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Supplementary Material

Characterisation and cross-amplification of sex-specific genetic markers in Australasian Egerniinae lizards and their implications for understanding the evolution of sex determination and social complexity

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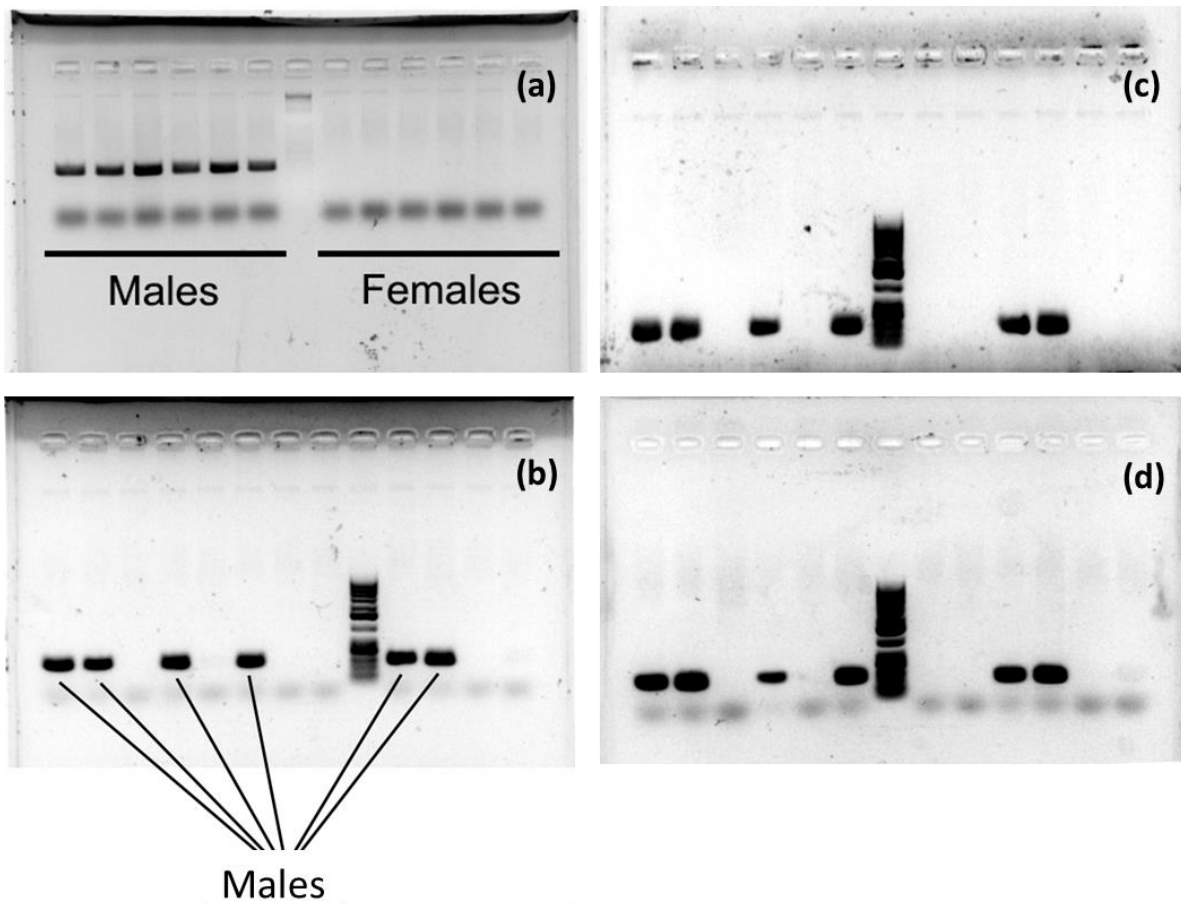
10 **Table S1.** Sequence information for the four Y-linked consensus loci

