Strong purifying selection in endogenous retroviruses in the saltwater crocodile (*Crocodylus porosus*) in the Northern Territory of Australia: Additional File 1

This file contains:

Supplementary Figure 1

Supplementary Figure 2

Supplementary Table 1

Supplementary Table 2

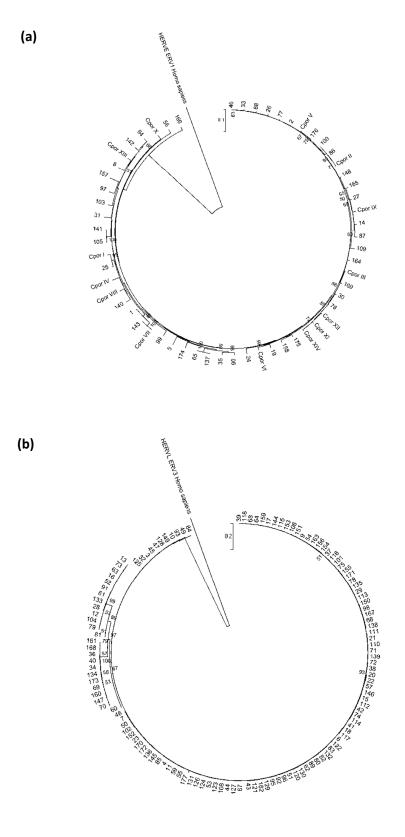
Supplementary Figures

(a)

