Figure S1. Multi-locus species phylogeny for the a) X-linked loci and b) autosomal loci. Posterior probabilities are mapped onto the branches, and the node bars represent the 95% highest posterior density for the node height. For the X-linked tree, *D. neotestacea* sequences were split into ST and SR.

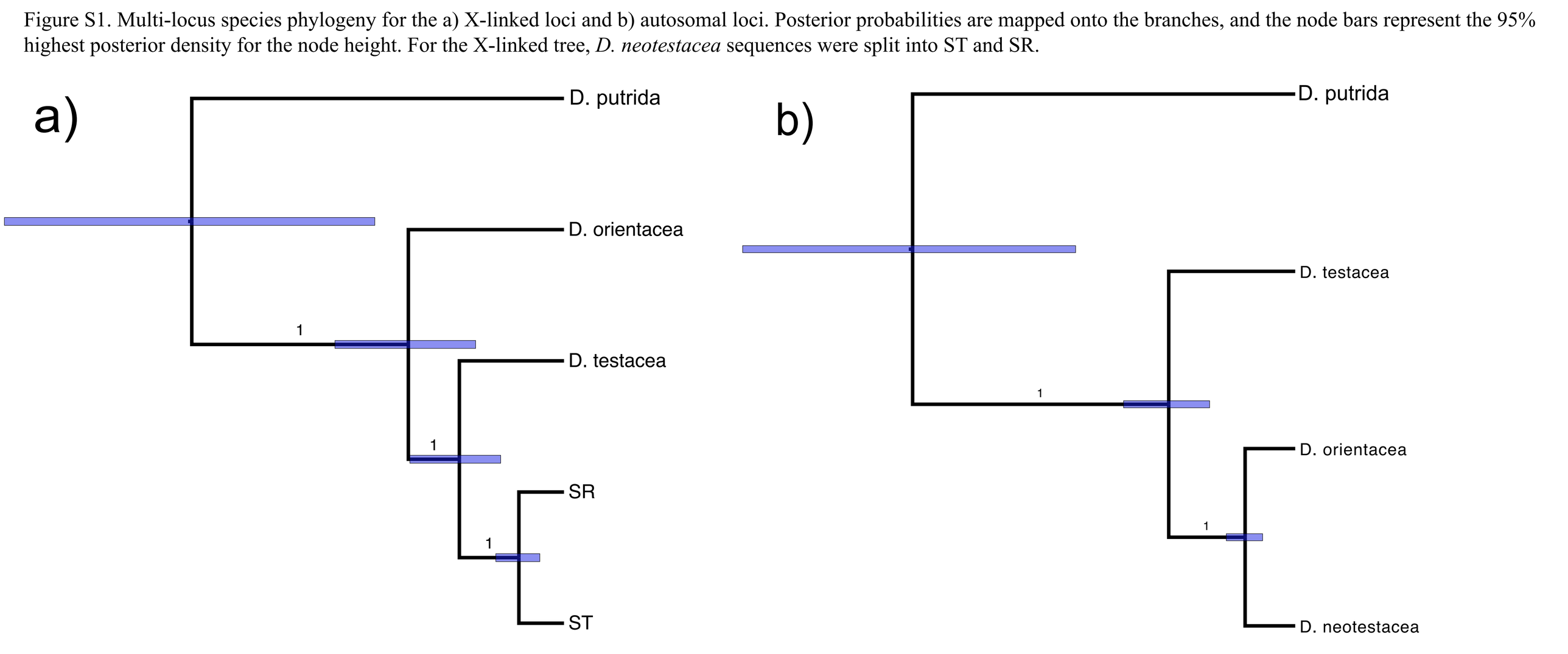
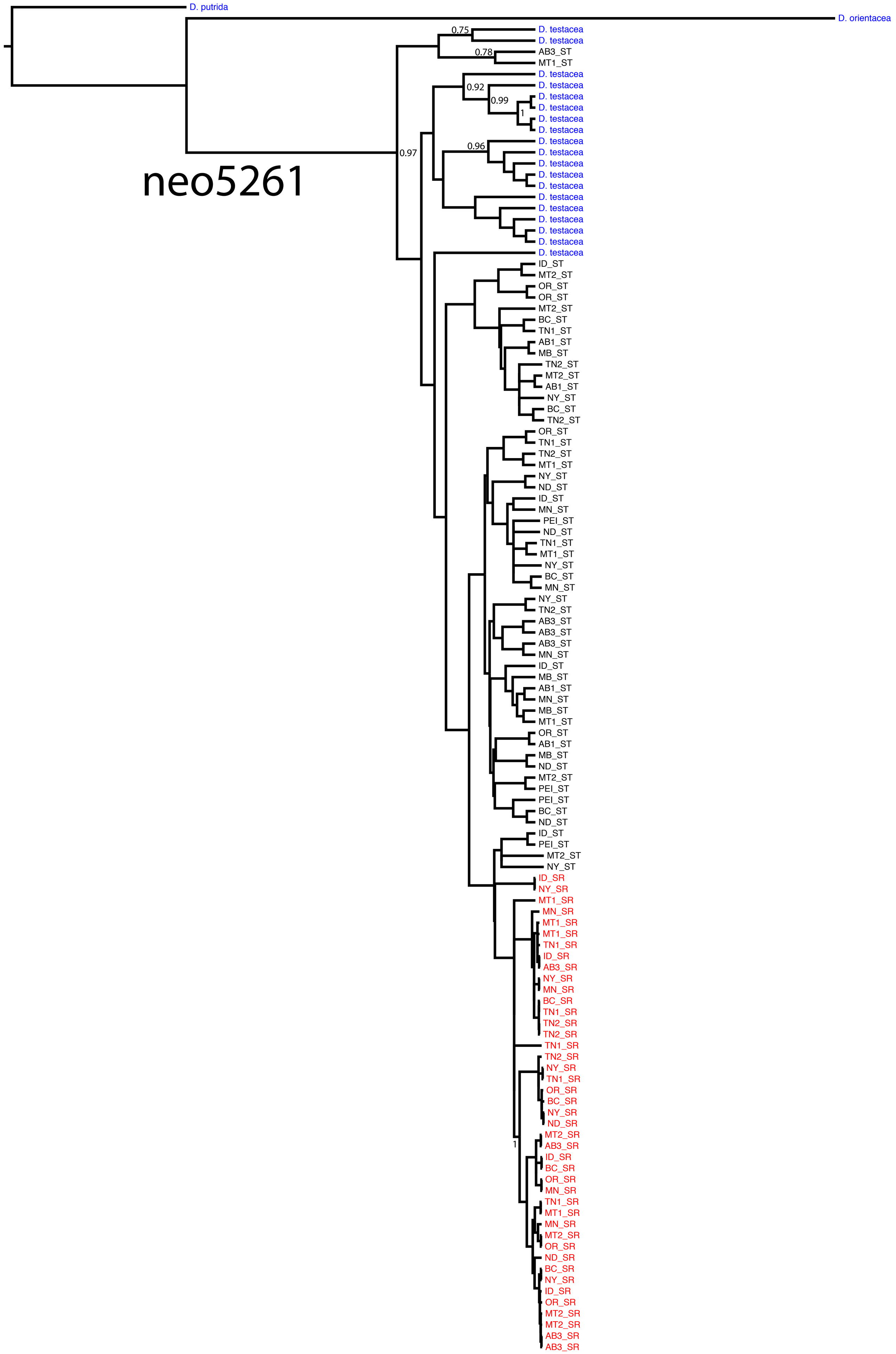
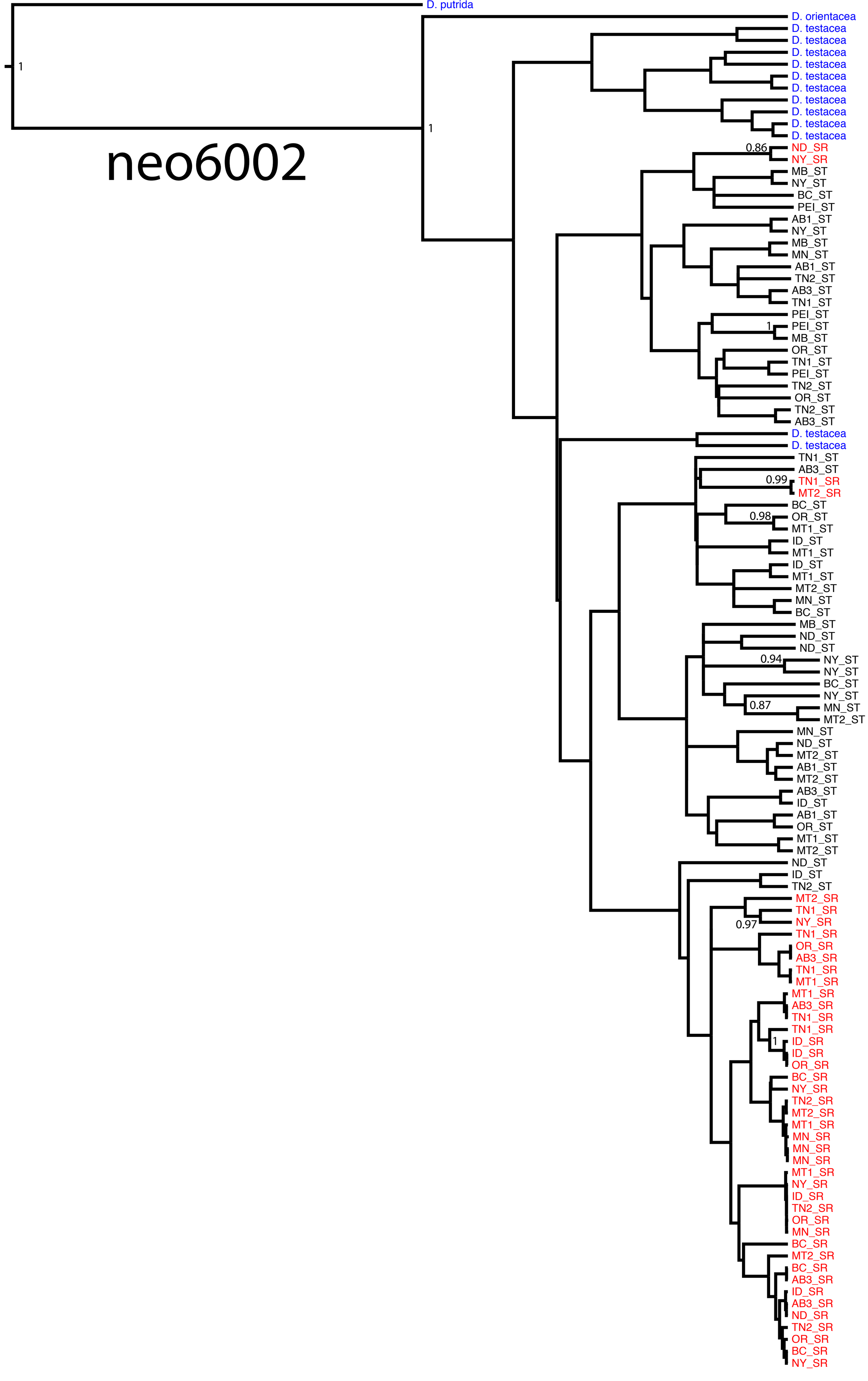


