

Supplementary Material – Favarini et al.

Table S1: Samples of *Lycalopex* individuals analyzed in this study.

Species	Geographic Origin	Sample Identification Number	Institution / Contact
<i>Lycalopex sechurae</i>	Peru	bPse-01 ^a , bPse02 ^a , bPse03 ^a , bPse04 ^a	F. Angulo, S. Cárdenas-Alayza
<i>Lycalopex culpaeus</i>	Central – Argentina	bPcu01 ^a , bPcu02 ^a	C. B. Kasper, M. F. Rodrigues
	Marcona – Peru	bPcu03 ^a , bPcu04 ^a , bPcu05 ^a , bPcu06 ^a	M. Roca, M. Cardeña, S. Cárdenas-Alayza
	Parque Nacional Nahuelbuta - Chile	bPcu07 ^a	W. Johnson
<i>Lycalopex griseus</i>	Western Argentina	bPgr01 ^a , bPgr02 ^a	C. B. Kasper, M. F. Rodrigues
	Parque Nacional Nahuelbuta - Chile	bPgr03 ^a , bPgr04 ^a , bPgr05 ^a , bPgr06 ^a	W. Johnson
	Central-Eastern Argentina	bPgr07 ^a , bPgr08 ^a	C. B. Kasper, M. F. Rodrigues
<i>Lycalopex gymnocercus</i>	Porto Alegre - Rio Grande do Sul (RS) - Brazil	bPgy50 ^a	J. Koeneman
	Camaquã (RS) – Brazil	bPgy52 ^a	C. B. Kasper, M. F. Rodrigues
	Capão do Leão (RS) - Brazil	bPgy53 ^a	C. B. Kasper, M. F. Rodrigues
	Bom Jesus (RS) - Brazil	bPgy55 ^a , bPgy58 ^a , bPgy73 ^a , bPgy77 ^a , bPgy80 ^a	C. B. Kasper
	Santa Catarina (SC) - Brazil	bPgy61 ^a , bPgy72 ^a	C. B. Kasper, M. Piccoli / Fundação Zoobotânica do RS
	Rio Grande (RS) - Brazil	bPgy62 ^a	C. B. Kasper
	Arroio Grande (RS) - Brazil	bPgy63 ^a , bPgy66 ^a	C. B. Kasper
	Jaguarão-Pelotas (RS) – Brazil	bPgy64 ^a	C. B. Kasper, M. F. Rodrigues

	Paraná – Brazil	bPgy67 ^a	M. H. N. Capão da Imbuia
	Alegrete (RS) – Brazil	bPgy83 ^a	C. B. Kasper, M. F. Rodrigues
	Eastern Argentina	bPgy84 ^a	C. B. Kasper, M. F. Rodrigues
<i>Lycalopex vetulus</i>	Goias (Go) - Brazil	bPve10 ^a	F. Grazziotin, A. Garda
	Maranhão (MA) - Brazil	bPve13 ^a , bPve14 ^a , bPve15 ^a	C. B. Kasper
	Piaui (PI) – Brazil	bPve16 ^a	C. B. Kasper
	Mato Grosso (MT) - Brazil	bPve307 ^a , bPve309 ^a , bPve310 ^a	CENAP/ICMBio
	Brazil	bPve18 ^a	CENAP/ICMBio
	São Paulo (SP) - Brazil	bPve322 ^a , bPve227 ^a , bPve328 ^a , bPve353 ^a	CENAP/ICMBio
<i>Lycalopex fulvipes</i>	Parque Nacional Nahuelbuta - Chile	bPfu01 ^a , bPfu02 ^a , bPfu03 ^a , bPfu04 ^a , bPfu05 ^a , bPfu006 ^a	W. Johnson

Table S2: Mitochondrial DNA segments amplified and sequenced from the genus *Lycalopex* in this study.