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94							LEHSFVYSPSCPV											
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103							LEHSFVYSPSCPV											
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(h)														
(b)		* 20	* (40 *	60	* 80	* (100	* 120	* 140	*	160	* 180	
		: VLLDTGAQVNVIKPTIPHQK												
		: VLVDTGAÇVNVIKPTIPHÇK												
		: VLLDTGAQVNVIKPTIPHQK : VLLDTGSCVNVIKPTIPHCK												
	121 138	: VLUDIGSQVNVIKPTIPHQK : VLVDIGACVNVIKPTIPHCK												
	145	: VLLDTGAQVNVIKPTIPHQK												
	146	: VLLDTGSCVNVIKPTIPHCK												
	149	: VLVDTGAQVNVIKPTIPHQK	-MNGRIWVEGINEAK	CADIVHFITEFÇGKHFE	LKALLGGQNLLGA	PTIRFMCLK*ILNSA	FPIAISENCOPEP.	HAPLLASTVPWKFR	SIPTKPEGRPAIRNLLP	GLLSEGKVVECAASHC	LSVGWGIPKPKAPDS	SRLVIDYRVANSCI	APIQYVTKYTL	: 178
	156	: VLVDTGAQVNVIKPTIPHQK	-MNQRIWVEGINEAK	EADIVHFITEFQGKHF	LKALLGGQNLLGA	PTIRFMCLK*ILNSA	FPIAISENGGPEP	HAPLLASTVPWKFR	SIPTKPEGRPAIRNLLP	GLLSEGKVVECAASHC	LSVGWGIPKPKAPDS	SRLVIDYQVANSCI	APIQYVTKYTL	: 178
	159	: VLLDTGAÇVNVIKPTIPHQK												
	167	: VLVDTGAQVNVIKPTIPHQK												
	170	: VLLETGAÇVNVIKPTIPHÇK : VLVETGAÇVNVIKPTIPHÇK												
	34	: VLVDIGAÇVNVIKPTIPHÇK												
		: VLVDTGAQASVVKPTIPHQK												
	40	: VLLDTGSCANVVKPTIPHCK												
	69	: VLVDTGAÇANVVKPTIPHÇK	-?NCRIWVEGINEAK	ADVTHFILEFOGTHFE	LKALLGGQNLLGT	PTIYFMRLKEILISA	FPVAISENCOPEP.	HAPLLVSTVP*KFR	PIPTKPEGRPAIRNLLP	SLLGGGKFVECAASRY	LSVGWDIPKPNTPGS	FHLVIDYRVANSLI	TPILYATK?TL	: 175
	70	: VLVDTGAÇANVVKPTIPHQK												
	161	: VLLDTGAÇANVVKPTIPHQK												
	28 52	: VLLDTGSÇANVVKPTIPHÇK : VLLDTGSÇANVVKPTIPHÇK												
	61	: VLVDTGAQANVVKPTIPHQK												
	73	: VLVDTGSQANVVKPTIPHQK												
	79	: VLLDTGACANVVKPTIPHCK												
	81	: VLVDTGAÇANVVKPTIPHQK	-TNQQIWVEGINEAK	CADVVHFIIEFQGKHFF	LKTLLGGQNLLGT	PTIRFVCLMKF*SLS	FL*?QGPEP	HAPLLVSTMPWKFR	SIPTKPEGRPAIRNLLF	GLLSEGKVVECAASHC	LSVGWGIPKPKAPDS	SRLVIDYWVANSCI	APIQYVTKYTL	: 172
		: VLVDTGSÇANVVKPTIPHQK												
	133	: VLVDTGSÇANVVKPTIPHÇK	-TNQQIWVEGINEAK	CADVVHFIIEFQGKHFF	LKTLLGGQNLLGT	PTIRFVCLMKF*SLS	FL*?QGPEP	HAPLLVSTMPWKFR	PIPTKPEGRHAIRNLLF	GLVSEGKVVECAASRY	?WGIPKPNAPGS	FRLVIDYRVANSHI	TPLQYATEHTL	: 168
		* 200		220 *	240	* 260								
	39	: PELIMIVPNSSCPWGTAIDL	KEMFCCIPI*DDCGI	INFSFEGCVYKWKVCPE				65						
	54	: PELIMIVPNSSYPWGTAIDL												
	82	: PELIMIVPNSSYPWGTAIDL	KEMFYQIPI*DDQGI	LNFSFEGCVYKWKVCPE	GYKNSP-AVSGPL	LQKTLDKVQIPDGGT	VLRYVDDLL : 2	65						
	121	: PELIMIVPNSSYPWGTAIDL												
	138	: PELIMIVPNSSYPWGTAVDL												
	145	: PELIMIVPNSSYPWGTAIDL : PELIMIVPNSSYPWGTAIDL												
	140	: PELIMIVPNSSYPWGTAIDL : PELIMIVPNSSYPWGTAIDL												
	156													
	159	: PELIMIVPNSSYPWGTAIDL												
	167	: PELIMIVPNSSYPWGTAIDL	KEMFCQIPL*DDQGI	INFSFEGCVYKWKVCPE	GYKNSP-AVSGPL	LOKTLDKVQIPDGGT	VLSYVDDLL : 2	64						
	170	: PELIMIVPNSSYPWGTAIDL												
		: PELIMIVPNSSYPWGTAIGL												
	34	: PELIMIVCNSSYP*GTAIDL												
	36 40	: PELIMIVCNSSYP*GTAIDL : PELIMIVCNSSYP*GTAIDL												
	69	: PELIMIVCNSSYP*GTAIDL												
	70	: PELIMIVCNSSYP*GTAIDL												
	161	: PELIMIVCNSSYP*GTAIDL												
	168	: PELIMIVCNSSYP*GTAIDL	KDMFYHIPLGDGQGII	INFSFKGRVYEWKVCPC	GYKNSP-TISGPV	LQKTLDKIQIPDGGT	VLSYVDDIL : 2	62						
	28	: PELIMIVHNSGYPWGAAIDP												
	52	: PELIMIVHNSGYPWGAAIDP												
	61	: PELIMIVHNSGYPWGAAIDP												
	73	: PELIMIVHNSGYPWGAAIDP												
	79 81	: PELIMIVHNSGYPWGAAIDP : PELIMIVPNSSYPWGTAIDL												
	91	: PELIMIVPNSSIPWGIAIDE												
		: PELIMIVHNSGYPWGAAIDP												
			RT2	RT3			RT4							
		1	R12	KT3			KT4							

Supplementary Figure 1: Alignments of putative amino acid translations of selected sequences from clades CERV1 (a) and CERV2 (b). Conserved retroviral motifs are bound by black boxes. The dark and light shaded boxes in (b) highlight the proposed sub-lineages within clade CERV2: the unshaded sequences are group CERV2a, dark grey are CERV2b, and lighter grey are CERV2c.