Figure S2. Individual gene trees. Trees were built in *BEAST (see Methods), and the posterior probabilities greater than 0.75 are mapped onto branches. Individual branches are labeled with population or province of origin (Table S1) and X-chromosome type. SR samples are noted in red text, ST samples are black, and outgroup samples are blue.

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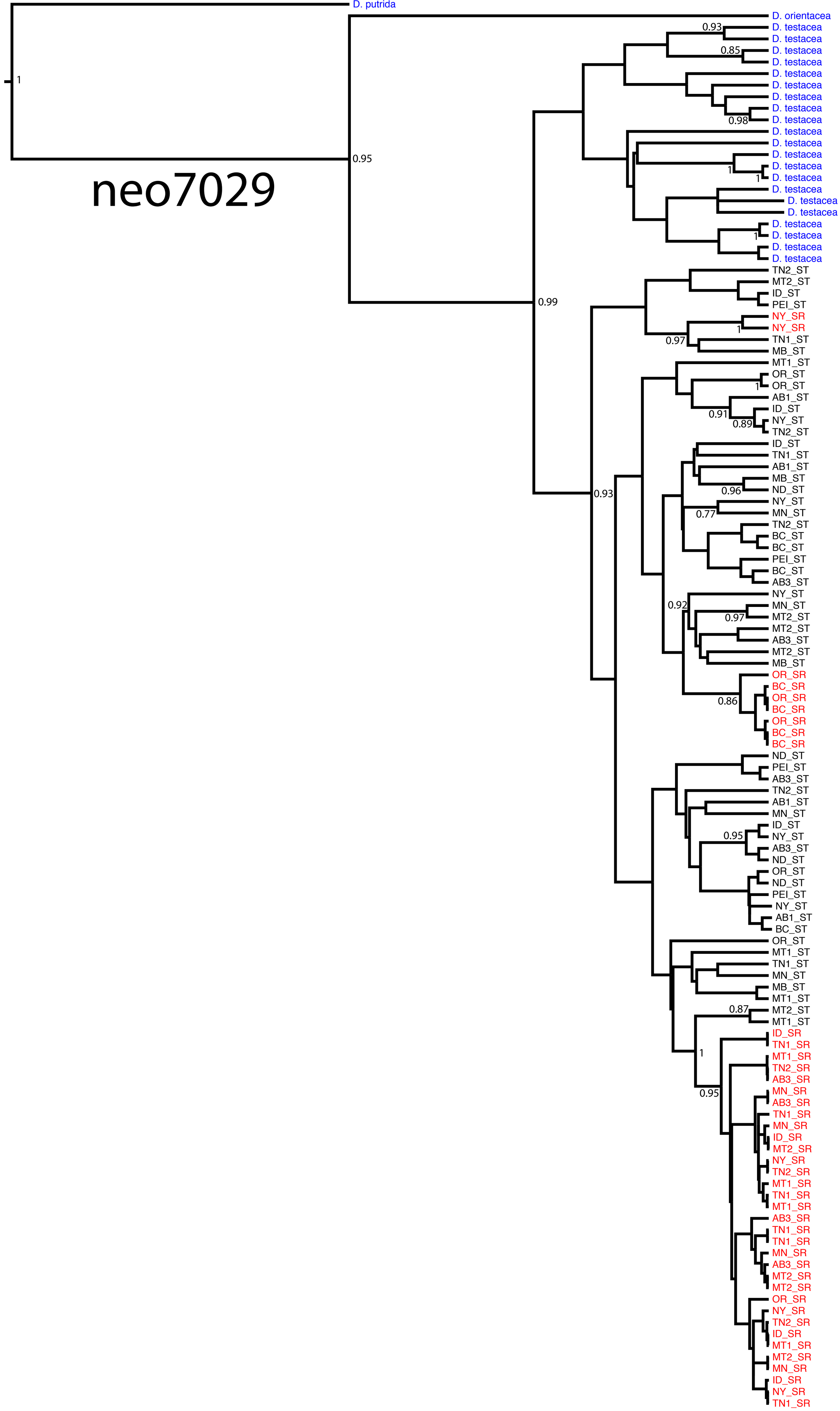
