Microsatellite markers for Australian temperate diadromous fishes *Pseudaphritis urvillii* (Bovichtidae) and *Lovettia sealii* (Galaxiidae).

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Abstract

Thirteen microsatellite loci were developed and characterised for two fishes from temperate Australia that exhibit atypical forms of diadromy. Cloning and sequencing of an enriched partial genomic library was used to develop seven highly polymorphic loci for the catadromous species *Pseudaphritis urvillii* (known as tupong or congolli). Mean number of alleles per locus was 16.5, and average observed and expected heterozygosity was 0.90 and 0.87 respectively. Six polymorphic markers characterised for the anadromous species *Lovettia sealii* (known as Tasmanian whitebait) included a mean of 12.3 alleles per locus and average observed and expected heterozygosity of 0.71 and 0.77 respectively. These microsatellites will be employed to understand regional patterns of recruitment, migration and stock structure.

The stream fish fauna of southern Australia includes many taxa with migratory life history strategies (~70%, Harris 1984). Many of these species undertake bidirectional movements between freshwater and marine habitats known as diadromy. The requirement for connectivity between stream habitats and the sea makes diadromous taxa vulnerable to barriers blocking their passage and consequently many taxa are threatened by human activities (McDowall 2008; Crook *et al.* 2010). *Pseudaphritis urvillii* (Valenciennes) and *Lovettia sealii* (Johnston) are both monotypic members of Australia's temperate diadromous fish fauna that exhibit atypical forms of diadromy. *Lovettia sealii* spends most of its life at sea around the coast of Tasmania before returning to the upper tidal reaches of rivers in spring to spawn. Larvae subsequently enter the marine environment, where development and growth is completed (Blackburn 1950). *L. sealii* was the subject of a significant commercial whitebait fishery in Tasmania that closed in 1974 in response to collapse of stocks, reopening in 1990 for recreational purposes under limited seasonal operation (Fulton 2000). *Pseudaphritis urvillii* has a catadromous life history that involves an unusual sexually dimorphic residence behaviour (Crook *et al.* 2010). Adult females reside in freshwater habitats and undertake downstream migration to the sea where it appears that spawning occurs with resident males (Crook *et al.* 2010). Dramatic population declines of juvenile *P. urvillii* migrating upstream were observed during a recent drought and attributed to failed recruitment due to limited access to spawning habitat (Zampatti *et al.* 2010).

The present study reports novel microsatellite loci that have been tested in populations of *P. urvillii* and *L. sealii*, and will be valuable for studies of stock structure, gene flow and parentage analyses. Microsatellites were developed following the enrichment procedure described by Real *et al.* (2009) with modifications to the cloning step outlined by Schmidt *et al.* (2011a). Probes used for microsatellite enrichment were AAGT₈, AAAT₈, AAAG₈, AAGG₆, AGAT₈, ATCT₈, ACAT₈, AAT₈, CA₁₃. For *P. urvillii*, eighteen primer sets were designed and trialled on a sample of 29 individuals collected from the Snowy River in eastern Victoria. Fluorescent labelling of PCR product was achieved by multi-tailed primer tagging as described in Real *et al.* (2009), and fragments were analysed using an Applied Biosystems 3010 sequencer. Seven loci produced clear fragment patterns in the size range expected from sequenced clones (Table 1). The seven loci were highly polymorphic with allele number per locus ranging from eight to 31. Analysis of genotype proportions using ARLEQUIN ver. 3.5.1.2 (Excoffier *et al.* 2005) showed one locus (purv008) deviated from expected Hardy-Weinberg proportions due to an excess of homozygotes (Table 1). This deviation may be

attributed to sampling error because analysis with MICRO-CHECKER ver. 2.2.3 (Van Oosterhout *et al.* 2004) did not detect evidence for null alleles, large allele dropout or stuttering artefacts. No statistical evidence for linkage-disequilibrium was detected between any pair of loci.

For *L. sealii*, eighteen primer sets were designed and trialled on a sample of 27 individuals collected from the Leven River, northern Tasmania. Six loci produced clear fragment patterns in the size range expected from sequenced clones (Table 1). The six loci were moderately polymorphic with allele number per locus ranging from three to 25 (Table 1). Genotype proportions were within expectations of Hardy-Weinberg equilibrium for each locus and there was no evidence for linkage-disequilibrium between any pair of loci. Analysis using MICRO-CHECKER did not detect evidence for null alleles, large allele dropout or stuttering artefacts.

