population. This procedure was repeated ten times for each population/locus combination. To determine the level of divergence between eastern and western Australia, we calculated the net mitochondrial average pairwise distance ($D_A$) between eastern and western Australia using ARLEQUIN 3.01.

*Intra-population analysis within eastern Australia*

To investigate the potential impacts of inbreeding and small population size on Kangaroo Island and Lake Wendouree (the populations with the two largest sample sizes in the southeast), we estimated theta from mtDNA haplotypes and both average pairwise relatedness and effective population size ($N_e$) using microsatellite data. Theta was calculated using DnaSp 5.10 (Librado & Rozas 2009). We calculated averaged pairwise relatedness (Queller & Goodnight 1989) and performed a permutation test (9999 permutations and 10000 bootstraps) using GENALEX 6. We estimated effective population size ($N_e$) from microsatellite data using the Linkage Disequilibrium Method (Bartley et al. 1992) implemented in the software NeESTIMATOR 1.3 (Peel et al. 2004). Finally, we tested for evidence of a recent bottleneck in either population using BOTTLENECK (Piry et al. 1999).

## Results

### *Mitochondrial sequences*

We found 13 variable sites (all transitions) and a single base pair indel in 15 distinct haplotypes (Figure 2A). Haplotype diversity (h) within populations varied between 0.250 and 0.692 and nucleotide diversity ($\pi$) varied between 0.00103 and 0.00557 (Table 1). In both cases, the lowest diversity was observed in Tasmania and the highest in western Australia.

No mitochondrial haplotypes were shared between eastern and western Australia. The two lineages were separated by a single transition (Fig. 2A). Thus, there was substantial genetic structure between eastern and western Australia for mtDNA ($\Phi_{ST}$ = 0.747; Table 2). In contrast, no significant structure was detected within eastern

Australia (Table 2). A low net average pairwise distance ($D_A$) of 0.36% separated the eastern and western lineages.

*Intron sequences*

We found six variable sites and a single base pair indel in ODC1 and six variable sites in FGB. Seven alleles were inferred for ODC1 (Figure 2B), and five alleles for FGB (Figure 2C). Neither intron deviated from Hardy-Weinberg equilibrium, and no evidence of linkage disequilibrium between loci was detected when populations were analysed separately or pooled together. Haplotype diversity (h) varied between 0.611 in western Australia and 0.770 on Lake Wendouree and nucleotide diversity ($\pi$) varied between 0.00415 on mainland eastern Australia and 0.00557 on Lake Wendouree for ODC1 (Table 1). For FGB, haplotype diversity (h) varied from 0.603 on Lake Wendouree to 0.723 in western Australia and nucleotide diversity ($\pi$) varied from 0.00341 in mainland eastern Australia to 0.00434 on Kangaroo Island (Table 1).

Many haplotypes were shared between populations for both ODC1 (Fig. 2B) and FGB (Fig. 2C). Genetic structure was detected between eastern and western Australia ($\Phi_{ST} = 0.193$; $F_{ST} = 0.157$; Table 2) for ODC1. Random resampling yielded significant $\Phi_{ST}$ ($P < 0.007$) in nine out of ten replicates. Within eastern Australia, no significant $\Phi_{ST}$ values were observed, but significant pairwise $F_{ST}$ values were observed between Lake Wendouree and both Kangaroo Island and mainland eastern Australia for ODC1 (Table 2). In both cases, random resampling yielded identical results. Although marginally significant $\Phi_{ST}$ and $F_{ST}$ were detected with FGB, no significant structure was detected with FGB at any level of analysis after Bonferroni correction (Table 2).

*Microsatellite loci*

None of the eight microsatellite loci deviated from Hardy-Weinberg equilibrium, and no evidence of linkage disequilibrium was observed. The average number of alleles per locus ranged from 7.0 in western Australia to 9.1 in Kangaroo Island, and the number of private alleles per population varied between 2 for mainland eastern Australia and 11 for Kangaroo Island (Table 3).

Using microsatellites, significant $F_{ST}$ values were observed between eastern and western Australia ($F_{ST} = 0.035$, $P = 0.001$; Table 4). All random samples from eastern Australia also yielded significant $F_{ST}$ (all $P < 0.002$) with western Australia. Within eastern Australia, we found significant pairwise $F_{ST}$ between Lake Wendouree and both Kangaroo Island ($F_{ST} = 0.050$, $P < 0.001$; Table 4) and mainland eastern Australia ($F_{ST} = 0.042$, $P < 0.001$; Table 4). In both cases, sub-sampling did not change the result. Kangaroo Island also differed from mainland eastern Australia ($F_{ST} = 0.018$, $P = 0.001$; Table 4). Identical results were obtained with ten random samples to control for uneven sample size (all $P < 0.02$).

*Intra-population analysis within eastern Australia*

Theta per site (Theta-W) estimated from mtDNA was significantly larger for Kangaroo Island (0.00373; 95% C.I.: 0.00314–0.00434) than for Lake Wendouree (0.00203; 95% C.I.: 0.00152–0.00254). Similarly, the effective population size of the Kangaroo Island population (118.7; 95% C.I.: 73.0–279.0) was larger than that of the Lake Wendouree population (31.2; 95% C.I.: 24.6–41.1). Furthermore, the Lake Wendouree population had a larger inbreeding coefficient ($F_{IS}$; 0.018) than did

Kangaroo Island (–0.055), average pairwise relatedness was higher in Lake Wendouree than on Kangaroo Island (0.053 vs 0.014; permutation test: $P < 0.05$), and in all but one microsatellite locus, heterozygosity was equal or larger in the Kangaroo Island population compared to Lake Wendouree. We found no evidence of a recent bottleneck in either the Kangaroo Island or Lake Wendouree population.