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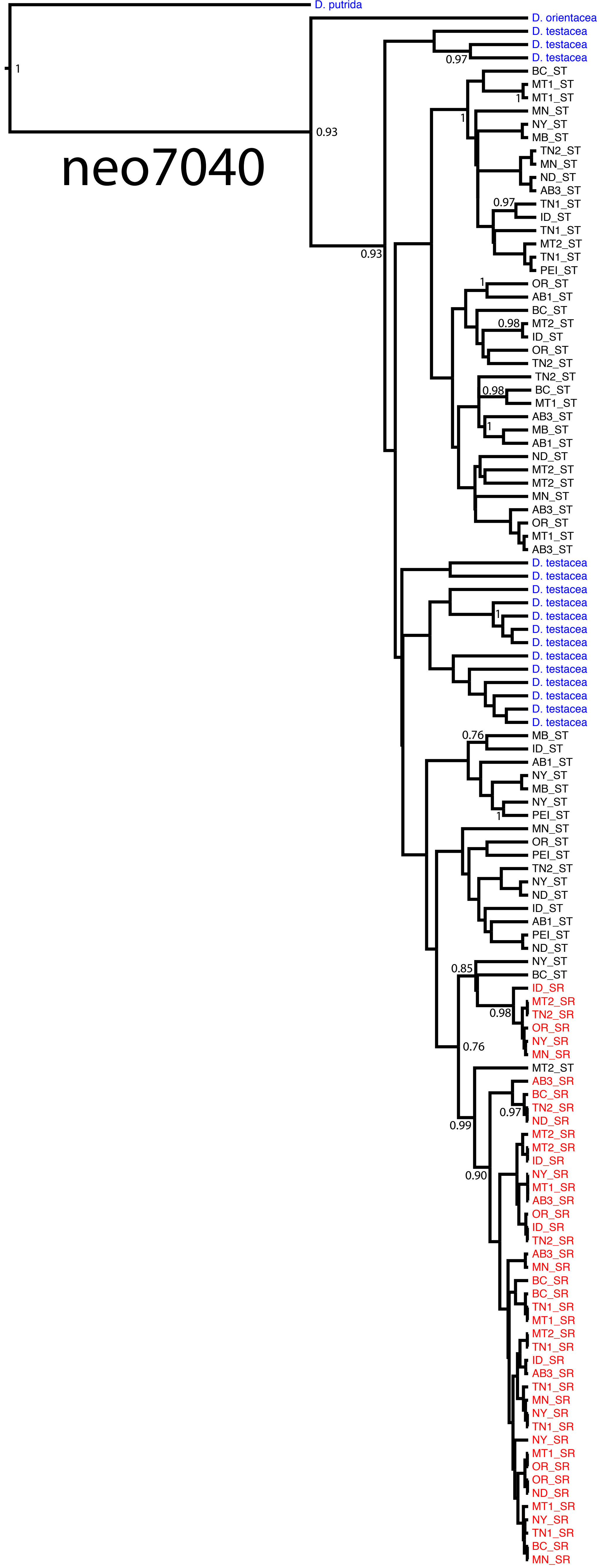
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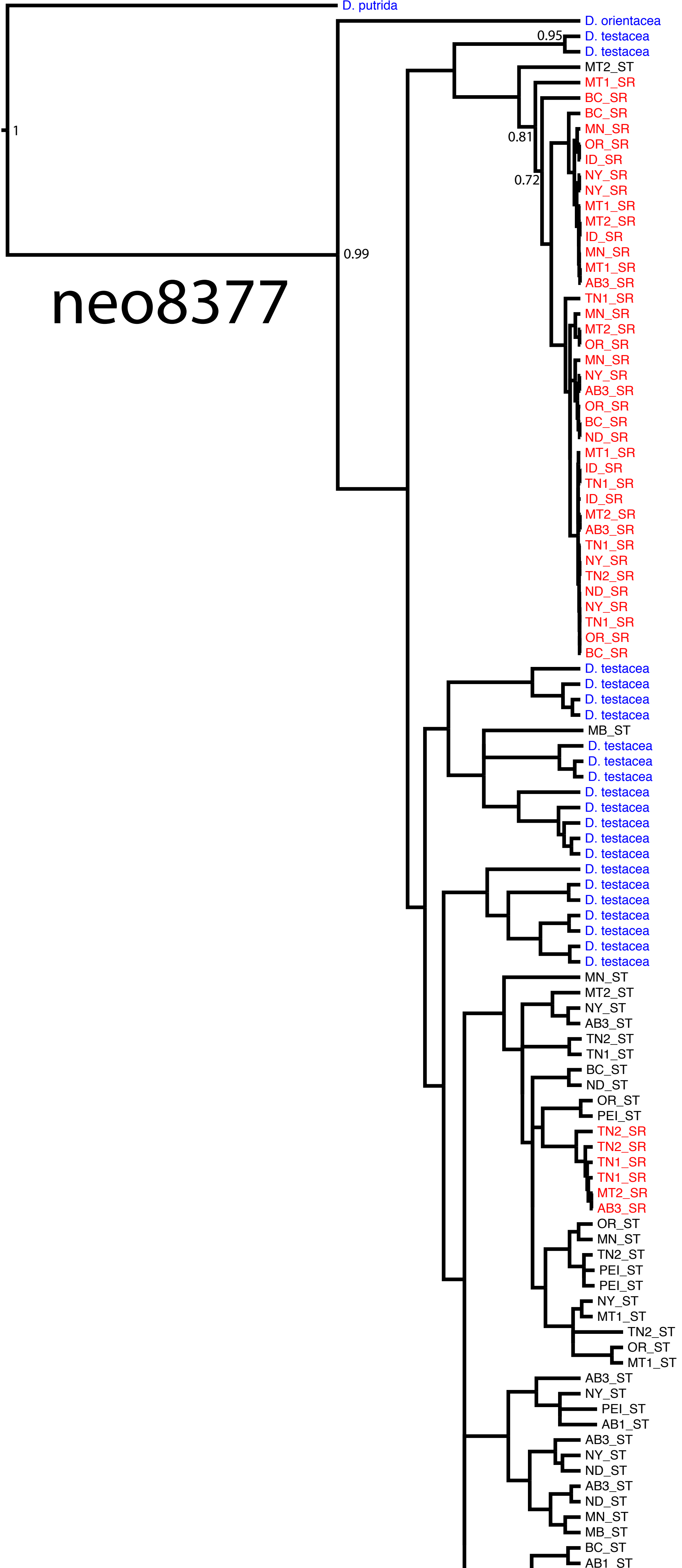
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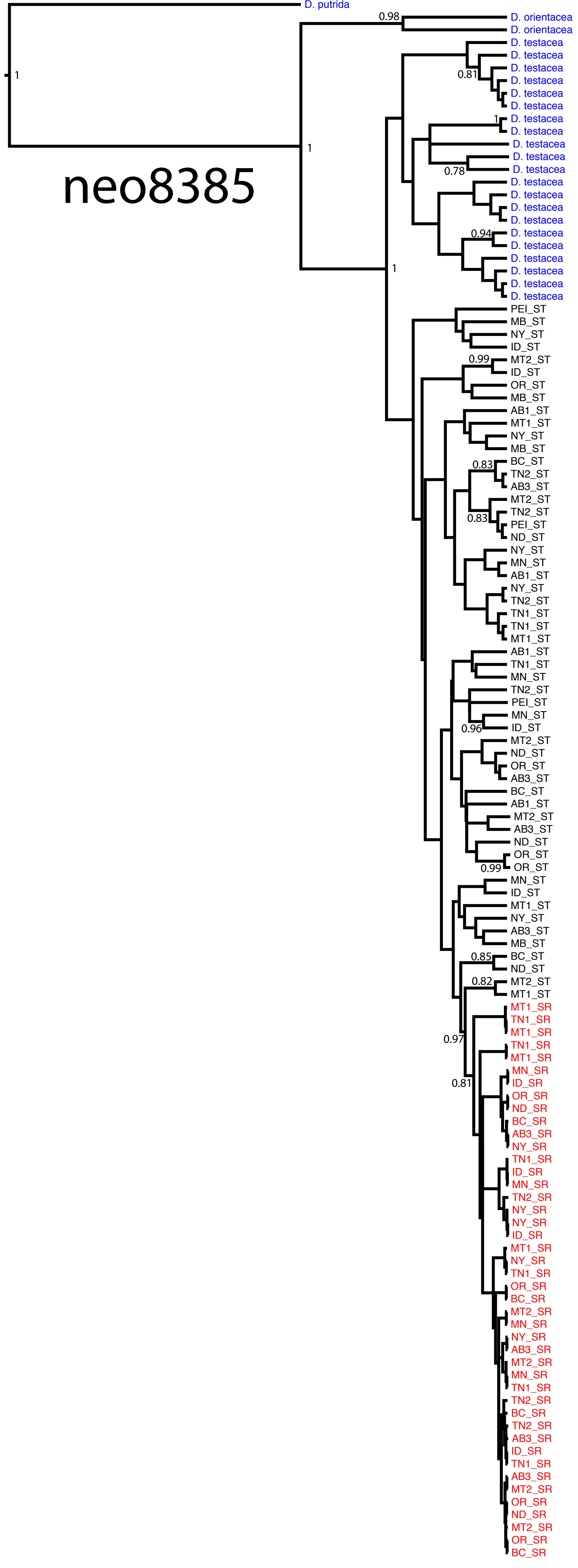
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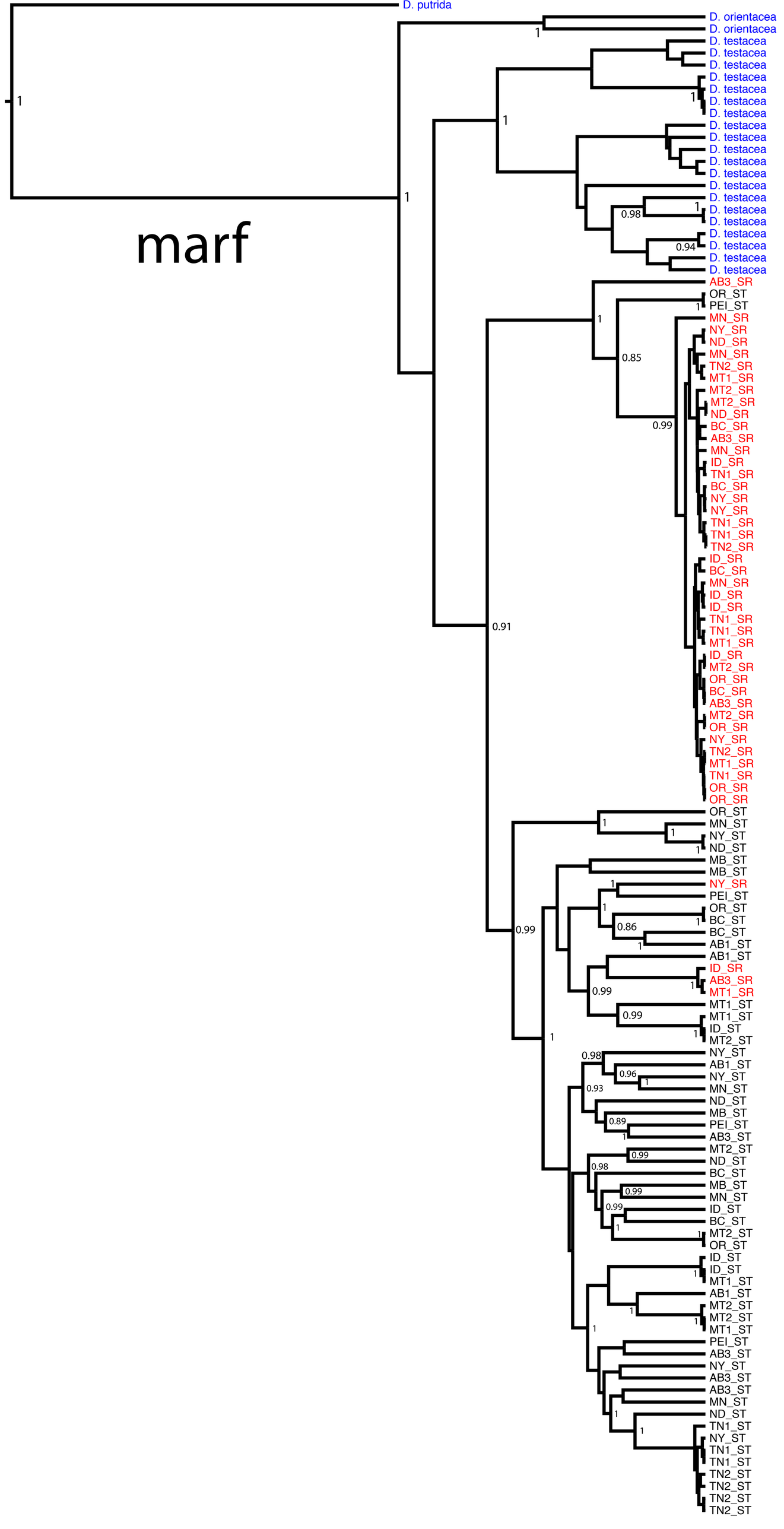
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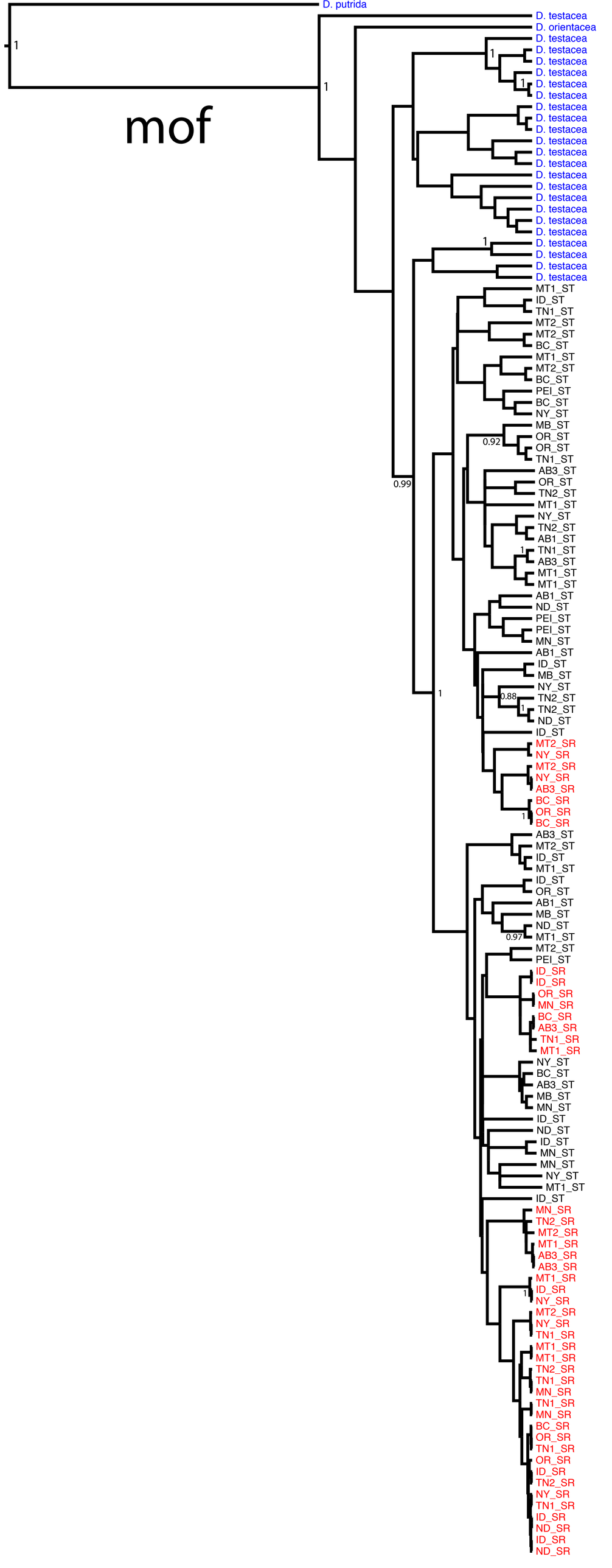
