

SUPPLEMENTARY MATERIAL

Multilocus coalescent species delimitation reveals widespread cryptic differentiation among Drakensberg mountain-living freshwater crabs (Decapoda : Potamonautes)

Ethel Emmarantia Phiri^A and Savel Regan Daniels^{A,B}

^ADepartment of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.

^BCorresponding author. Email: srd@sun.ac.za

Table S1. The molecular markers and primer pairs used in this study with their respective polymerase chain reaction conditions. Temperatures in bold under PCR profile indicate the annealing temperatures. The final extension was at 72 °C for 10 minutes (7 min. for DecapANT). * Protein coding. ** Exon-priming, intron-crossing

Molecular markers	Product size (pb)	PCR	Primer sequence (5'-3' direction)	PCR profile	Reference
Mitochondrial (mt)					
12S rRNA	353	12Sai	AAACTAGGATTAGATAACCCTATTAT	95 °C (5 min.), [95 °C (30 s),	Kocher <i>et al.</i> , (1989)
		12Sb	GAGAGTGACGGCGATGTGT	50 °C (40 s), 72 °C (1 min.)] x 34	
16S rRNA					
16S rRNA	536	16Sa-1471	ACTTGATATATAATTAAAGGGCCG	95 °C (5 min.), [95 °C (30 s),	Palumbi <i>et al.</i> , (1991)
		16Sb-1472	CTGGCGCCGCTCTGAACCAAATC	50 °C (40 s), 72 °C (1 min.)] x 34	
*Cytochrome oxidase subunit I	625	LCOI-1490 HCOI-2198	GGTCAACAAATCATAAAGATATTG TAAACTCAGGGTACCAAAAAATCA	94 °C (4 min.), [94 °C (30 s), 42 °C (40 s), 72 °C (45 s)] x 36	Folmer <i>et al.</i> , (1994)

Nuclear (nu)

28S rRNA	620	28Sa-modified 28Sb	GACCCGTCTTGAARCACCGA GAAGGAACCAGCTAC	TCG-	94 °C (4 min.), [94 °C (45 s), 50 °C (1 min.), 72 °C (1 min.)] x 40	Jesse <i>et al.</i> , (2010)
**Adenine nucleotide transporter	455	DecapANT-F DecapANT-R	CCTCTTGAYTTCGCKCGAAC TCATCATGCGCCTACGCAC		94 °C (3 min.), [94 °C (30 s), 60 °C (30 s), 72 °C (30 s)] x 35	Teske & Beheregaray, (2009); Teske <i>et al.</i> , (2009)
*Phosphoenolpyruvate carboxykinase	493	PEPCK-for PEPCK-rev	GTAGGTGACGACATTGCYTG- GATGAAGAACCAAGTTGACGTGGAA- GATC		94 °C (3 min.), [94 °C (30 s), 60 °C (45 s), 72 °C (1 min. 30 s)] x 35	Tsang <i>et al.</i> , (2008)

Table S2. Model parameters for each locus used in this study, where (a) represents the best-fit model for the Bayesian analyses as obtained in jModelTest v. 2.1.3 and (b) is nucleotide substitution models as obtained in MEGA5 v. 2.2 (* for Maximum Likelihood analyses only, obtained from MEGA 5 v. 2.2)

Gene fragment	Model	Base pair frequencies (%)	Gamma (G) distribution parameter	Proportion invariable (I) sites
12S rRNA	(a) TPM3uf + G (nst = 6; -lnL = 879.95; AIC = 1975.91)	A = 36.98 C = 8.63 G = 18.35 T = 36.04	0.013	N/A
	(b) T92 + I; -ln L = 899.90; AIC = 2009.10)	A = 36.12 C = 13.88 G = 13.88 T = 36.12	N/A	0.779
16S rRNA	(a) TPM2uf + G (nst = 6; -lnL = 1722.17; AIC = 3660.35)	A = 37.32 C = 9.50 G = 16.39 T = 36.79	0.208	N/A

	(b) TN93 + I; -lnL = 1741.12; AIC = 3697.10)	A = 37.70	N/A	0.660
		C = 9.90		
		G = 17.0		
		T = 35.4		
COI	(a) TIM2 + G + I (nst = 6; -lnL = 2446.72; AIC = 5113.44)	A = 28.81	1.095	0.603
		C = 19.05		
		G = 15.30		
		T = 36.84		
	(b) TN93 + G + I (-lnL = 2463.18; AIC = 5143.11)	A = 29.30	0.720	0.570
		C = 19.30		
		G = 15.80		
		T = 35.60		
28S rRNA	(a) TIM3 + G (nst = 6; -lnL = 1263.51; AIC = 2745.01)	A = 14.95	0.157	N/A
		C = 31.15		
		G = 34.89		
		T = 19.02		
	(b) T92 (-lnL = 1286.30; AIC = 2779.40)	A = 16.89	N/A	N/A
		C = 33.11		
		G = 33.11		
		T = 16.89		
DecapANT	(a) TPM2uf + G (nst = 6; -lnL = 844.78; AIC = 1903.556)	A = 23.34	N/A	N/A
		C = 20.82		
		G = 26.24		
		T = 29.60		
	(b) K2 (-lnL = 849.85; AICc = 1904.80)	A = 25.00	N/A	N/A
		C = 25.00		
		G = 25.00		

		T = 25.00		
PEPCK	(a) HKY + G + I (nst = 2; -lnL = 1059.05; AIC = 2334.09)	A = 19.51	0.396	0.824
		C = 35.73		
		G = 27.99		
		T = 16.77		
	(b) T92 + G + I (-lnL = 1261.09; AIC = 2733.11)	A = 18.20	3.590	0.930
		C = 31.80		
		G = 31.80		
		T = 18.20		
*cmtDNA	T92 + G (-lnL = 5220.92; AIC = 10642.13)	A = 34.67	0.129	N/A
		C = 15.33		
		G = 15.33		
		T = 34.67		
*cnuDNA	T92 + G + I (-lnL = 3677.55; AIC = 7565.40)	A = 19.80	0.430	0.830
		C = 30.20		
		G = 30.20		
		T = 19.80		
*cmtDNA + cnuDNA	T92 + G + I (-lnL = 10430.85; AIC = 21071.84)	A = 27.30	0.760	0.750
		C = 22.70		
		G = 22.70		
		T = 27.30		