## Discussion

### *Genetic diversity*

We observed low mtDNA genetic diversity (h = 0.25–0.69 %, $\pi = 0.10–0.56$) in Musk Ducks as compared to two other species of Australian waterfowl, Grey Teal (*Anas gracilis*; h = 0.99 %, $\pi = 1.4$) and Chestnut Teal (*Anas castanea*; h = 0.97 %, $\pi = 1.3$; Joseph et al. 2009). This suggests recent population decline or long-term low effective population size for Musk Ducks possibly exacerbated by their highly polygynous lek mating system (Johnsgard & Carbonell 1996). Although our microsatellite data do not suggest a recent bottleneck event, Musk Ducks were previously more widely distributed (Worthy 2002). Alternatively, the low genetic diversity of Musk Duck may be due to their relatively smaller population size. At an estimated 20,000–50,000 (Wetlands International 2006), the global population of Musk Ducks is much lower than the estimate of >1 million for Grey Teal or 105,000 for Chestnut Teal (Wetlands International 2006).

### *Genetic structure*

We found no shared mtDNA haplotypes and substantial genetic structure between eastern and western Australia. The $\Phi_{ST}$ for the 5'end of the mtDNA control region of Musk Ducks was greater than that observed using the same marker between eastern and western populations of North American Wood Ducks (*Aix sponsa*; $\Phi_{ST}$: 0.31; (Peters et al. 2005) or between North American and Eurasian Mallards (*Anas platyrhynchos* $\Phi_{ST}$: 0.41–0.0.50; (Kulikova et al. 2005). In contrast, haplotype sharing was extensive between eastern and western Australia for both ODC1 and FGB. While ODC1 displayed significant genetic structure, FGB did not. Within eastern Australia, no significant $\Phi_{ST}$ were detected between any two populations for ODC1, but significant pairwise $F_{ST}$ was detected for both ODC1 and microsatellites between Lake Wendouree and both Kangaroo Island and mainland eastern Australia. This suggests that genetic drift rather than mutation may be responsible for differentiation within eastern Australia. Our results yielded no evidence of mtDNA gene flow across the Nullarbor Plain. Although our sampling in Western Australia was limited, the sampling in eastern Australia was extensive and presumably sufficient to detect moderately low levels of shared mtDNA haplotypes if introgression had occurred west to east.

In contrast to the mtDNA, the nuclear introns showed numerous shared alleles. Similar results are evident for other waterfowl species (e.g. Sonsthagen *et al.* 2009). Such contrasts between mitochondrial and nuclear markers may result from high female philopatry and/or incomplete lineage sorting (e.g. Funk & Omland 2003). Female Musk Ducks may exhibit higher natal site fidelity as male Musk Ducks are more often sighted at sea (McCracken 1999). While we cannot rule out gene flow across the Nullarbor mediated by males, east-west movement is likely to be limited because we found significant, albeit small, $F_{ST}$ values using microsatellite markers. Alternatively, the lack of differentiation in nuclear introns may be the result of incomplete lineage sorting. Because its effective population size is four times larger, nuclear DNA requires longer to sort to reciprocal monophyly (Moore 1995; Palumbi et al. 2001). Thus, although eastern and western Musk Duck populations have been isolated for an extended period of time, and have attained reciprocally monophyletic mtDNA, they likely have not been isolated long enough to have reciprocally monophyletic nuclear DNA at these loci.

### *Possible causes of genetic differentiation at Lake Wendouree*

The Kangaroo Island and Lake Wendouree populations differ markedly in their ecology. For example, the Kangaroo Island population exhibited seasonal movements (McCracken 1999), whereas the Lake Wendouree population, prior to the lake drying out in 2005, was sedentary (Thomas & Wheeler 1983). This difference may have influenced the genetic structure of these two populations. Compared to Kangaroo Island, the Lake Wendouree population had larger $F_{IS}$, larger average pairwise relatedness, lower theta and effective population size and lower heterozygosity. This suggests that the Lake Wendouree population was both smaller and more inbred than was the Kangaroo Island population. Although immigration to Lake Wendouree was probably taking place, our sample may have been biased toward resident birds, which were easy to capture because they were habituated to take food from humans (Biro & Dingemase 2009). Since genetic drift occurs more rapidly in small populations (Nei & Takahata 1993), the genetic differentiation we observed between Lake Wendouree and the rest of eastern Australia may thus have been the result of drift resulting from small population size and inbreeding.

*Timing of isolation*

The average mtDNA pairwise distance between the eastern and western Australia populations was low at 0.36%. Based on a rate of divergence of 9.7% per million years for the 5’ end of the mtDNA control region in ducks (Peters et al. 2005), divergence between the two lineages is recent and likely dates to the late Pleistocene. This is considerably more recent than the late Pliocene aridification of the Nullarbor that has been suggested to have led to the initial differentiation of the southern Australian avifauna (Cracraft 1986). In Australia, the Pleistocene was characterised by major fluctuations in precipitation regime (Ayliffe et al. 1998). The availability of surface water during periods of increased precipitation could have allowed wetland connectivity through the arid interior and favoured east-west dispersal of Musk Ducks. Musk Ducks are known to disperse long distances to colonise ephemeral wetlands in the arid zone (Frith 1967; Brooker et al. 1979; Marchant & Higgins 1990; Todd 1997). The last wet period occurred between 55 and 35 kyr ago and was characterised by lower temperature and higher lake levels in the semi-arid region of southeastern Australia (Bowler et al. 1986; Bowler & Teller 1986; Nanson et al. 1992; Miller et al. 1997). The amount of surface water may have been higher during these wet periods and thus perhaps connectivity was increased at that time. Since the end of the last wet period, 35 kyr bp, the Nullarbor has been drier, restricting movement between isolated populations that are now differentiated.