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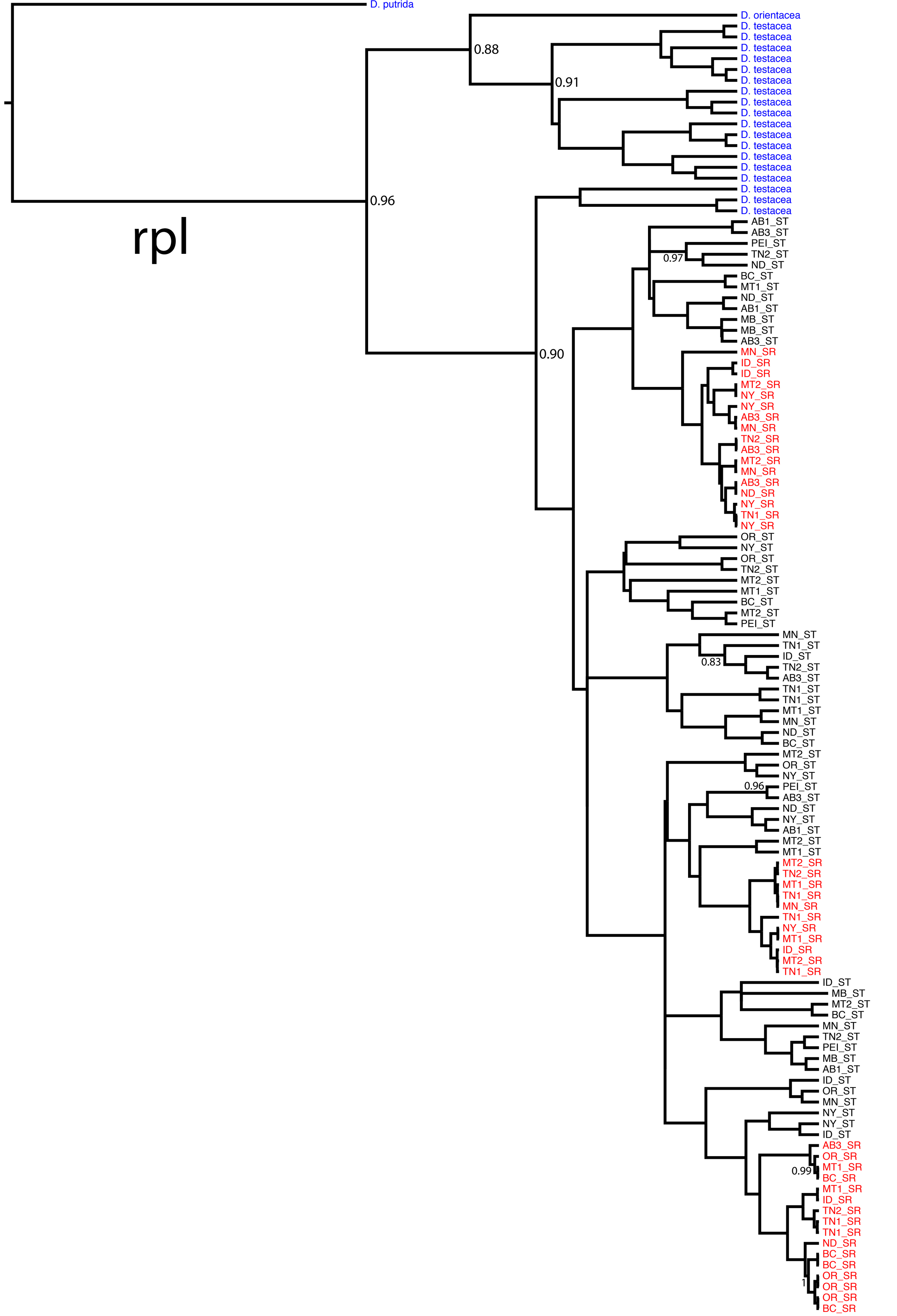
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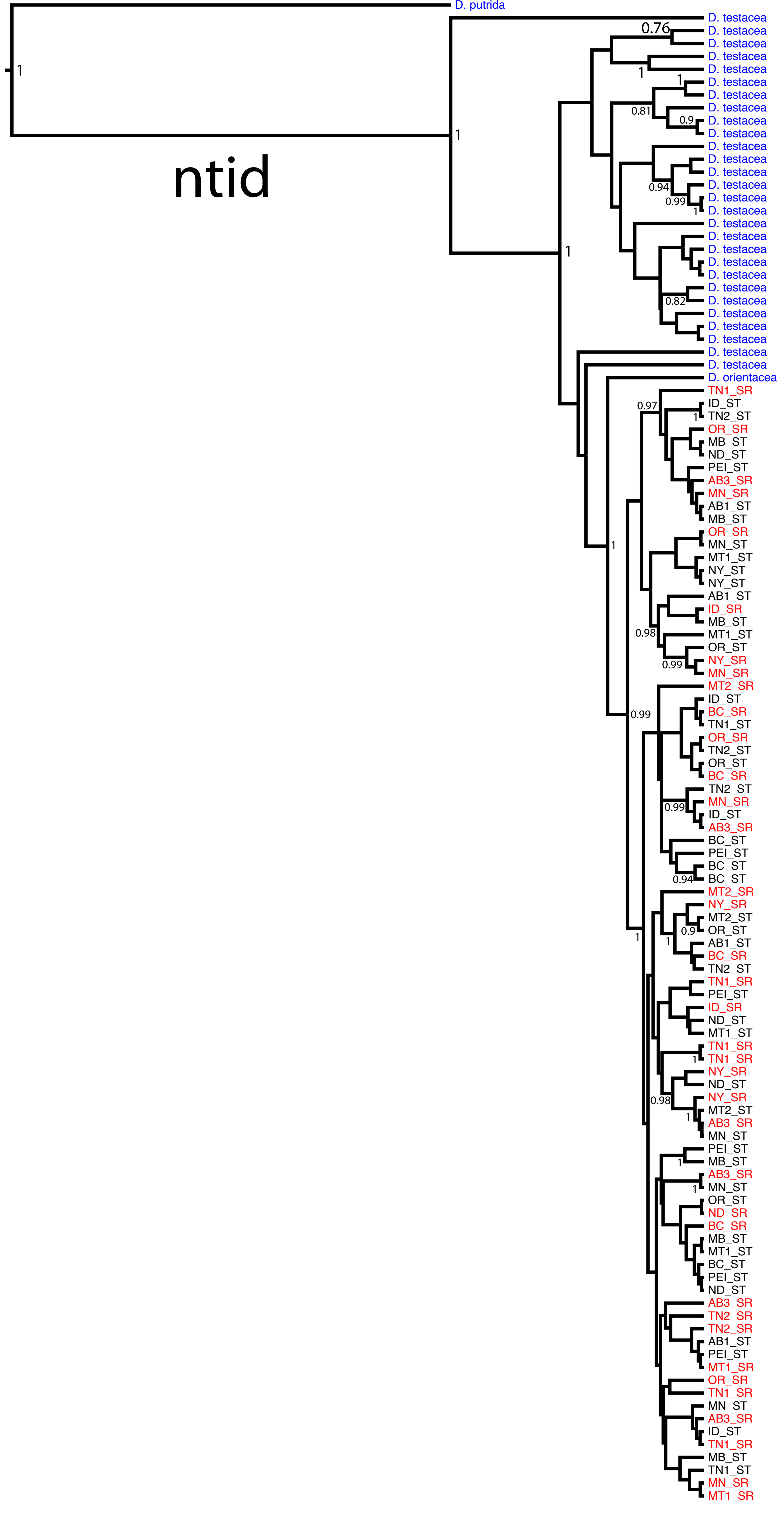
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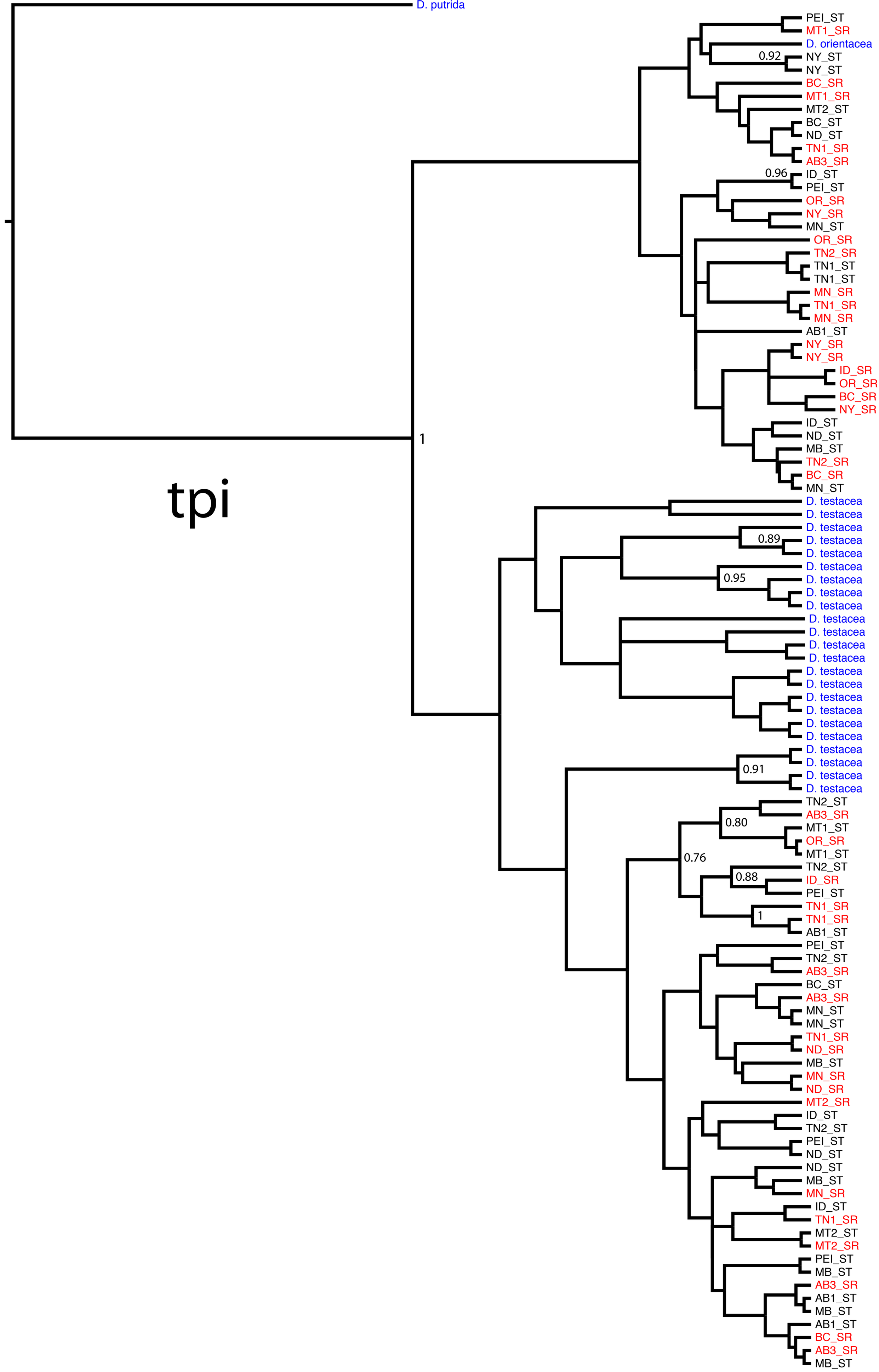
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Figure S3. Linkage disequilibrium between SNPs considering ST and ST samples separately. Shown are R^2 values between pairs of SNPs within and between each marker, using parsimony informative sites only. All sites included in the analyses are pictured. R^2 is represented as a heat map, with the lightest grey = 0 and the darkest grey = 1. *mof* and *rpl* were unable to be mapped, have been added to the end of the alignment. Some markers are not represented in the SR-only matrix due to a lack of parsimony informative sites.