Segment	PCR/sequencing Primers	Reference
<i>Cytocrome oxidase c subunit I (COI)</i>	LCO1490 / HCO2198	Folmer et al (1994)
Control region (D-Loop)	MTLPRO2 / CCR-DR1	Tchaicka et al. (2007)
<i>Cytochrome b (cyt-b)</i>	Cytb-DF1 / Cytb-DR1	This study
	L14724/ H15494	Irwin et al. (1991)
	L15162/ H15915	
ND5	ND5-DF1 / ND5-DR1	Trigo et al. (2008)
7mt	mtDNA7H / mtDNA7L	Delisle & Strobeck (2002)
8mt	mtDNA8H / mtDNA8L	Delisle & Strobeck (2002)

Table S3. Mitochondrial PCR/sequencing primers generated from the genus *Lycalopex* in this study.

Segment	Primer	Primer (5' – 3')
<i>Cytb</i>	Cytb-DF1	TCTCACATGGAATTTAACCATGA
	Cytb-DR1	GAATTTTCAGCTTTGGGTGCT
7mt	7mti-R1	CAAGTAATAGATACTCCGGAGGCTAG
	7mti-F2	ACCATACCCCTATCGTACAAAAAG
	7mti-R2	CATGGGGTCAAAACCATATT
	7mti-F3	CCGCTGCATGATATTGACA
8mt	8mti-R1	CTACTAGGAGTGGGAGGGATCCT
	8mti-F2	ACCACTATTAGCACTTACAACATGACT
	8mti-R2	AGTACGGCTATGGATTCGTTC
	8mti-F3	GTAGCGGTTCTTATTCAAACACC
9mt	9mti-F2	GCAAATACAGCTGCCCTACAAGC

Table S4: *Lycalopex* mitochondrial DNA data set features. N: Number of sequenced individuals; L: Length of the sequenced segment; V: variable sites; PI: parsimony-informative sites; EM: evolutionary model estimated for each partition.

Partition	N	L (bp)	Full data set		Ingroup only		EM
			V	PI	V	PI	
COI&III	59	715*	180	154	53	44	HKY+G
ND3	38	347	104	77	29	24	HKY+G
ND4L	60	297	82	71	27	21	HKY+I
ND4	65	1372	417	360	165	124	HKY+I+G
ND5	66	1197	369	319	146	112	GTR+G
Cytb	64	1139	316	255	135	96	GTR+I
CR	63	596**	153	129	103	87	HKY+I+G
tRNAs	63	337	50	34	17	12	HKY+I+G

Concatenated	56	6000	1671	1399	675	520	GTR+I+G
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**COI* spanned 676 bp and *COIII* spanned 39 bp.

**After exclusion of 40 bp presenting ambiguous alignment.

Table S5: Estimation of the times of origin (time to the most recent common ancestor – TMRCA) for genus *Lycalopex*, as well as each of the analyzed species, with the mtDNA data set.

	TMRCA						
	<i>Lycalopex</i>	<i>L. vetulus</i>	<i>L. sechurae</i>	<i>L. gymnocercus</i>	<i>L. griseus</i>	<i>L. culpaeus</i>	<i>L. fulvipes</i>
Mean	1.17	0.17	0.09	0.39	0.21	0.35	0.06
95% HPD							
lower	0.88	0.12	0.05	0.29	0.14	0.25	0.03
95% HPD							
upper	1.52	0.23	0.13	0.51	0.29	0.47	0.10

Table S6: Number of sequences generated here per species of *Lycalopex* for each nuclear gene segment.

Species	Gene						
	<i>ATP7AE3</i>	<i>CHRNA1</i>	<i>CYP1A1</i>	<i>FES</i>	<i>GHR</i>	<i>TCP1</i>	<i>VTN</i>
<i>Lycalopex culpaeus</i>	3	8	6	2	1	6	4
<i>Lycalopex fulvipes</i>	5	7	6	3	2	8	4
<i>Lycalopex griseus</i>	4	9	9	4	2	9	5
<i>Lycalopex gymnocercus</i>	3	7	7	2	4	6	6
<i>Lycalopex sechurae</i>	2	4	5	1	2	3	4
<i>Lycalopex vetulus</i>	5	4	5	3	4	4	8
<i>Cerdocyon thous</i>	0	4	2	4	2	3	2
Total	22	43	40	19	17	39	33

Table S7: Accession numbers for nuclear sequences downloaded from GenBank for genus *Lycalopex*.