The Nullarbor is a well defined isolating barrier (Cracraft 1986; Ford 1987). It was formed in the mid-Miocene (11-15 my bp) when the sea retreated (Wasson 1982). It is characterised by mallee and shrub vegetation and thus constitutes a strong geographical dispersal barrier for mesic species of southeastern and southwestern

Australia (Ford 1971; Specht 1981). Similarly to our results, phylogeographic work on Australian magpies (*Gymnorhina tibicen*; Toon et al. 2007) and Southern Emu-wrens (*Stipiturus malachurus*; Donnellan et al. 2009) revealed divergent monophyletic lineages on either sides of the Nullarbor. This pattern is not limited to birds and has also been observed in other vertebrates (Spencer et al. 2001; Chapple et al. 2004; Keogh et al. 2005).

### *Conservation implications*

The taxonomic level (i.e. species, subspecies, population) to target for conservation efforts is often debated. Moritz (1994) advocated independent management of monophyletic populations, whereas others have suggested that even in the absence of genetic differentiation, ecologically distinct populations should be preserved (Crandall et al. 2000). Although their mtDNA lineages are not highly differentiated, our data demonstrate that the eastern and western mtDNA haplotype groups are monophyletic, and other studies have demonstrated that eastern and western populations differ in display behaviour (Robinson & Robinson 1970; McCracken et al. 2002). Accordingly, the two Musk Duck populations satisfy both criteria of evolutionarily significant units. Our data are also consistent with Mathews' (1914, 1927) split of Musk Ducks into eastern (*B. l. menziesi*) and western (*B. l. lobata*) subspecies.

Musk Duck populations appear stable in Western Australia (Saunders & Ingram 1995) and Tasmania (S. Blackhall, unpublished data), but they have decreased in mainland eastern Australia (e.g. Parker et al. 1985; Davey 1989; Paton et al. 1994), where they are now listed as vulnerable in Victoria (Victorian Department of Sustainability and Environment 2007) and rare in South Australia (Robinson et al. 2000). This decrease corresponds with habitat loss through decreased rainfall and wetland drainage. About one third of Victoria's wetlands have been drained since European settlement (Anonymous 1988b) and 60% of wetlands in coastal New South Wales have also been lost (Goodrick 1970). Musk Ducks may also be threatened by the introduction of the European carp (*Cyprinus carpio*), with whom they compete for food, in river systems (Paton et al. 1994; McCracken 2005). A management plan for protection of Musk Ducks has not been completed to date, probably because they appear stable in Western Australia (Saunders & Ingram 1995). Our results indicate that the eastern and western Musk Duck populations are genetically distinct, and that wildlife agencies should consider managing them separately.

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**Table 1.** Genetic diversity estimates for the mtDNA control region, ornithine decarboxylase (ODC1), and beta-fibrinogen (FGB) including sample size (N), number of haplotypes (H), number of private haplotypes (Pri), haplotype diversity (h ± S.E.), and nucleotide diversity ($\pi$ ± S.E.) for western Australia (WA), Kangaroo Island (KI), Tasmania (TAS), mainland eastern Australia (SE), and Lake Wendouree (LW).

| Pop | N | H | Pri | h ± S.E. | $\pi$ |
|---|---|---|---|---|---|
| | | | | Mitochondrial control region | |
| WA | 16 | 4 | 4 | 0.692 ± 0.018 | 0.00557 ± 0.00102 |
| KI | 47 | 7 | 4 | 0.537 ± 0.010 | 0.00388 ± 0.00044 |
| TAS | 8 | 2 | 0 | 0.250 ± 0.064 | 0.00103 ± 0.00052 |
| SE | 49 | 6 | 4 | 0.419 ± 0.012 | 0.00311 ± 0.00037 |
| LW | 33 | 2 | 0 | 0.409 ± 0.013 | 0.00338 ± 0.00048 |
| | | | | ODC1 | |
| WA | 14 | 5 | 1 | 0.611 ± 0.018 | 0.00556 ± 0.00067 |
| KI | 46 | 6 | 1 | 0.728 ± 0.003 | 0.00489 ± 0.00033 |
| SE | 25 | 5 | 0 | 0.668 ± 0.008 | 0.00415 ± 0.00040 |
| LW | 34 | 5 | 0 | 0.770 ± 0.003 | 0.00557 ± 0.00043 |
| TAS | 1 | 2 | 0 | — | — |
| | | | | FBG | |
| WA | 13 | 5 | 0 | 0.723 ± 0.011 | 0.00381 ± 0.00050 |
| KI | 43 | 5 | 0 | 0.655 ± 0.003 | 0.00434 ± 0.00030 |
| SE | 12 | 3 | 0 | 0.627 ± 0.010 | 0.00341 ± 0.00048 |
| LW | 34 | 4 | 0 | 0.603 ± 0.005 | 0.00382 ± 0.00030 |
| TAS | 2 | 2 | 0 | — | — |

**Table 2.** Pairwise $\Phi_{ST}$ values and $F_{ST}$ values for mtDNA and introns (*P*-values in parenthesis). Bold text indicates a significant comparison after Bonferroni correction.

| Pairwise comparison | $\Phi_{ST}$ | $F_{ST}$ |
|---|---|---|
| Mitochondrial DNA control region (mtDNA) | | |
| Eastern vs western Australia | **0.747 (<0.001)** | **0.509 (<0.001)** |
| Tasmania vs mainland eastern Australia | -0.042 (0.769) | -0.013 (0.487) |
| Tasmania vs Kangaroo Island | 0.047 (0.219) | 0.057 (0.183) |
| Kangaroo Island vs mainland eastern Australia | 0.046 (0.036) | 0.025 (0.092) |
| Kangaroo Island vs Lake Wendouree | -0.025 (0.980) | -0.015 (0.639) |
| Lake Wendouree vs mainland eastern Australia | 0.041 (0.068) | 0.008 (0.253) |
| Ornithine decarboxylase (ODC1) | | |
| Eastern vs western Australia | **0.193 (<0.001)** | **0.157 (0.002)** |
| Kangaroo Island vs mainland eastern Australia | -0.001 (0.406) | -0.004 (0.611) |
| Kangaroo Island vs Lake Wendouree | 0.019 (0.078) | **0.057 (0.002)** |
| Lake Wendouree vs mainland eastern Australia | 0.022 (0.095) | **0.091 (0.003)** |
| Beta-fibrinogen (FBG) | | |
| Eastern vs western Australia | -0.002 (0.373) | 0.057 (0.027) |
| Kangaroo Island vs mainland eastern Australia | 0.035 (0.037) | 0.041 (0.018) |
| Kangaroo Island vs Lake Wendouree | 0.046 (0.018) | 0.040 (0.118) |
| Lake Wendouree vs mainland eastern Australia | 0.081 (0.030) | 0.030 (0.039) |