Supplementary Figure 2: Neighbour joining trees based on the nucleotide alignments of *C. porosus* sequences from the CERV1 (a) and CERV2 (b) clades. Outgroups are human ERV sequences extracted from the Repbase database and from the same general ERV classes as these sequences. Numbers outside the circles indicate the haplotypes included in each tree. Smaller numbers within the circle represent bootstrap support values greater than 50%.

Supplementary Tables

Supplementary Table 1: Predicted recombinant and parental sequences as determined by RDP

Recombinant	Breakpoint Positions		Major Parental		Probability that the detected sequence is a recombinant ^c								
Sequence	Begin	End	Sequence ^a	Sequence ^b	RDP	GENECONV	Bootscan	Maxchi	Chimaera	SiSscan	3Seq		
CERV1-All													
1	289	906	148	Cpor_VII	0.0230	NS	0.0234	0.0001	0.0107	0.0000	0.0000		
Csia_IV	753	872	164	142	0.0301	NS	0.0306	NS	NS	NS	0.0344		
Cnil_I			164	142	Trace evi	dence only							
CERV1-porosus	;												
1	289	869	175	Cpor_VII	0.0005	NS	0.0005	0.0001	0.0021	NS	0.0000		
CERV2-All													
81	23	296	53	52	0.0001	0.0020	0.0001	0.0001	0.0001	0.0000	0.0000		
Amis_II	698	1	Asin_IV	Ppal_II	0.0398	NS	NS	NS	NS	NS	0.0215		
Amis_I			Asin_IV	Ppal_II	Trace evi	dence only							
CERV2-porosus	;												
81	23	296	53	52	0.0001	0.0013	0.0001	0.0001	0.0001	0.0000	0.0000		
60	16	191	162	34	0.0329	NS	NS	NS	NS	NS	0.0003		

^a Major Parent: Parent contributing the larger fraction of sequence ^b Minor Parent: Parent contributing the smaller fraction of sequence ^c NS: No significant P-value was recorded for this recombination event using this method

Supplementary Table 2: PAML analysis showing parameter estimates, average d_N/d_s , and significance of the pairwise test comparisons

Hn	Model	Average d _N /d _S	PAML parameter estimates	InL	LRT test statistic	df	p value
CER	2V1						
H0	M0: One ratio	0.4904	ω = 0.49042	-6936.0525	273.3946	4	< 0.001
H1	M3: Discrete	0.5187	p0 = 0.63002 p1 = 0.36034 p2 = 0.00964 ω0 = 0.14883 ω1 = 0.97228 ω2 = 7.73167	-6799.3552			
H2	M1a: Nearly neutral	0.4344	p0 = 0.66860 p1 = 0.33140P2 ω0 = 0.15403 ω1 = 1.00000	-6845.3939	92.0213	2	< 0.001
H3	M2a: Positive selection	0.5234	p0 = 0.64094 p1 = 0.34945 p2 = 0.00962 ω0 = 0.15461 ω1 = 1.00000 ω2 = 7.78576	-6799.3832			
H4	M7: Beta	0.4217	p = 0.37192 q = 0.50996	-6848.2309	96.8512	2	< 0.001
H5	M8: Beta & ω	0.4973	p0 = 0.99019 p = 0.40522 q = 0.54097 (p1 = 0.00981) ω = 7.46662	-6799.8053			
CER	XV2						
H0	M0: One ratio	0.5539	ω = 0.55393	-4029.5600	413.1006	4	< 0.001
H1	M3: Discrete	0.6898	p0 = 0.79438 p1 = 0.18634 p2 = 0.01928 ω0 = 0.14019 ω1 = 1.66189 ω2 = 13.93868	-3823.0097			
H2	M1a: Nearly neutral	0.3057	p0 = 0.76252 p1 = 0.23748 ω0 = 0.08951 ω1 = 1.00000	-3921.6374	178.2283	2	< 0.001
H3	M2a: Positive selection	0.5864	p0 = 0.72223 p1 = 0.25559 p2 = 0.02218 ω0 = 0.10433 ω1 = 1.00000 ω2 = 11.51932	-3832.5232			
H4	M7: Beta	0.3349	p = 0.17755 q = 0.35268	-3923.0792	164.9022	2	< 0.001
H5	M8: Beta & ω	0.6217	p0 = 0.97733 p = 0.21801 q = 0.39323 (p1 = 0.02267) ω = 12.04341	-3840.6281			