Species	<i>CHRNA1</i>	<i>CYP1A1</i>	<i>FES</i>	<i>GHR</i>	<i>VTN</i>
<i>Lycalopex culpaeus</i>	DQ239440.1	DQ239448.1	DQ239456.1	DQ239464.1	DQ239480.1
<i>Lycalopex fulvipes</i>	DQ239441.1	DQ239449.1	DQ239457.1	DQ239465.1	DQ239481.1
<i>Lycalopex griseus</i>	AY885319.1	AY885343.1	AY885366.1	AY885390.1	AY885414.1
<i>Lycalopex gymnocercus</i>	EF106987.1 and AY885320.1	AY885344.1	AY885367.1	AY885391.1	AY885415.1

<i>Lycalopex sechurae</i>	AY885321.1	AY885345.1	AY885368.1	AY885392.1	AY885416.1
<i>Lycalopex vetulus</i>	DQ239442.1 and EF106988.1	DQ239450.1	DQ239458.1	DQ239466.1	DQ239482.1
<i>Cerdocyon thous</i>			AY885364.1; EF107038.1; EF107041.1 and EF106982.1	AY885388.1	-
		AY885341.1	EF107040.1		

Table S8: List of the nuclear segments and primers used in this study for genus *Lycalopex*.

Gene	Gene Name	Reference
ATP7A - exon 3	<i>ATPase, Cu++ transporting, alpha polypeptide</i> , exon 3	This study. F: 5'aaaaatgcaactattatttatgacccta3' R: 5' taattcgctgaacaccttgc 3'
CHRNA1	<i>Cholinergic receptor, nicotinic alpha polypeptide precursor 1</i> , intron 8	Lyons et al. (1997)
CYP1A1	<i>Cytochrome P-450</i> , intron 3 to exon 6	Venta et al. (1996)
FES	<i>Feline sarcoma protooncogene</i> , intron 14	Venta et al. (1996)
GHR	<i>Growth hormone receptor</i> , intron 9 to exon 10	Venta et al. (1996)
TCP1	<i>T-complex protein 1, alpha subunit</i>	Lyons et al. (1997)
VTN	<i>Vitronectin</i> , intron 4	Jiang et al. (1998)

Table S9: Parameters employed for the *BEAST analyses.

Parameter	Setting
Chain length*	200.000.000
Spacing between data samples	20.000
Species tree prior	Species tree: Yule process
Population Size Model	Piecewise linear & constant root
Clock Model	Strict Clock
Base frequencies	Empirical
Substitution Model	HKY for <i>ATP7AE3</i> , <i>GHR</i> , <i>TCP1</i> and <i>VTN</i>
	HKY+I for <i>CHRNA1</i> , <i>CYP1A1</i> and <i>FES</i>

* The initial 10% of each MCMC run was discarded as burn-in.

References for the Supplementary Material

Jiang, Z. Priat, C. Galibert, F. 1988 Traced orthologous amplified sequence tags (TOASTs) and mammalian comparative maps. *Mamm. Genome* **9**, 577–587. (doi: 10.1007/s003359900821)

Lyons, L. A. Laughlin, T. F. Copeland, N. G., Jenkins, N. A., Womack, J. E., O'Brien, S. J. 1997 Comparative anchor tagged sequences (CATS) for Integrative mapping of mammalian genomes. *Nature Genetics* **15** 47–56. (doi:10.1038/ng0197-47)

Venta, P. J. Brouillette, J. A. Yuzbasiyan-Gurkan, V. Brewer, G. J. 1996 Gene-specific universal mammalian sequence-tagged sites: application to the canine genome. *Biochem. Genet.* **34**,321–341. (doi: 10.1007/BF02399951).