**Table 3.** Genetic diversity estimates for eight microsatellite loci, including number of individuals (N), number of alleles (A), allelic richness (Rs), observed ($H_O$) and expected heterozygosity ($H_E$), $F_{IS}$ for western Australia (WA), Kangaroo Island (KI), mainland eastern Australia (SE) and Lake Wendouree (LW), and average number of alleles ($N_A$) and private alleles ($P_{VA}$), and $F_{IS}$ summed over all loci.

| | | Populations | | | | | |
|---|---|---|---|---|---|---|---|
| Locus | | WA | KI | SE | LW | All | Mean |
| Blm2 | N | 13 | 39 | 12 | 34 | 98 | |
| | A | 7 | 11 | 8 | 9 | 15 | 8.8 |
| | Rs | 6.490 | 7.076 | 7.725 | 7.850 | 8.342 | |
| | $H_O$ | 0.85 | 0.87 | 0.83 | 0.82 | | |
| | $H_E$ | 0.79 | 0.80 | 0.82 | 0.87 | | |
| | $F_{IS}$ | -0.075 | -0.091 | -0.021 | 0.052 | | |
| Blm3 | N | 11 | 39 | 10 | 34 | 94 | |
| | A | 6 | 8 | 6 | 7 | 8 | 6.8 |
| | Rs | 5.723 | 6.342 | 6.000 | 5.450 | 6.266 | |
| | $H_O$ | 0.64 | 0.85 | 0.80 | 0.71 | | |
| | $H_E$ | 0.66 | 0.81 | 0.79 | 0.79 | | |
| | $F_{IS}$ | 0.038 | -0.045 | -0.019 | 0.105 | | |
| Blm4 | N | 12 | 36 | 12 | 34 | 94 | |
| | A | 8 | 12 | 10 | 12 | 16 | 10.5 |
| | Rs | 7.639 | 8.539 | 9.266 | 9.025 | 9.599 | |
| | $H_O$ | 0.75 | 0.92 | 1.00 | 0.88 | | |
| | $H_E$ | 0.83 | 0.87 | 0.85 | 0.88 | | |
| | $F_{IS}$ | 0.096 | -0.053 | -0.176 | 0.000 | | |
| Blm5 | N | 12 | 39 | 12 | 34 | 97 | |
| | A | 8 | 8 | 7 | 7 | 11 | 7.5 |
| | Rs | 7.599 | 6.074 | 6.665 | 5.846 | 6.839 | |
| | $H_O$ | 0.83 | 0.82 | 0.92 | 0.82 | | |
| | $H_E$ | 0.79 | 0.78 | 0.82 | 0.79 | | |
| | $F_{IS}$ | -0.053 | -0.057 | -0.119 | -0.038 | | |
| Blm7 | N | 13 | 39 | 12 | 34 | 98 | |
| | A | 6 | 8 | 6 | 4 | 10 | 6.0 |
| | Rs | 5.308 | 5.452 | 5.333 | 3.160 | 4.782 | |
| | $H_O$ | 0.85 | 0.77 | 0.67 | 0.35 | | |
| | $H_E$ | 0.69 | 0.66 | 0.64 | 0.41 | | |
| | $F_{IS}$ | -0.222 | -0.169 | -0.038 | 0.135 | | |
| Blm9 | N | 13 | 39 | 12 | 34 | 98 | |
| | A | 8 | 10 | 10 | 8 | 11 | 9.0 |
| | Rs | 7.615 | 8.210 | 9.284 | 6.580 | 8.289 | |
| | $H_O$ | 0.92 | 0.90 | 0.83 | 0.79 | | |
| | $H_E$ | 0.82 | 0.87 | 0.85 | 0.81 | | |
| | $F_{IS}$ | -0.122 | -0.037 | 0.016 | 0.014 | | |

|  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|
| Blm11 | N | 12 | 39 | 12 | 34 | 97 |  |
|  | A | 7 | 9 | 7 | 6 | 9 | 7.3 |
|  | Rs | 6.601 | 6.612 | 6.496 | 4.670 | 6.418 |  |
|  | $H_O$ | 0.75 | 0.90 | 0.83 | 0.71 |  |  |
|  | $H_E$ | 0.72 | 0.79 | 0.75 | 0.63 |  |  |
|  | $F_{IS}$ | -0.038 | -0.132 | -0.116 | -0.124 |  |  |
|  |  |  |  |  |  |  |  |
| Blm12 | N | 12 | 38 | 12 | 34 | 96 |  |
|  | A | 6 | 7 | 6 | 9 | 10 | 7.0 |
|  | Rs | 5.954 | 5.253 | 5.663 | 6.689 | 6.710 |  |
|  | $H_O$ | 0.83 | 0.71 | 0.75 | 0.88 |  |  |
|  | $H_E$ | 0.80 | 0.73 | 0.74 | 0.82 |  |  |
|  | $F_{IS}$ | -0.048 | 0.021 | -0.014 | -0.083 |  |  |
| $N_A$ |  | 7.0 | 9.1 | 7.5 | 7.7 |  |  |
| $P_{VA}$ |  | 4 | 11 | 2 | 6 |  |  |
| $F_{IS}$ All |  | -0.009 | -0.055 | -0.017 | 0.018 |  |  |