Y-linked consensus loci	Sequences
2	<p>AATTCAGCACAGGACACTGCTGTTGTGAATAAAGTGGGCATCAGGGGAACAAATGAGTTACTATTTTGT GGACCAAGGTCCAGGGTGATCCACAGGCTTACAGAAACAGAGAGAAAATGGGAAGGTGGCAGGAGATG GGAGGGAGNNNNNNNNNACTTGTGATGTTAGGGAGATGATACCCTGGATGACCAGGATAACTAATGG AAAATGTTGGTTTCCATAATCTCGTAGCAGAACTGACACCCTGTTACCAACTGTCATCCTGAACCATCTTC AGCTGCAGGGTGGTGCCAGATTTATACCG</p>
12	<p>AATTCTTCTAAACTAAAATTCCTATAGGTAAGAACAGTGGAATATACTGAGTGGTTTATGTGTCTGACTC TATTATCTCTACAGTTTCTGGCTGCATGTAACATCCATTCCATATGTCTTTTAAAATACTGAGAATTGTGT TATCTCTTCATCTCCCCCTGCACTATAGTGCTGAAGAGTGTAACAAGTTTGCTGGGCTCTGAGGGACTTC ACCAGGTTGTTCCG</p>
14	<p>AATTCATACTGGCCAGCACTGTAGATGTC CCTCAGATGCTATTCTTTCCCATGCTCAGCCCTTTAATTATC CTTTTCAGCCTTGTGGACAAATCGCTCTCTGTAGGTTCCCTTTATTCATAATTTCTGAGTTCTAAGGGCCAT CTCAGAGTGGCAGTGGGAAAATTTGTTGGACTGGGTAAGAGGACTCTGGAGAATTATCTATATTCTCCAG AGAGCCG</p>
18	<p>AATTCGGAACGGAGGGGACAGCGCTACAGGAGGTAGCCATGGCCAGATGGTAGTGGACGAAGCGGCAA CAAAGGCGGATGACAAATAGACGACAGCCGAGTGGAAAGTATTTATTTCTACGCACGCGGATACTCGC ATAAGCTCCACCCCCTGTGCATCAGAGACTGAATGCAGGAGCGCCTCTCAAATCGCGGTGCTGGCAGA ACATGAAGCCCATTCAAATTGCTCCGTTTGAGGCAGCGGAGGTGGGACTTCCG</p>

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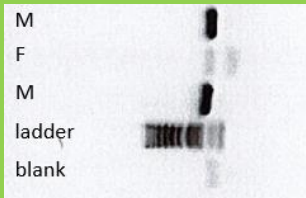

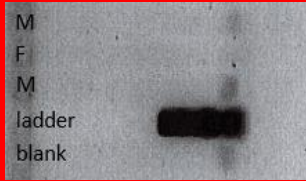
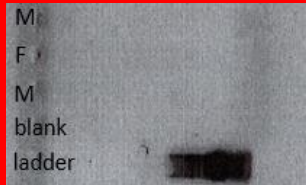
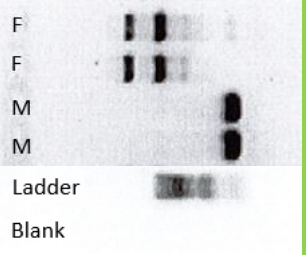
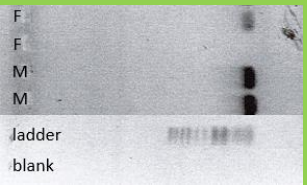
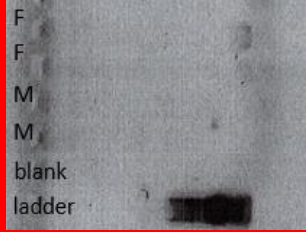
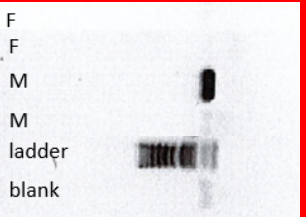
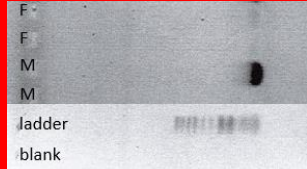
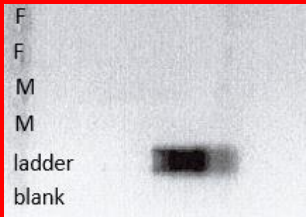
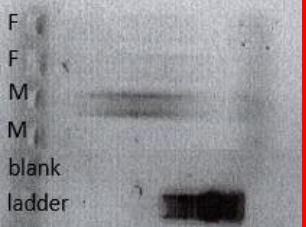
15 **Figure S1:** Gel images for the tests of the four primer pairs on 6 male and 6 female *Liopholis*
16 *whitii*. Figures refer to the gel results from primer pairs 2 (a), 12 (b), 14 (c) and 18 (d). Sexes
17 are grouped together on the gel set for primer set 2 but distributed across the gel for the other
18 primer pairs (male location the same for each set as indicated in Figure 1b).