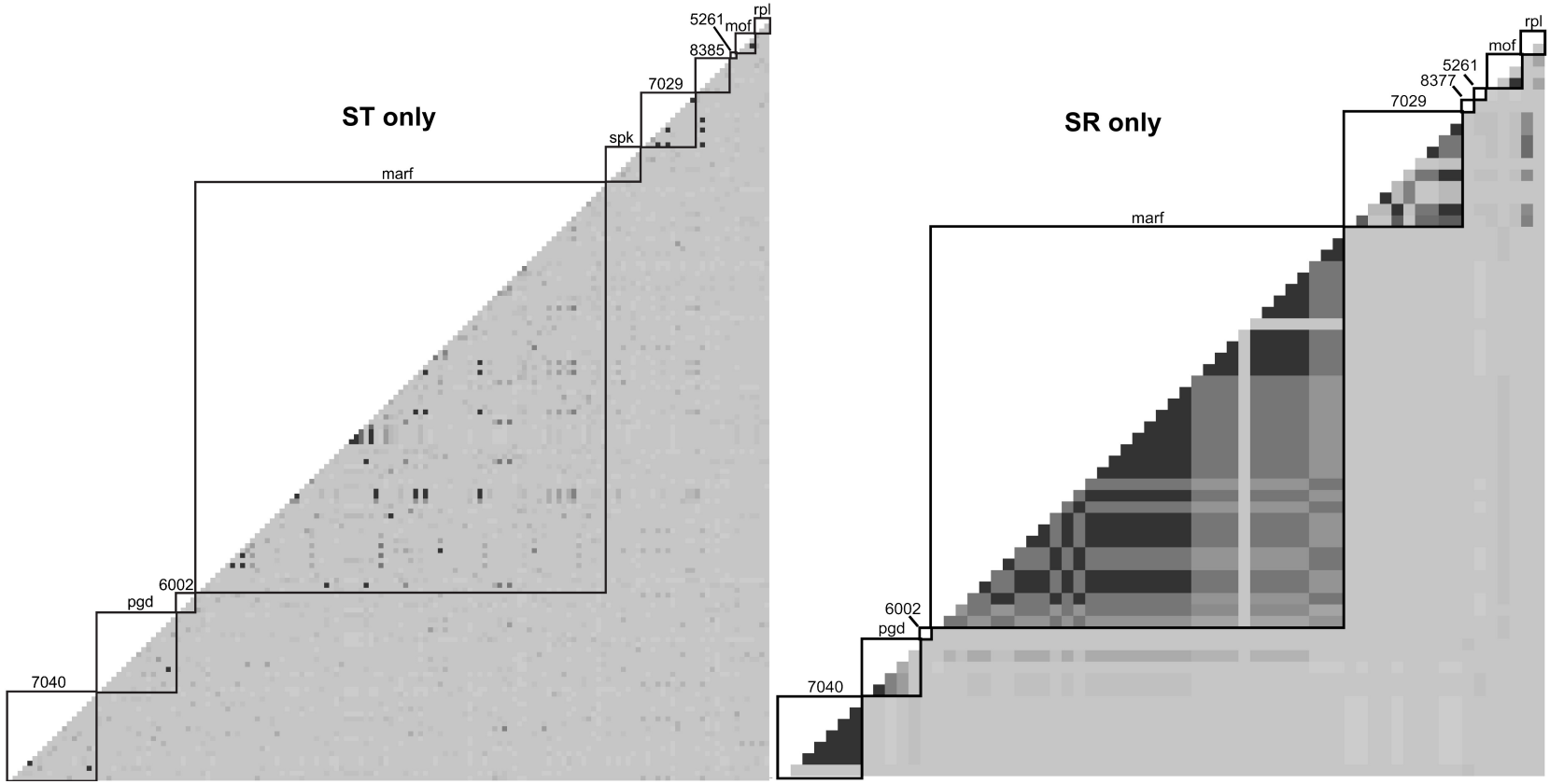
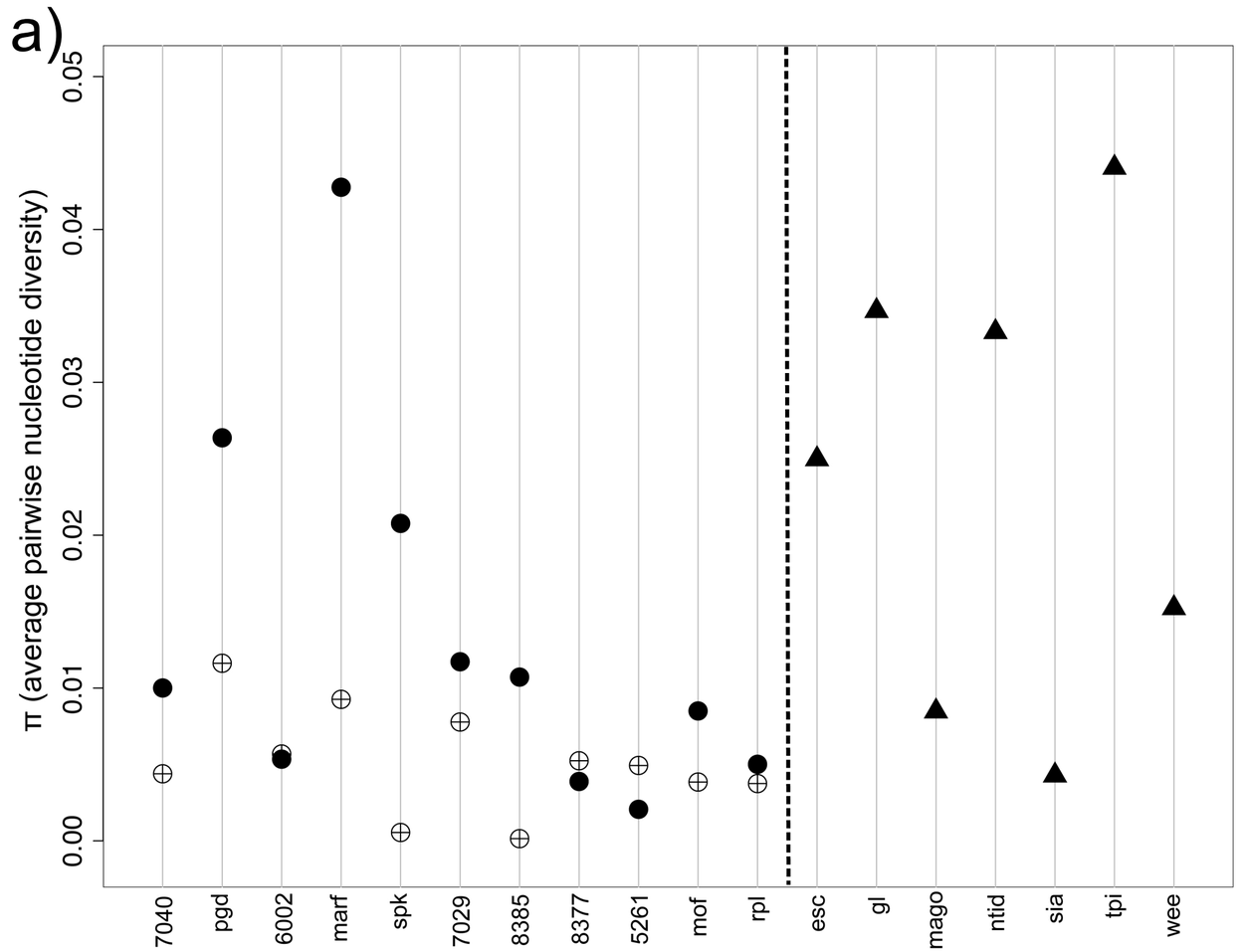
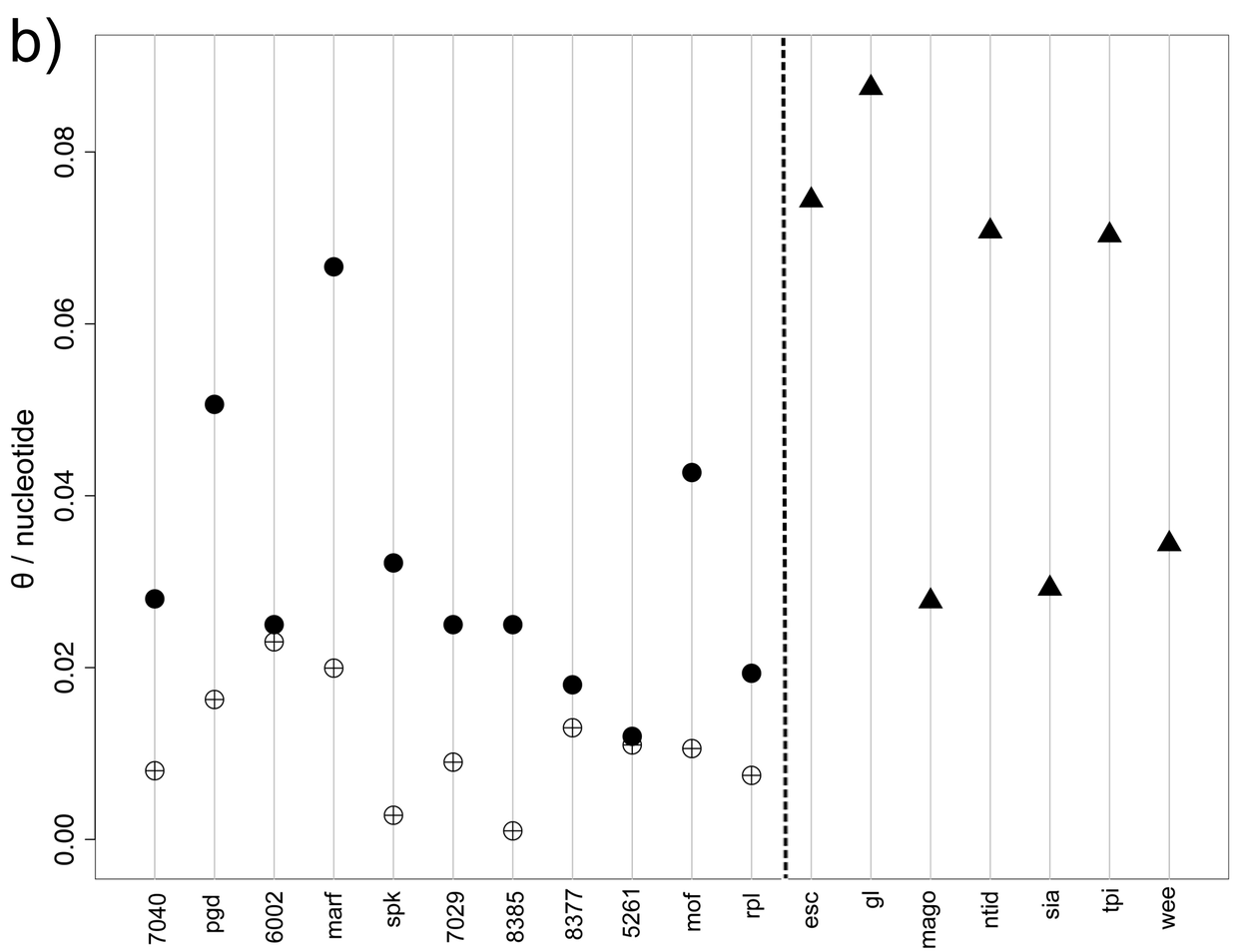