Table 4. Pairwise $F_{ST}$ for microsatellite loci (*P*-values in parenthesis). Bold text indicates a significant comparison after Bonferroni correction.

| Pairwise comparison | $F_{ST}$ |
|---|---|
| Eastern vs western Australia | **0.035 (0.001)** |
| Kangaroo Island vs mainland eastern Australia | **0.018 (0.001)** |
| Kangaroo Island vs Lake Wendouree | **0.050 (<0.001)** |
| Lake Wendouree vs mainland eastern Australia | **0.042 (<0.001)** |

**Figure 1.** Geographic distribution of sampling sites within the fivepopulations of Musk Ducks. The shaded area in the southern part of the continent corresponds to the Nullarbor Plain.

**Figure 2.** Unrooted haplotype networks for (A) mtDNA, (B) ODC1 and (C) FGB sequences. Open circles represent samples from eastern Australia, black circles samples from western Australia. KI: Kangaroo Island; EA: mainland eastern Australia; LW: Lake Wendouree; TA: Tasmania; WA: Western Australia. Open squares represent ancestral haplotypes that were not sampled.

**Appendix 1.** Localities for the specimens used in this study

| Catalogue number [a] (Field number) *Band number* | Sex | Date | Locality |
|---|---|---|---|
| Western Australia | | | |
| ANWC 50286 | M | 31 May 2004 | Lake Carabundup, MW of Mt Baker, WA, 34°28’S, 117°18’E |
| ANWC 50287 | M | 31 May 2004 | Lake Carabundup, MW of Mt Baker, WA, 34°28’S, 117°18’E |
| ANWC 50288 | F | 31 May 2004 | Lake Carabundup, MW of Mt Baker, WA, 34°28’S, 117°18’E |
| ANWC 50289 | F | 31 May 2004 | Lake Carabundup, MW of Mt Baker, WA, 34°28’S, 117°18’E |
| ANWC 50384 | M | 7 June 2004 | Lake Namming, c. 15km S of Cataby, WA, 30°54’S, 115°35’E |
| ANWC 50385 | M | 7 June 2004 | Lake Namming, c. 15km S of Cataby, WA, 30°54’S, 115°35’E |
| UAM 11882 | M | 19 November 2000 | Coolgardie, WA, 33°27’S, 121°44’E |
| UAM 15016 | M | 20 November 2000 | Warden Lake, WA, 33°49’S, 121°53’E |
| UAM 22312 | M | 21 November 2000 | Warden Lake, WA, 33°49’S, 121°53’E |
| WAM A18748 | F | 14 January 1940 | Torbay, WA, 35°02’S, 117°38’E |
| WAM A4333 | M | 29 March 1933 | Harvery Estuary, WA, 32°42’S, 115°41’E |
| WAM A7419 | M | 1 June 1954 | Floreat Park, WA, 31°56’S, 115°47’E |
| WAM A36076 | M | 4 October 2005 | Cracker Swamp, Coldat, WA, 30°54’S, 115°35’E |
| WAM A36077 | M | 4 October 2005 | Cracker Swamp, Coldat, WA, 30°54’S, 115°35’E |
| (PJG 290) *132-20301* | M | 1 October 2005 | Lake Joondalup, Perth, WA, 31°45’S, 115°47’E |

| | | | |
|---|---|---|---|
| (PJG 294) *132-20302* | M | 1 October 2005 | Lake Joondalup, Perth, WA, 31°45’S, 115°47’E |
| | | | <u>Kangaroo Island</u> |
| LSUMZ 34096 *131-88701* | M | 11 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34097 *131-88702* | M | 11 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34098 *131-88703* | M | 11 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34099 *131-88704* | F | 12 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34100 *131-88705* | F | 12 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34101 *131-88706* | F | 12 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34102 *131-88707* | F | 15 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34103 *131-88708* | M | 15 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34104 *131-88709* | F | 15 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34105 *131-88710* | F | 15 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34106 *131-88711* | M | 16 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34107 *131-88712* | M | 16 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34108 *131-88713* | M | 22 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34109 *131-88714* | F | 23 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34110 *131-88715* | F | 23 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34111 *131-88716* | M | 25 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |

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|---|---|---|---|
| LSUMZ 34112 *131-88717* | M | 25 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34113 *131-88718* | M | 25 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34114 *131-88719* | M | 26 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34115 *131-88720* | M | 26 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34764 *131-88721* | M | 26 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34765 *131-88722* | F | 26 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34767 *131-88724* | M | 27 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34768 *131-88725* | M | 27 September 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34769 *121-41101* | F | 23 October 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34770 *121-41102* | F | 24 October 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34771 *131-88727* | M | 25 October 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34772 *131-88728* | M | 27 October 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34773 *131-88729* | M | 4 November 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34774 *121-41103* | F | 8 November 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34775 *121-41104* | F | 10 November 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34776 *121-41105* | F | 11 November 1995 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34777 *121-41106* | F | 27 September 1996 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |
| LSUMZ 34778 *131-88730* | M | 28 September 1996 | Murray Lagoon, Kangaroo Island, SA, 35°55’S, 137°24’E |