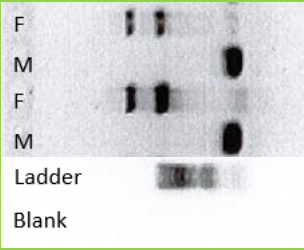

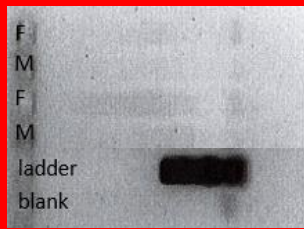
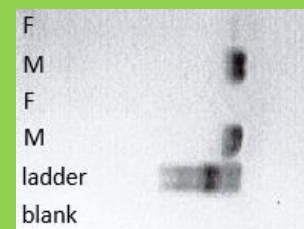
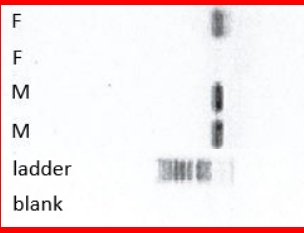
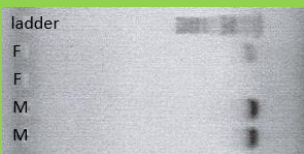
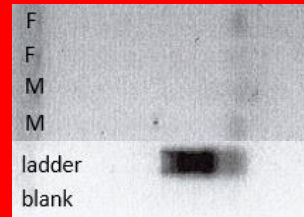
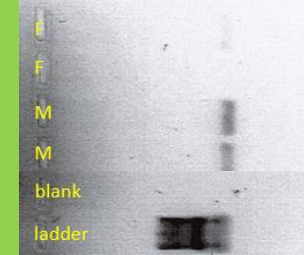
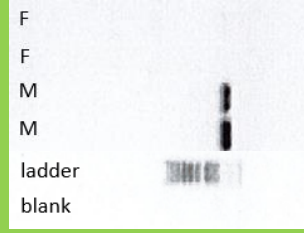

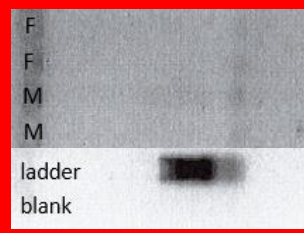
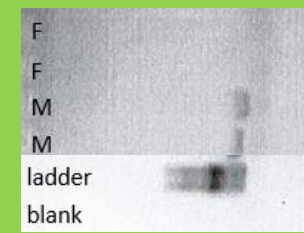
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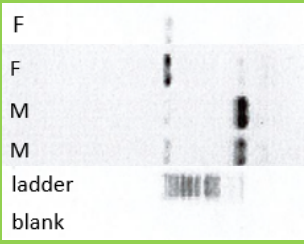