Figure S4. Polymorphism and frequency spectrum estimates for each individual marker, using only silent sites. On the X-chromosome, estimates for ST (filled circles) and SR (open circles) were calculated separately. Estimates for the autosomes (triangles) were calculated using all samples. Panel (a) shows π, panel (b) shows Watterson's θ, and panel (c) shows Tajima's D. Markers are presented in their ST map order. mof and rpl were unable to be mapped and have been added to the end.





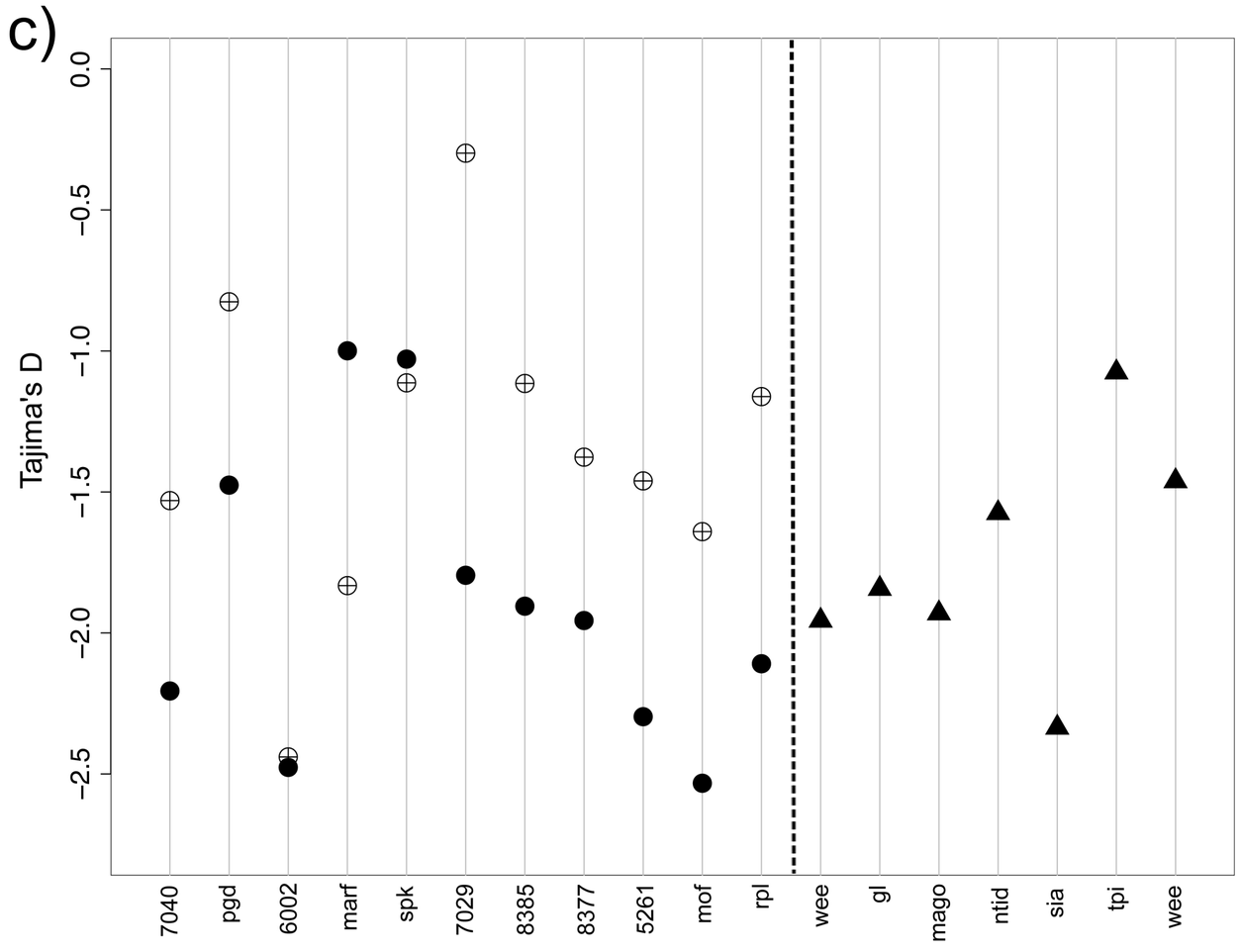


Table S1. All of the populations used in this study, and the number of unique ST and SR males sampled from each. Not all individuals were sequenced at all loci. A subset of individuals random with respect to X-chromosome status was chosen for sequencing at autosomal loci.

Population	Collection location	SR samples	ST samples	Autosomal samples
AB1	Winston Churchill, AB	0	4	2
AB3	Jasper, AB	4	4	3
BC	Vancouver, BC	4	4	4
ID	Coeur d'Alene, ID	6	8	3
MB	The Pas, MB	0	4	3
MN	Bemidji, MN	4	4	4
MT1	Columbia Falls, MT	5	8	3
MT2	St. Regis, MT	4	5	3
ND	Minot, ND	2	4	3
NY	Rochester, NY	6	5	3
OR	MacKenzie Bridge, OR	4	4	4
PEI	Charlottetown, PEI	0	4	3
TN1	Gatlinburg, TN	6	3	4
TN2	Clingmans Dome, TN	3	4	3

Table S2. Molecular population genetic summary statistics for each locus. A) Statistics for *D. neotestacea* samples. S (segregating sites) for total and silent sites is presented. M (mutations), π, θ, and D for silent sites only includes synonymous sites in open reading frames and all noncoding sites. π NS is using nonsynonymous polymorphism only. ρ is population recombination rate ($2N_er$) divided by the number of nucleotides in the marker. For the autosomal markers, all statistics were calculated from the combined set of SR and ST individuals. ρ and ZnS could not be calculated for some markers due to a lack of segregating sites. Bolded D values are less than the expected D in 95% of 10,000 simulations. Da is percent divergence between ST and SR.

	Marker	X-chromosome	N	Total Sites	S	Silent sites	M silent	S silent	π silent	π NS	θ silent	D silent	ρ	ZnS	π_a/π_s	K_a/K_s	Da
X-linked	neo5261	ST	61	183	10	183	10	10	0.002		0.012	**-2.297**	0.000	0.178			0.274
		SR	45		7		7	7	0.005		0.011	-1.461	0.000	0.054			
	neo6002	ST	57	190	19	190	21	19	0.005		0.025	**-2.477**	0.300	0.035			0.388
		SR	44		19		19	19	0.006		0.023	**-2.440**	0.000	0.060			
	neo7029	ST	57	369	39	369	41	39	0.012		0.025	**-1.796**	0.081	0.036			0.722
		SR	42		13		13	13	0.008		0.009	-0.299	0.008	0.317			
	neo7040	ST	57	515	56	515	59	56	0.010		0.028	**-2.206**	0.179	0.029			0.474
		SR	43		18		18	18	0.004		0.008	**-1.531**	0.050	0.106			
	neo8385	ST	55	319	32	319	32	32	0.011		0.025	**-1.905**	0.444	0.029			0.745
		SR	44		1		1	1	0.000		0.001	-1.115	0.000	NA			
	neo8377	ST	56	72	5	72	6	5	0.004		0.018	**-1.956**	0.266	0.000			0.993
		SR	44		4		4	4	0.005		0.013	-1.377	NA	0.502			
	marf	ST	57	1063	186	670.34	206	184	0.043	0.005	0.067	-0.999	0.122	0.037	0.195	0.424	1.581
		SR	48		73	844.49	74	71	0.009	0.001	0.020	**-1.832**	0.008	0.252	0.577	0.282	
	mof	ST	65	576	30	128.36	26	26	0.009	0.000	0.043	**-2.533**	0.000	0.041	0.044	0.065	0.002
		SR	47		7	128.34	6	6	0.004	0.000	0.011	**-1.641**	0.000	0.049	0.038	0.064	
	pgd	ST	56	569	38	128.98	30	28	0.026	0.002	0.051	**-1.476**	0.146	0.023	0.064	0.069	-0.010
		SR	43		18	127.7	9	9	0.012	0.001	0.016	**-0.826**	0.000	0.284	0.092	0.068	
	rpl	ST	57	302	11	123.33	11	11	0.005	0.000	0.019	**-2.109**	0.024	0.051	0.00	0.00	0.008
		SR	44		4	123.33	4	4	0.004	0.000	0.007	-1.162	0.066	0.004	0.00	0.000	
	spk	ST	56	382	13	81.17	12	12	0.021	0.000	0.032	-1.029	0.298	0.015	0.006	0.021	0.041
		SR	45		1	81.17	1	1	0.001	0.000	0.003	-1.113	NA	NA	0.00	0.022	
Autosomal	esc		82	370	29	78.32	29	27	0.025	0.001	0.074	**-1.956**	0.130	0.023	0.020	0.002	
	gl		80	402	52	96.96	42	39	0.035	0.002	0.087	**-1.843**	0.065	0.023	0.060	0.059	
	mago		78	324	14	57.15	7	7	0.010	0.001	0.025	**-1.429**	0.086	0.058	0.109	0.500	
	ntid		88	527	50	123.42	44	41	0.033	0.003	0.071	**-1.575**	0.068	0.027	0.138	0.110	
	sia		82	400	13	96.33	14	13	0.004	0.000	0.029	**-2.336**	0.000	0.015	0.024	0.084	
	tpi		80	347	33	83.28	29	29	0.044	0.001	0.070	-1.011	0.245	0.043	0.00	0.000	
	wee		84	285	14	58.14	10	10	0.015	0.001	0.034	**-1.462**	0.561	0.027	0.048	0.003	

Table S2. Molecular population genetic summary statistics for each locus. B) Statistics for *D. testacea* samples. All analyses included both synonymous and nonsynonymous sites. M is the number of mutations; S is the number of segregating sites. Bolded D values are less than the expected D in 95% of 10,000 simulations. ρ is population recombination rate ($2N_er$) divided by the number of nucleotides in the marker.