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|---|---|---|---|
| LSUMZ 34779 *131-88731* | M | 18 October 1996 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34780 *131-88732* | M | 18 October 1996 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34781 *131-88733* | M | 20 October 1996 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34782 *131-88734* | M | 21 October 1996 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34783 *131-88735* | M | 5 October 1997 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34784 *131-88736* | M | 12 October 1997 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34785 *121-41108* | F | 14 October 1997 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34786 *131-88737* | M | 15 October 1997 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34787 *131-88738* | M | 17 October 1997 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34788 *131-88739* | M | 18 October 1997 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| LSUMZ 34789 *131-88740* | M | 19 October 1997 | Murray Lagoon, Kangaroo Island, SA, 35°55'S, 137°24'E |
| SAMA B46336 | U | 18 September 1990 | Nepean Bay, Kangaroo Island, SA, 36°39'S, 137°42'E |
|  |  |  | <u>Tasmania</u> |
| AMNH 734151 | M | November 1914 | Colebrook, TAS, 42°32'S, 147°21'E |
| ANWC 47623 | F | 11 March 1989 | Hobart, TAS, 42°52'S, 147°19'E |
| QVM:1963/2/28 | F | 17 April 1961 | Moulting Lagoon, TAS, 42°02'S, 148°10'E |
| QVM:1969/2/7 | F | 1 March 1969 | Flinders Island, TAS, 39°51'S, 147°54'E |
| QVM:1969/2/8 | M | 9 March 1969 | Needles, TAS, 41°32'S, 146°33'E |

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|---|---|---|---|
| QVM:1988/2/102 | M | 16 September 1988 | Painted Post, Greens Beach, TAS, 41°05’S, 146°44’E |
| TM B2080 | F | 1800s | Richmond, TAS, 42°44’S, 147°26’E |
| TM B3360 | F | 7 March 1971 | Sanford Lagoon, TAS, 42°56’S, 147°29’E |
| | | | Mainland South East |
| AMS O.42017 | M | 1 October 1962 | Keepit Dam, Tamworth, NSW, 30°52’S, 150°30’E |
| AMS O.45232 | F | 13 December 1974 | Barren Box Swamp, Griffith, NSW, 34°09’S, 145°49’E |
| AMS O.45233 | M | 13 December 1974 | Barren Box Swamp, Griffith, NSW, 34°09’S, 145°49’E |
| AMS O.45465 | F | 30 August 1975 | Myall Lakes, NSW, 32°25’S, 152°22’E |
| AMS S.720 | M | 4 September 1899 | Sydney, NSW, 33°46’S, 150°46’E |
| ANWC 10793 | F | 24 September 1964 | Barren Box Swamp, Griffith, NSW, 34°09’S, 145°49’E |
| ANWC 15647 | M | 28 August 1964 | Barren Box Swamp, Griffith, NSW, 34°09’S, 145°49’E |
| ANWC 15648 | F | 29 August 1964 | Barren Box Swamp, Griffith, NSW, 34°09’S, 145°49’E |
| ANWC 15654 | F | No collection date | Barren Box Swamp, Griffith, NSW, 34°09’S, 145°49’E |
| ANWC 22655 | M | 6 June 1905 | Lake Cowal, NSW, 33°30’S, 147°22’E |
| ANWC 22658 | F | 12 June 1905 | Stranger Pond, Bonython, ACT, 35°26’S, 149°05’E |
| ANWC 50091 | M | 18 February 2004 | Deadmans Creek, E of Mathoura, NSW, 35°50’S, 144°56’E |
| ANWC 50099 | F | 19 February 2004 | Duck Lagoon, Moira State Forest, NSW, 35°52’S, 144°52’E |
| ANWC 50180 | F | 26 March 2004 | Mullawoolka Basin, Tonga Station, NSW, 30°29’S, 143°47’E |

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| BBM B.lobata1 | F | 1 February 1981 | Bool Lagoon, SA, 37°07’S, 140°41’E |
| BBM B.lobata2 | M | 23 February 1997 | Bool Lagoon, SA, 37°07’S, 140°41’E |
| BBM B.lobata3 | M | 23 February 1997 | Bool Lagoon, SA, 37°07’S, 140°41’E |
| NMV B.5102 | M | 11 July 1951 | King's Billabong, Mildura, VIC, 34°14’S, 142°13’E |
| NMV B.5188 | U | No collection date | Middle Park Beach, Port Phillip Bay, VIC, 37°51’S, 144°57’E |
| NMV B.7738 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.7739 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.7742 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.7743 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.7744 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.7746 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.7747 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.7748 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.7749 | U | 17 October 1961 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.9078 | M | 6 October 1967 | Little Ranker Ck, Keera Stn, VIC, 34°16’S, 141°43’E |
| NMV B.13775 | M | 2 March 1985 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.18358 | M | 19 March 1988 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV B.19134 | M | 14 March 1987 | Lake Martin, VIC, 35°05’S, 143°36’E |

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|---|---|---|---|
| NMV B.19198 | M | 12 November 1986 | Beechworth, VIC, 36°21’S, 146°41’E |
| NMV B.19227 | F | 14 March 1987 | Lake Martin, VIC, 35°05’S, 143°36’E |
| NMV B.19229 | F | 12 November 1986 | Beechworth, VIC, 36°21’S, 146°41’E |
| NMV B.19288 | M | 14 March 1987 | Lake Martin, VIC, 35°05’S, 143°36’E |
| NMV B.25089 | M | 14 March 1987 | Lake Murdeduke, VIC, 38°10’S, 143°54’E |
| NMV B.31571 | F | 26 April 1993 | Lake Bael Bael, VIC, 35°31’S, 143°44’E |
| NMV MV785 | U | 19 March 1989 | Kerang, VIC, 35°43’S, 143°55’E |
| NMV W15085 | M | No collection date | Lake Purrumbete, near Colac, VIC, 38°16’S, 143°13’E |
| QMO 666 | M | No collection date | Kalbar, QLD, 27°56’S, 152°37’E |
| QMO 9335 | F | 16 June 1962 | Lake McKenzie, Fraser Island, QLD, 25°27’S, 153°04E |
| SAMA B23004 | M | 1 December 1942 | Tailem Bend, SA, 41°05’S, 146°44’E |
| SAMA B23710 | F | 17 April 1947 | Tailem Bend, SA, 41°05’S, 146°44’E |
| SAMA B23883 | M | 13 March 1947 | Lake Albert, SA, 35°37’S, 139°18’E |
| SAMA B25005 | F | 1 December 1942 | Tailem Bend, SA, 41°05’S, 146°44’E |
| SAMA B38100 | F | 2 September 1979 | Merbein, VIC, 34°10’S, 142°03’E |
| SAMA B48312 | F | 30 January 1984 | Ulbana Res., Colwell, Eyre Peninsula, SA, 33°32’S, 136°57’E |
| (PJG 054) | U | 21 March 2005 | Kitty Miller Wetland, Philip Island, VIC, 38°30’S, 145°10’E |
| (PJG 231) *132-20261* | M | 5 September 2004 | Western Treatment Plant, Werribee, VIC, 38°00’S, 144°34’E |