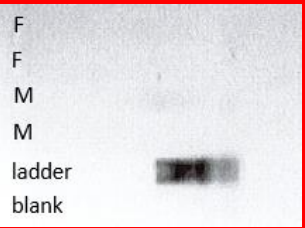
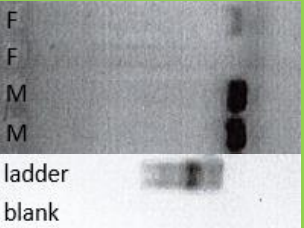
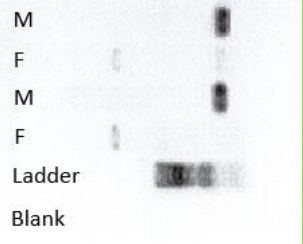
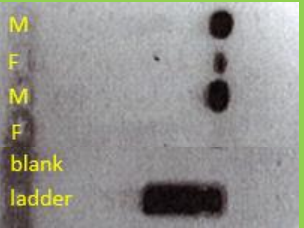
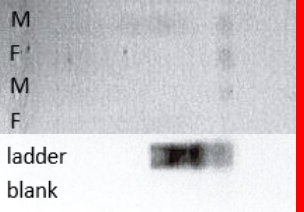

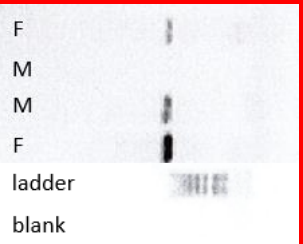


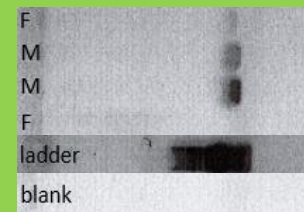
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	Primer Pair 2		Primer Pair 12		Primer Pair 14		Primer Pair 18	
<i>Egernia depressa</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	56.8 - 59	59	60.5	60.5	55 - 60.5	NA	55 - 60.5	NA
	Notes: Bands for males, no bands for females		Notes: Bands for males, no bands for females		Notes: No bands		Notes: No bands	
<i>Egernia kingii</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	55.4 - 59	55.4 - 59	60.5	60.5	NA	NA	55 - 60.5	NA
	Notes: Bands for males, non-target band (<1000 bp) for female		Notes: Bands for males, putative band for one female		NA		Notes: No bands	
<i>Egernia richardi</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	56.8 - 59	NA	59 - 60.5	NA	55 - 60.5	NA	55 - 60.5	NA
	Notes: Band for one male, no band for the other male or females.		Notes: Band for one male, no band for the other male or females.		Notes: No bands		Notes: No bands	

	Primer Pair 2		Primer Pair 12		Primer Pair 14		Primer Pair 18	
<i>Egernia cunninghami</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	55 – 60.5	55 - 59	60.5	60.5	55 – 60.5	NA	58.5 – 60.5	60.5
	Notes: Bands for males, non-target band (<1000 bp) for female		Notes: Bands for males, no bands for females		Notes: No bands		Notes: Bands for males, no bands for females	
<i>Egernia striolata</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	59	NA	55.0 – 60.5	58.3	55 – 60.5	NA	55 – 60.5	60.5
	Notes: Bands for males, band (250 bp) for one female		Notes: Bands for males, putative band for one female		Notes: No bands		Notes: Bands for males, no bands for females	
<i>Egernia stokesii</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	59	59	59 – 60.5	59	55 – 60.5	NA	58.5 – 60.5	60.5
	Notes: Bands for males, no bands for females		Notes: Bands for males, no bands for females		Notes: No bands		Notes: Bands for males, no bands for females	

	Primer Pair 2		Primer Pair 12		Primer Pair 14		Primer Pair 18	
<i>Cyclodomorphus venustus</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	58 – 59	59	60.5	60.5	55 – 60.5	NA	58.5 – 60.5	58.5 – 60.5
	Notes: Bands for males, non-target band (<1000 bp) for female		Notes: Bands for males, no bands for females		Notes: No bands		Notes: Bands for males, no bands for females	
<i>Cyclodomorphus melanops</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	59	59	60.5	60.5	55 – 60.5	NA	58.5 – 60.5	58.5 – 60.5
	Notes: Bands for males, non-target bands (<1000bp) for female		Notes: Bands for males, no bands for females		Notes: No bands		Notes: Bands for males, no bands for females	
<i>Tilapia rugosa</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	58 – 59	59	58.3 – 60.5	58.3 – 60.5	55 – 60.5	NA	58.3 – 60.5	NA
	Notes: Bands for males, no bands for females		Notes: Bands for males, no bands for females		Notes: No bands		Notes: No bands	