Little is known about the importance of inter-river migration via the sea in Australia's temperate diadromous fish fauna. Gene flow between rivers is expected to be high in amphidromous taxa due to mixing of the juvenile phase in the marine environment, as observed in the threatened Australian Grayling, *Prototroctes maraena* (Schmidt *et al.* 2011b). However, anadromous and catadromous migration strategies may be associated with more restricted patterns of genetic structure (*e.g.* Shaddick *et al.* 2011). The microsatellite loci described here will be used to quantify inter-river patterns of genetic structuring in two temperate diadromous taxa that are of conservation concern and are important to recreational fisheries. The highly polymorphic nature of these markers means they would also be valuable for analyses of within-river recruitment patterns. Analysis using GENELEX ver. 6.2 (Peakall & Smouse 2006) showed the multilocus probability of identity (*i.e.* the probability that two random individuals share same multilocus genotype) was 5.4×10^{-12} for *P. urvilli* ; and 1.6×10^{-8} for *L. sealii*. This high variation provides power (approaching 1.0 for the two datasets presented here) for excluding parents in kinship and parentage studies.

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Table 1. Microsatellite loci for *Pseudaphritis urvillii* and *Lovettia sealii*.

Species/ Locus	Primer sequence (5'-3')	Repeat motif	5'-Tail*	N	N _A	Size Range†	H _O /H _E	P _{HWE}	F _{IS}	GenBank Accession
<i>Pseudaphritis urvillii</i>										
purv002	F: TCGGGCTCCTTTGAACAGTGTGACT R: TGTCATTAGAGAGCAGGCTGGCGG	GT ₁₉	2	29	13	141-173	0.86/0.82	0.82	-0.051	KC012455
purv004	F: AGCTGCAATGTACTGCTCACTCA R: TGGACTTCCTTGGGACCTTAGCA	ATGT ₃₂	3	29	31	181-317	0.97/0.96	0.62	-0.002	KC012456
purv005	F: AGTAGTCAGTGACCCATAACATCCCT R: GCCTCTGTCCCGCTGTTCTGC	ATGT ₁₄	3	27	14	161-217	1.00/0.92	0.92	-0.088	KC012457
purv008	F: AGACCCACAAAGCCACACACCTG R: GCTGGTGAATGTCCTGGCTGGCA	ATAG ₁₉	1	25	18	258-350	0.84/0.94	0.02	0.104	KC012458
purv010	F: GTGGCAGTCGAGCGAGGCCATT R: CGCGTGCGGTGACAGACGTT	TGG ₁₁	4	28	8	115-160	0.79/0.74	0.87	-0.066	KC012452
purv011	F: CCCGACAGCACCTGGACCTTTGAGA R: AGAGTCGCTGTCCACATTCGTGGAG	ATAC ₄₈	3	26	21	314-414	0.96/0.95	0.31	-0.015	KC012453
purv013	F: TGCTACTCCCGAAGCTGCCTGTAC R: AATCCTGTGGGAGAGAGTCAGCCTG	GT ₄ (AT)GT ₁₁	1	27	11	174-194	0.85/0.78	0.47	-0.092	KC012454
<i>Lovettia sealii</i>										
lsea009	F: GTTGGTTTAGCAGCATGTGCAATCA R: GTACCCTGCCACACGCCCAAT	ATGT ₁₀	4	24	9	110-154	0.71/0.79	0.41	0.101	KC012459
lsea012	F: GTTGGTTTAGCAGCATGTGCAATCA R: GTACCCTGCCACACGCCCAAT	ACAT ₁₈	2	27	25	168-272	0.93/0.97	0.56	0.043	KC012462
lsea013	F: CCCTTCATGACCATGGGTGT R: GTCGCTTTATCGCACTGTGG	AGAT ₅ (....)AGAT ₁₃	4	26	3	125-149	0.42/0.35	0.63	-0.222	KC012464
lsea019	F: AGGGTGTACCCTGCCATACG R: TGGAGCAGAATCATGACATTGAC	ATGT ₇	2	26	10	170-202	0.69/0.83	0.49	0.169	KC012463
lsea021	F: AGGGTGTACCCTGCCATACG R: TTTGGGGGTTTTCAAACATTC	ATGT ₆ (ATTT)ATGT ₄	1	24	8	186-242	0.71/0.75	0.06	0.053	KC012460
lsea023	F: TTGCAGACGCTAGCAAACAT R: TCTTTGCCCATCTGAAGACC	ACAT ₁₇	3	26	19	220-274	0.81/0.94	0.13	0.101	KC012461

N: number of individuals genotyped; N_A : number of alleles per locus; H_O/H_E : observed and expected heterozygosity; P_{HWE} : P -value for exact test for Hardy-Weinberg proportions; F_{IS} : inbreeding coefficient; * code for 5'-tail added to forward primer (see Real *et al.* 2009); † fragment size includes 20bp primer tail.