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| (PJG254) | U | 17 August 2005 | Western Treatment Plant, Werribee, VIC, 38°00’S, 144°34’E |
| (PJG 308) | U | 30 October 2005 | Western Treatment Plant, Werribee, VIC, 38°00’S, 144°34’E |
| (PJG 321) | M | 4 July 2005 | Bool Lagoon, SA, 37°07’S, 140°41’E |
| (PJG 331) | M | 28 December 2005 | Western Treatment Plant, Werribee, VIC, 38°00’S, 144°34’E |
| (PJG 341) | M | 16 June 1988 | Port Augusta, SA, 32°29’S, 137°46’E |
| | | | Lake Wendouree |
| (PJG 200) *121-49551* | F | 1 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 201) *121-49552* | F | 24 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 202) *121-49553* | F | 10 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 203) *121-49554* | F | 24 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 204) *121-49555* | F | 14 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 205) *121-49556* | F | 24 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 206) *121-49557* | F | 12 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 207) *121-49558* | F | 24 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 208) *121-49559* | F | 20 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 209) *121-49560* | F | 20 August 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 210) *121-49561* | F | 3 December 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 211) *121-49562* | F | 27 August 2004 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |

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|---|---|---|---|
| (PJG 212) *121-49563* | F | 4 December 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 213) *121-49564* | F | 3 December 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 214) *121-49565* | F | 3 December 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 215) *121-49566* | F | 25 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 216) *121-49567* | F | 14 October 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 217) *121-49568* | F | 4 December 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 218) *121-49569* | F | 16 October 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 219) *121-49570* | F | 14 October 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 220) *121-49571* | F | 7 December 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 221) *132-20251* | F | 29 August 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 222) *132-20252* | M | 29 August 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 223) *132-20253* | M | 6 October 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 224) *132-20254* | F | 11 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 225) *132-20255* | M | 13 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 226) *132-20256* | F | 11 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 227) *132-20257* | M | 31 August 2004 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 228) *132-20258* | M | 7 October 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 229) *132-20259* | F | 11 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |

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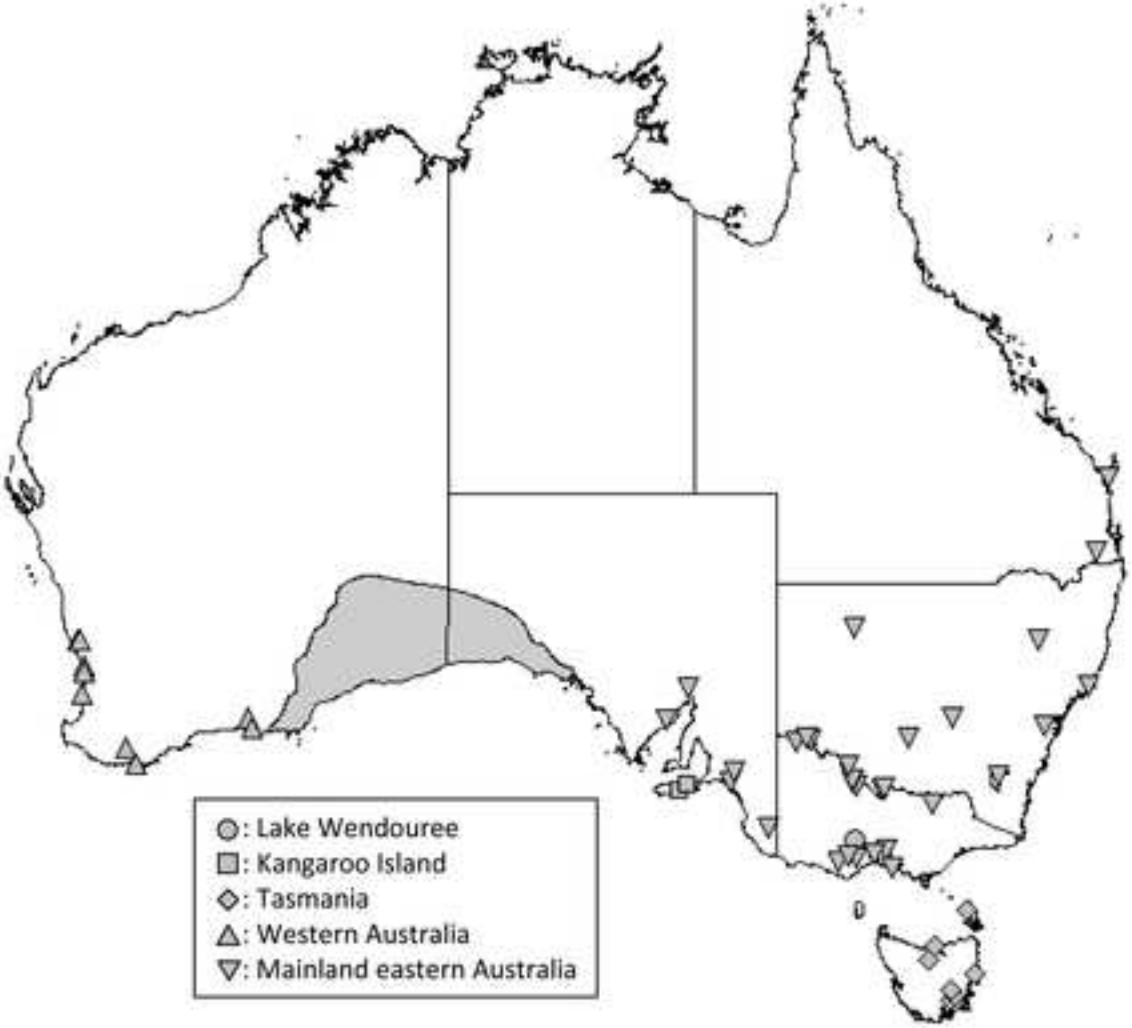
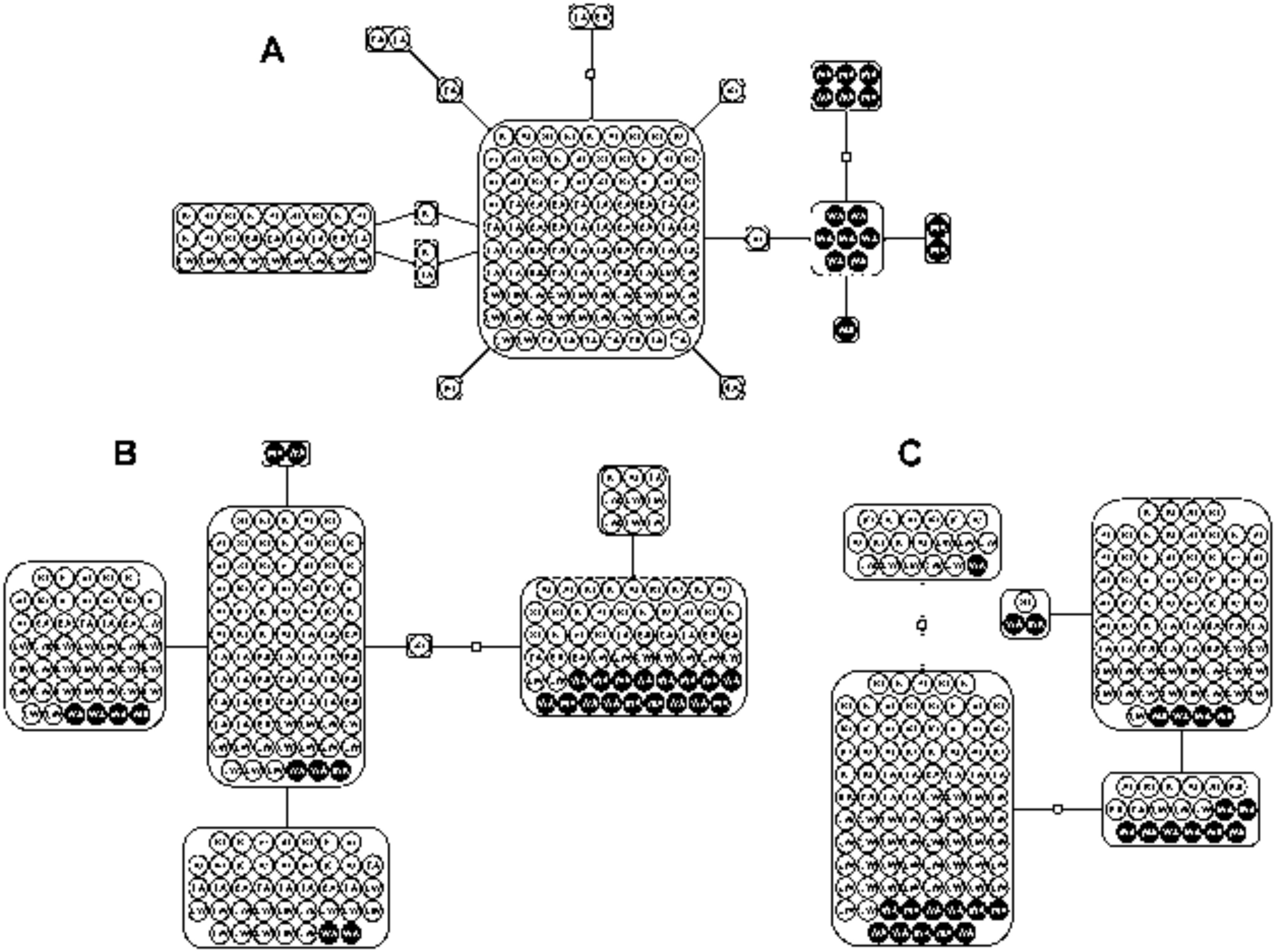
|---|---|---|---|
| (PJG 230) *132-20260* | M | 7 November 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 232) *140-52811* | M | 1 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 233) *140-52819* | M | 13 September 2003 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |
| (PJG 234) *121-49572* | F | 4 November 2004 | Lake Wendouree, Ballarat, VIC, 37°33’S, 143°50’E |

[a] Catalogue number for vouchered specimens from the American Museum of Natural History (AMNH), the Australian Museum (AMS), CSIRO Sustainable Ecosystems, Australian National Wildlife Collection (ANWC), the Bourne’s Bird Museum (BBM), Louisiana State University Museum of Natural History (LSUMZ), Museum Victoria (NMV), the Queensland Museum (QMO), the Queen Victoria Museum and Art Gallery (QVM), The South Australian Museum (SAMA), the Tasmanian Museum (TM), the University of Alaska Museum (UAM) and the Western Australian Museum (WAM).


○: Lake Wendouree
□: Kangaroo Island
◇: Tasmania
△: Western Australia
▽: Mainland eastern Australia


A
B
C