	Marker	**N**	**Total sites**	**M**	**S**	**π**	**θ**	**D**	**ZnS**	**ρ**
X-linked	neo5261	19	175	14	12	0.017	0.027	-1.429	0.126	0.218
	neo6002	12	191	6	5	0.007	0.010	-1.225	0.060	9.857
	neo7029	21	352	19	19	0.010	0.017	-1.621	0.056	NA
	neo7040	16	513	28	27	0.012	0.017	-1.264	0.073	3.264
	neo8377	21	72	3	3	0.016	0.017	-0.137	0.061	NA
	neo8385	21	312	9	7	0.004	0.009	**-2.018**	0.004	NA
	marf	20	244	7	7	0.004	0.008	-1.692	0.177	0.002
	mof	23	575	17	15	0.006	0.007	-1.063	0.104	0.114
	pgd	16	560	27	26	0.010	0.014	-1.420	0.192	0.017
	rpl	18	269	9	9	0.005	0.010	-1.561	0.140	0.024
	spk	22	382	11	11	0.004	0.008	-1.768	0.193	0.009
Autosomal	esc	25	369	13	12	0.006	0.009	-1.197	0.072	0.859
	gl	23	402	29	27	0.017	0.018	-0.551	0.103	0.189
	mago	21	326	6	5	0.003	0.005	-1.778	0.003	NA
	ntid	28	407	29	29	0.009	0.009	**-1.823**	0.116	0.012
	sia	20	399	2	2	0.001	0.001	-1.513	0.003	NA
	tpi	23	347	13	13	0.006	0.010	-1.499	0.088	0.140
	wee	21	285	9	9	0.008	0.009	-0.461	0.115	0.186

Tables S3. Forward and reverse PCR primers used to amplify sequences for each marker. All primers are presented in 5' to 3' orientation.

Marker	Primer	Sequence
neo5261	F	GAAGCAACAACAAAAGCC
	R	AATGAGGCAAGGTCCCACTG
neo6002	F	TCTAAATGCACAAATCCCAGC
	R	CACGACTGCGTAATACTTCACC
neo7029	F	AGCACATGGCACAGATGTTAG
	R	GAAGGATACAAGAGACGTCAGC
neo7040	F	CAAACAACAATTGCAACGTG
	R	GTGTGCACACATTTCCATACC
neo8377	F	TGGACAATTGTTGTGGACTG
	R	AACAACATCATTCGCATTCG
neo8385	F	AGAGCTTTAATGTGCTGGCA
	R	CCCAACTGAAAGTGAATTG
marf	F	CCCAACATCTTCATCCTGAACAAYMGNTGGGA
	R	GCGGACTGGGAGATGCAYTCYTCRAA
mof	F	CAGAAGCGRCGCTACGA
	R	TAKGCCCAATAGCTGCGATA
pgd	F	ATYGATGGYGGCAACTC
	R	CNCGCATWAGCATRAAKCCYTG
rpl	F	CMRVGSCCACAAGACCWCSAARRTC
	R	CRTGRGTCTGRGCCTTCC
spk	F	AAVATGCCBARYATYAARYTGCARTC
	R	CTTCTCCTCRCACCAYTCRTTC
esc	F	GGCCATCAACGAGCTGAARTTYCAYCC
	R	TTCCAGCACACGATGGCRTTYTCRCA
gl	F	TTTCGATTGCGGCGGNTGYTTYGA
	R	GCCGTGGTGCATGGTCATRTTCAT
mago	F	CCACAAGGGCAAGTTCGGNCAYGARTT
	R	CACTTCAGGTCCTGCACCARRTARTARAA
ntid	F	GGGCCGCATCTTCGARCAYAARTGG
	R	TGGAGGGGTAGGTGTTCCARCARTA
sia	F	TCGAGTGCCCCGTGTGYTTYGAYTA
	R	GAAGTGGAAGCCGAAGCAGSWYTGCATCAT
tpi	F	CAACTGGAAGATGAAYGGIGACC
	R	TTCTTGGCATAGGCGCACATYTG
wee	F	GCCTGGGCCGAGGAYGAYCAYATG
	R	TCACGTGGCCCAGGTCNCCDATYTT

Table S4. Genetic differentiation per marker. Significance for K_{ST} and S_{nn} were calculated in DnaSP using 1000 randomized permutations. Italics indicate $p < 0.05$, bold indicates $p < 0.01$, and bold italics indicate $p < 0.001$. For the X-linked markers, the number of segregating sites unique to ST/SR and shared between ST and SR are presented.

	Marker	Geographic K_{ST}	Geo S_{nn}	ST-SR K_{ST}	ST-SR S_{nn}	SR only sites	ST only sites	Shared sites
X-linked	neo5261	0.0069	0.06537	***0.286***	***0.7624***	6	10	0
	neo6002	0.01212	0.07205	***0.272***	***0.84451***	10	13	5
	neo7029	**0.11563**	**0.11476**	***0.260***	***0.82035***	4	28	9
	neo7040	-0.02985	0.06235	***0.265***	***0.95568***	7	51	11
	neo8377	0.01795	0.07495	***0.524***	***0.86763***	3	3	1
	neo8385	0.00955	0.07416	***0.381***	***0.95946***	0	31	0
	marf	0.02777	0.08879	***0.249***	***0.97087***	29	155	41
	mof	*0.02616*	**0.10742**	*0.007*	**0.55057**	4	24	2
	pgd	-0.01186	0.09386	***0.169***	***0.93777***	11	29	7
	rpl	**0.04427**	0.08124	**0.022**	***0.5894***	3	10	1
	spk	0.00158	0.06184	***0.074***	***0.73976***	1	11	0
Autosomal	esc	*0.04437*	***0.16071***	-0.003	0.50313			
	gl	-0.02099	0.06884	0.005	*0.59407*			
	mago	*0.0632*	**0.11973**	0.004	0.55051			
	ntid	-0.01788	0.07167	-0.004	0.46653			
	sia	0.01782	0.08351	0.003	0.52953			
	tpi	*0.05581*	*0.11495*	-0.001	0.45123			
	wee	*0.014*	0.08582	-0.001	0.4705			

Table S5. Evidence of gene conversion and/or double crossover events between SR and ST. Start and end positions are the nucleotide positions within the concatenated alignment of all X-linked markers, and the start and end loci are which markers contain these positions. The tract length is also within the concatenated alignment.

Recipient sample	Start position	End position	Tract length (bp)	Source	Recipient	Start marker	End marker
TN2_27|SR	45	3435	3296	ST	SR	neo7040	neo8377
MT2_27|SR	121	3435	3219	ST	SR	neo7040	neo8378
BC_28|SR	45	2885	2753	ST	SR	neo7040	neo7030
OR_6|SR	121	2885	2677	ST	SR	neo7040	neo7031
OR_27|SR	672	2885	2144	ST	SR	pgd	neo7032
BC_8|SR	672	2885	2144	ST	SR	pgd	neo7033
NY_27|SR	672	2224	1490	ST	SR	pgd	marf
NY_29|SR	330	1829	1449	ST	SR	neo7040	marf
MN_5|SR	121	1491	1344	ST	SR	neo7040	marf
AB3_30|SR	672	2047	1318	ST	SR	pgd	marf
ID_1|SR	121	672	534	ST	SR	neo7040	pgd
MT1_30|SR	1491	2047	508	ST	SR	marf	marf
ID_28|SR	1491	2047	507	ST	SR	marf	marf
AB3_27|SR	1851	2047	175	ST	SR	marf	marf
NY_28|SR	1152	1164	13	ST	SR	neo6002	neo6002
MT2_11|ST	831	1605	753	SR	ST	pgd	marf
MT2_8|ST	2761	3435	667	SR	ST	neo7029	neo8379
MN_2|ST	2168	2761	593	SR	ST	marf	neo7029
OR_10|ST	1152	1605	433	SR	ST	neo6003	marf
OR_9|ST	1813	2172	329	SR	ST	marf	marf
MT2_8|ST	141	330	184	SR	ST	neo7040	neo7040
ID_3|ST	1705	1766	62	SR	ST	marf	marf
BC_4|ST	1705	1766	62	SR	ST	marf	marf
PEI_8|ST	1764	1813	50	SR	ST	marf	marf
MT2_10|ST	1766	1813	48	SR	ST	marf	marf
MT2_9|ST	1766	1813	48	SR	ST	marf	marf
MT1_13|ST	1766	1813	48	SR	ST	marf	marf
ND_1|ST	2168	2172	5	SR	ST	marf	marf
OR_7|ST	1764	1766	3	SR	ST	marf	marf
NY_4|ST	1764	1766	3	SR	ST	marf	marf
AB3_1|ST	1764	1766	3	SR	ST	marf	marf
BC_1|ST	1868	1870	3	SR	ST	marf	marf
AB3_4|ST	1868	1870	3	SR	ST	marf	marf