



## PCR amplification, sequencing, and *in silico* analysis of holandric genes *TSPY* and *SRY* in Nili Ravi buffalo

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	SRY_NF_Final	JX668002 Buffalo 920Nili-Ravi	JX667999 Buffalo 717Nili-Ravi	JX667999 Buffalo Kundi	OQ259332 Buffalo	F_546413 Buffalo Murr	AY341337 Buffalo	DQ417872 Buffalo	DO119747 Buffalo	EU594189 Cattle	DQ888702 WT_Deer	AY079142 E_Bison	AY079141 Bison	Z30321 E_Bison	EU647257 Yak	AY079146 Cattle	EU016229 Buffalo	KF038141 E_Deer	KF038140 Deer	MH680934 S_Deer	EF100132 W_Deer	KC337274 Moose	
1																							
2	SRY_NF_Final																						
3	JX668002 Buffalo 920Nili-Ravi	0.00																					
4	JX667999 Buffalo 717Nili-Ravi	0.00	0.00																				
5	JX667999 Buffalo Kundi	0.00	0.00	0.00																			
6	GQ259332 Buffalo	0.00	0.00	0.00	0.00																		
7	F_546413 Buffalo Murr	0.00	0.00	0.00	0.00	0.00																	
8	AY341337 Buffalo	0.00	0.00	0.00	0.00	0.00	0.00																
9	DQ417872 Buffalo	0.01	0.01	0.01	0.01	0.01	0.01	0.01															
10	DQ119747 Buffalo	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00														
11	EU294189 Cattle	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03													
12	DQ888702 WT_Deer	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.02												
13	AY079142 E_Bison	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.02											
14	AY079141 Bison	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.02	0.00										
15	Z30321 E_Bison	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.02	0.00	0.00									
16	EU547257 Yak	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.01	0.03	0.01	0.01	0.01								
17	AY079146 Cattle	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.01	0.03	0.01	0.01	0.01	0.01							
18	EU016229 Buffalo	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.07	0.06	0.07	0.07	0.07	0.07	0.07						
19	KF038141 E_Deer	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03					
20	KF038140 Deer	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.08				
21	MH680934 S_Deer	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.00			
22	EF100132 W_Deer	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.01	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.07	0.02	0.02		
23	KC337274 Moose	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.02	0.04	0.04	0.04	0.04	0.04	0.04	0.08	0.03	0.03	0.03	0.03	

Supplementary Fig. 1. Pair-wise distance (below diagonal) and the respective standard errors (above diagonal) of the *SRY* gene have been displayed in a matrix form. Created using MEGA7 software (dark to light colour indicates less to high pair-wise distance).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	
1																					
2	S1P1_Final_seq																				
3	EU032586 Buffalo TSPY	0.01																			
4	DQ923704 Buffalo wTspy	0.02	0.03																		
5	EU350952 Cattle TSPY	0.18	0.20	0.19																	
6	XM_018045416 Goat TSPY	0.94	0.98	0.84	0.86																
7	MT768536 Sheep TSPY	0.12	0.13	0.14	0.19	0.85															
8	JQ303256 Ind_Cow TSPY	0.86	0.86	0.80	0.79	0.14	0.87														
9	EF432554 D_yak TSPY	0.86	0.86	0.80	0.79	0.13	0.87	0.00													
10	XM_020903635 Wl_deer TSPY	1.03	1.08	0.97	0.97	0.19	0.98	0.13	0.13												
11	XM_043460210 Ek TSPY	0.95	0.99	0.87	0.93	0.22	0.92	0.14	0.14	0.12											
12	XM_032474735 Camel TSPY	1.01	1.01	1.01	0.99	0.36	1.02	0.23	0.23	0.31	0.35										
13	XM_035725886 Cs_lion TSPY	1.03	1.07	1.00	0.98	0.36	1.04	0.26	0.27	0.35	0.37	0.18									
14	XM_033420189 orca TSPY	1.04	1.09	1.00	1.02	0.31	1.02	0.22	0.23	0.28	0.33	0.14	0.15								
15	XM_030856992 Lfp_whale TSPY	1.01	1.05	0.96	1.00	0.31	0.98	0.22	0.22	0.29	0.33	0.14	0.14	0.01							
16	XM_029233063 Narwhale TSPY	1.04	1.09	0.97	1.03	0.33	1.01	0.23	0.24	0.30	0.34	0.15	0.14	0.02	0.02						
17	XM_027102774 Ws_dolphin TSPY	1.02	1.07	0.98	1.02	0.31	1.00	0.21	0.22	0.29	0.33	0.14	0.14	0.00	0.01	0.02					
18	XM_036862929 Blue-whale TSPY	1.03	1.08	0.99	1.03	0.31	1.01	0.22	0.23	0.29	0.33	0.14	0.16	0.03	0.02	0.03	0.02				
19	XM_043897488 Red-deer TSPY	0.86	0.93	0.86	0.82	0.33	0.76	0.23	0.24	0.28	0.30	0.28	0.26	0.25	0.25	0.25	0.24	0.25			
20	XM_042926830 Lion TSPY	1.03	1.03	0.98	0.98	0.38	0.97	0.24	0.25	0.35	0.37	0.20	0.14	0.17	0.16	0.17	0.16	0.17	0.17	0.31	

Supplementary Fig. 2. Pair-wise distance (below diagonal) and the respective standard errors (above diagonal) of the *TSPY* gene have been displayed in a matrix form. Created using MEGA7 software (dark to light colour indicates high to less pair-wise distance).