Molecular diversity of molluscs (Glacidorbidae) from Kangaroo Gully and Drummond Nature Reserve, Western Australia

Report to *Adrian Pinder, Department of Parks and Wildlife*
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Summary

Ten specimens of land snail collected from two localities were lodged into the Western Australian Museum collection by Adrian Pinder*, Department of Parks and Wildlife* (see Appendix 1 for specimen and locality details). The main objective of the WA Museum's Molecular Systematics Unit (MSU) was to use COI DNA barcoding to assess genetic diversity among six specimens, three from each location. There are currently no existing DNA sequences of *Glacidorbis occidentalis* in the Western Australian Museum's DNA database or on the public DNA database, GenBank or Barcode of Life database. However the queried sequences were compared to two publicly available sequences from congeners *Glacidorbis hedleyi* (JQ228467.1; Golding, 2012) from Boyd River, Blue Mountains, NSW, Australia and *G. rusticus* (FJ917284.1; Dinapoli and Klussmann-Kolb, 2010) from Wilsons Promontory, Vic, Australia.

DNA was extracted and DNA barcoding sequences (CO1) were amplified by PCR in the MSU and sequenced at the Australian Genomic Research Facility (AGRF) Perth node. DNA sequences were BLASTED against the Western Australian Museum DNA database and publically accessible GenBank.

Results

COI DNA barcodes (630 base pairs) were amplified and successfully sequenced from all six specimens. DNA sequences are provided in Appendix 2. An unrooted network was generated for the six specimens (Figure 1) as well as a rooted phylogeny with *Glacidorbis hedleyi* and *G. rusticus* as outgroups (Figure 2). Number of polymorphisms in each population, the number of fixed differences between two poputions and the net divergence between the two populations (Da) were calculated in DNAsp (Librado and Rozas; 2009) (see Table 1). A pairwise distance matrix shows the percentage of identical base pairs between each pair of taxa, including outgroups, in Table 2.

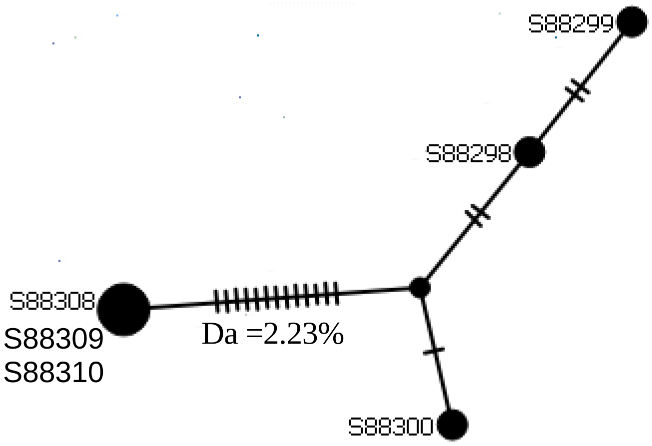

Figure 1. An unrooted network (Neighbour Joining) of six barcoded specimens, displaying the number of nucleotide substitutions between each haplotype.