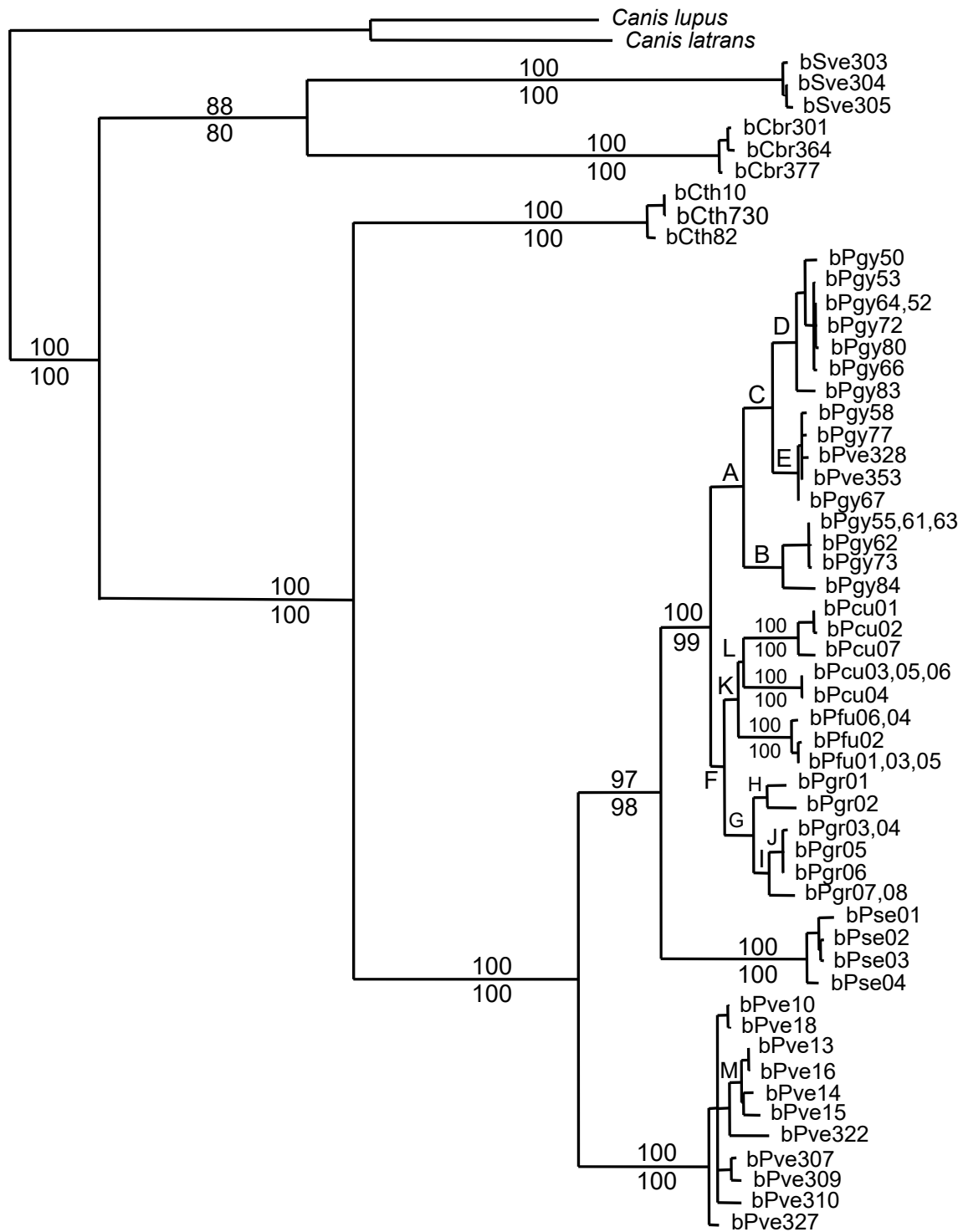


Figure S1. Maximum parsimony phylogeny of genus *Lycalopex*. Strict consensus of 270 equally parsimonious trees (length: 2889) retrieved with PAUP*. Values above and below branches represent bootstrap support computed with PAUP and TNT, respectively. Support values for lettered nodes are given in Table 5. See Figure 2 and Table 1 for sample identification codes.