	Primer Pair 2	Primer Pair 12	Primer Pair 14	Primer Pair 18				
<i>Tiliqua adelaidensis</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	55.4 - 59	55.4 - 59	59 - 60.5	59	55 - 60.5	NA	58.5 - 60.5	58.5 - 60.5
	Notes: Bands for males, non-target band (<1000 bp) for female	Notes: Bands for males, no bands for females	Notes: No bands	Notes: Bands for males, putative band for one female				
<i>Tiliqua scincoides</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	55 - 60.5	59	58 - 60.5	59	55 - 60.5	NA	58.5 - 60.5	58.5 - 60.5
	Notes: Bands for males, non-target band (<1000 bp) for female	Notes: Bands for males, putative band for one female	Notes: No bands	Notes: Bands for males, putative band for one female				
<i>Liopholis inornata</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	55 - 60.5	NA	55.4 - 60.5	55.4 - 60.5	55 - 60.5	NA	58.5 - 60.5	59
	Notes: Band for only one male, bands for females. All bands <1000bp.	Notes: Bands for males, no bands for females	Notes: No bands	Notes: Bands for males, putative band for one female				

	Primer Pair 2		Primer Pair 12		Primer Pair 14		Primer Pair 18	
<i>Lissolepis coventryi</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	55 – 60.5	NA	55 – 60.5	NA	55 – 60.5	55 – 59.0	55 – 60.5	NA
	Notes: Bands for one male (>1000 bp) and the one female with an additional band for the female		Notes: Band for one male, no band for the other male or females.		Notes: Bands for males, putative band for female		Notes: No band for males, putative band for the female	
<i>Eutropis multifasciata</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	55 – 60.5	NA	55.4 – 60.5	NA	55 – 60.5	NA	55 – 60.5	NA
	Notes: No bands		Notes: No bands		Notes: No bands		Notes: No bands	
<i>Carinascincus ocellatus</i>								
	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
	55 – 60.5	NA	55 – 60.5	NA	55 – 60.5	NA	55 – 60.5	NA
	Notes: No bands		Notes: No bands		Notes: No bands		Notes: No bands	

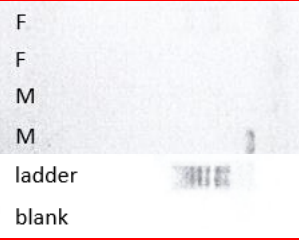
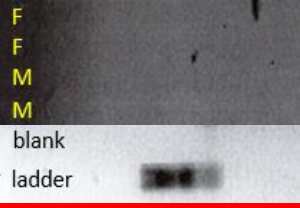
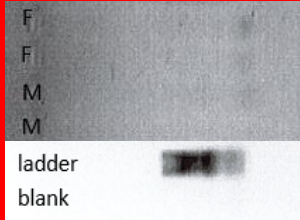

		Primer Pair 2		Primer Pair 12		Primer Pair 14		Primer Pair 18	
<i>Carinascincus greenii</i>									
		Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)	Ta tested (°C)	Ta amplified (°C)
		55 – 60.5	NA	55 – 60.5	NA	55 – 60.5	NA	55 – 60.5	NA
		Notes: No bands		Notes: No bands		Notes: No bands		Notes: No bands	

Figure S2: Gel images for the tests of the four primer pairs on the 13 species other than *L. whitii* within the Egernia group and the additional 3 species outside the Egernia group (subfamilies Mabuyinae and Eugongylineae). Sexes are indicated on each picture (‘M’ for males, ‘F’ for females). Each picture is associated to a ladder and a blank. All samples were run on a row including 23 other samples, a ladder and a blank. Background colour between some samples and their associated ladder differ because of different exposure across the gel. Green background indicates species/primer pairs that allowed for successful distinction between the sexes and therefore cross amplification. Red background indicates species/primer pairs where cross amplification failed.