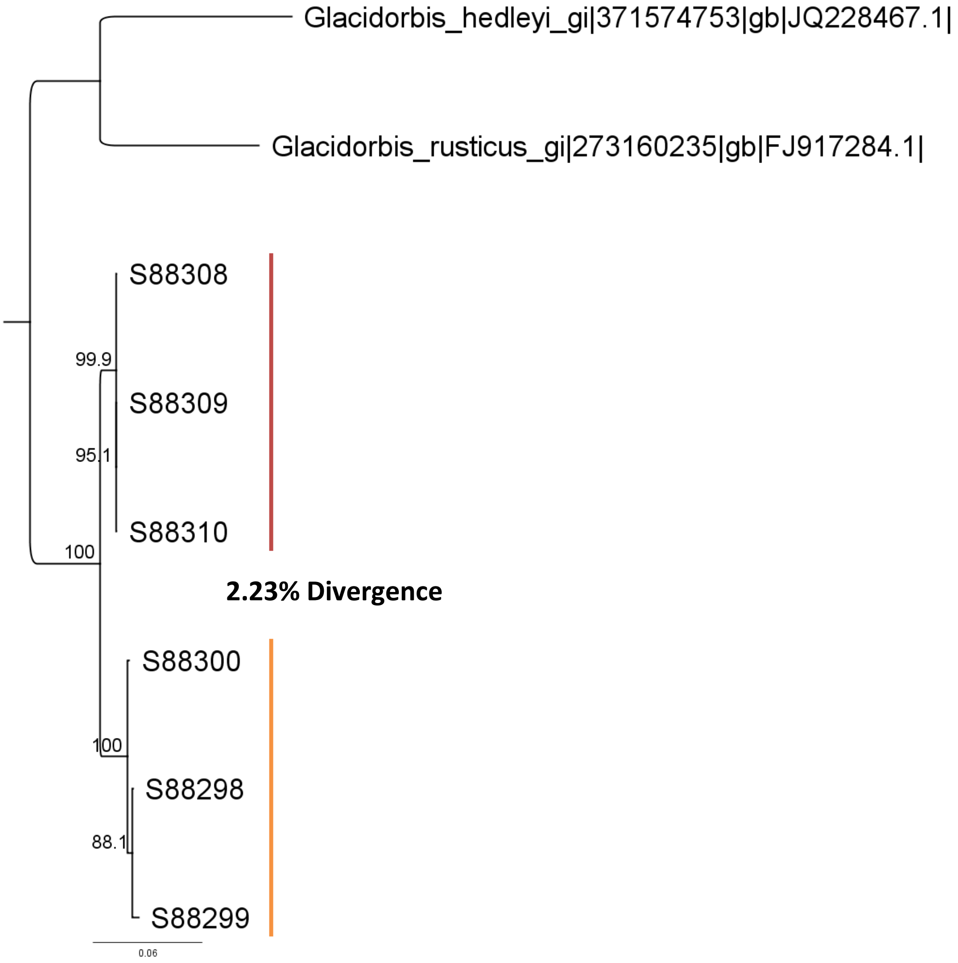

Figure 2. Neighbour joining consensus tree (bootstraps above nodes)

Table 1. Genetic diversity and divergence summary statistics.

Genetic diversity within population	
Number of polymorphic sites (Kangaroo Gully)	4
Nucleotide diversity, Pi (Kangaroo Gully)	0.42%
Number of polymorphic sites (Drummond Nature Reserve)	0
Nucleotide diversity, Pi (Drummond Nature Reserve)	0.00%
Divergence between populations	
Number of fixed differences	14
Average number of nucleotide differences between populations	15.333
Number of net nuc. subs. per site between populations, Da	2.23%

Table 2. Pairwise distance matrix (% identity) for 577 base pairs COI (slightly reduced dataset because of available data for outgroups- but notably there were no polymorphisms present in bases that were excluded).

	G. hedleyi	***G. rusticus***	**S88298**	**S88300**	**S88299**	**S88308**	**S88309**	**S88310**
G. hedleyi		83.189	82.842	82.842	82.842	83.016	83.016	83.016
G. rusticus	83.189		83.709	83.882	83.536	84.922	84.922	84.922
S88298	82.842	83.709		99.653	99.653	97.4	97.4	97.4
S88300	82.842	83.882	99.653		99.307	97.574	97.574	97.574
S88299	82.842	83.536	99.653	99.307		97.054	97.054	97.054
S88308	83.016	84.922	97.4	97.574	97.054		100	100
S88309	83.016	84.922	97.4	97.574	97.054	100		100
S88310	83.016	84.922	97.4	97.574	97.054	100	100	

References

Dinapoli, A., and A. Klussmann-Kolb. 2010. The long way to diversity – Phylogeny and evolution of the Heterobranchia (Mollusca: Gastropoda). Molecular Phylogenetics and Evolution 55:60-76.

Golding, R. E. 2012. Molecular phylogenetic analysis of mudflat snails (Gastropoda: Euthyneura: Amphiboloidea) supports an Australasian centre of origin. Molecular Phylogenetics and Evolution 63:72-81.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Appendix 1. Specimen data for six queried *Glacidorbis* specimens

Reg. No.	Family	Genus	Species	Latitude	Longitude	Site	Substrate	Habitat
S88298	Glacidorbidae	*Glacidorbis*	*occidentalis*	32°8'54.76"S	116°7'21.27"E	Kangaroo Gully just upstream of confluence with Canning River near Mundaring Weir, 3.8km south-east of Araluen Botanical Park	Sand/gravel	Stream
S88299	Glacidorbidae	*Glacidorbis*	*occidentalis*	32°8'54.76"S	116°7'21.27"E	Kangaroo Gully just upstream of confluence with Canning River near Mundaring Weir, 3.8km south-east of Araluen Botanical Park	Sand/gravel	Stream
S88300	Glacidorbidae	*Glacidorbis*	*occidentalis*	32°8'54.76"S	116°7'21.27"E	Kangaroo Gully just upstream of confluence with Canning River near Mundaring Weir, 3.8km south-east of Araluen Botanical Park	Sand/gravel	Stream
S88301	Glacidorbidae	*Glacidorbis*	? *occidentalis*	31°19'32.50"S	116°24'11.73"E	Drummond Nature Reserve, south-east claypan	Clay	Wetland (claypan)
S88302	Glacidorbidae	*Glacidorbis*	? *occidentalis*	31°19'32.50"S	116°24'11.73"E	Drummond Nature Reserve, south-east claypan	Clay	Wetland (claypan)
S88303	Glacidorbidae	*Glacidorbis*	? *occidentalis*	31°19'32.50"S	116°24'11.73"E	Drummond Nature Reserve, south-east claypan	Clay	Wetland (claypan)

Appendix 2. COI DNA sequence data for queried *Glacidorbis* specimens

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>S88299
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