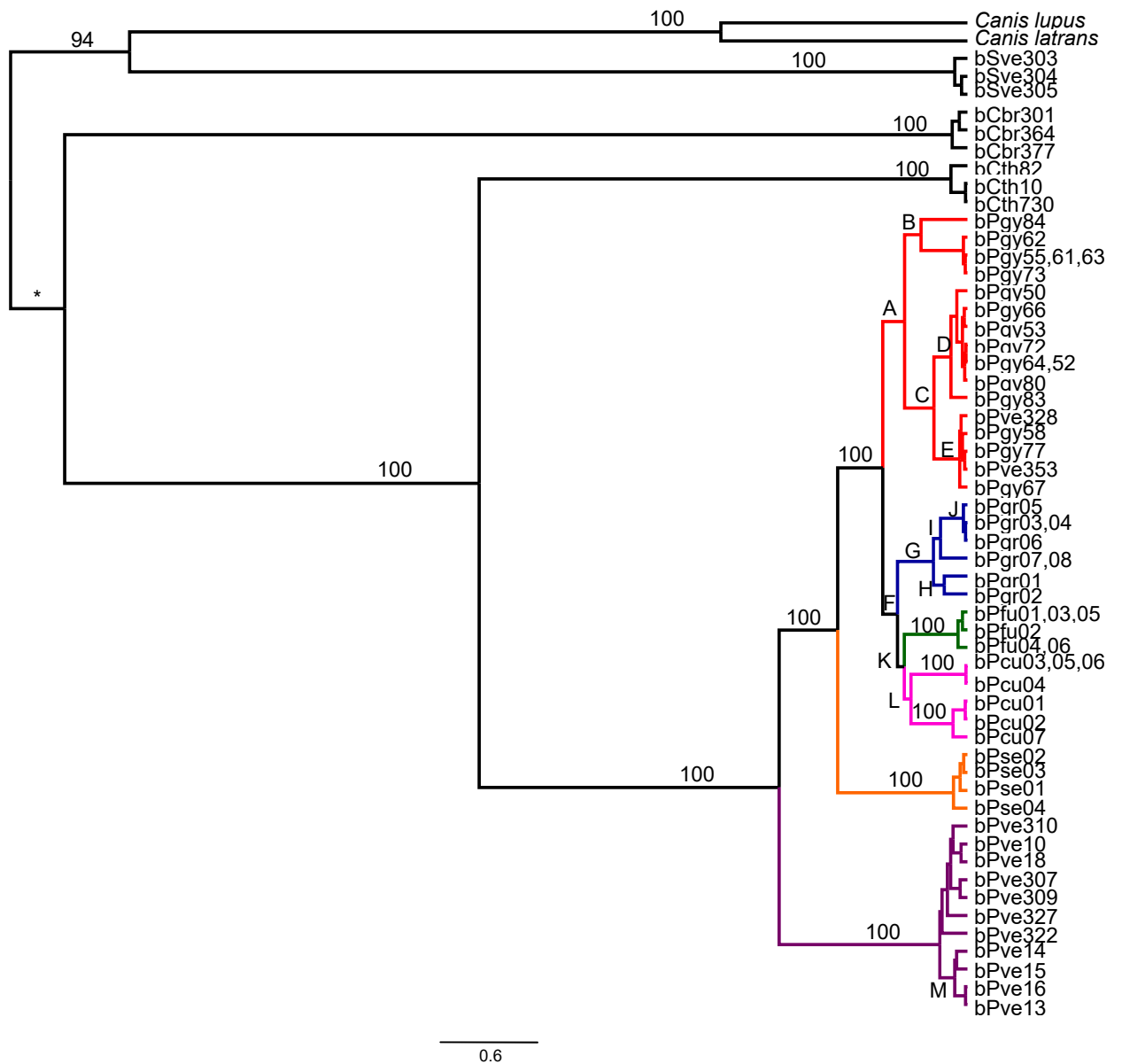


Figure S2. Bayesian phylogeny of South American foxes of genus *Lycalopex*, generated with Beast 1.6.0. Values above branches indicate the Bayesian posterior probability (expressed as percentages) of the clade defined by the adjacent node. The asterisk indicates a posterior probability below 0.50. Support values for lettered nodes are given in Table 5. Species-level branches are colored as in Figure 2.