

## Supplementary material

### DNA extraction method

Method previously described for *C. elegans* (Mello and Fire, 1995), with some modification as described by Min-Ho Lee and Sudhir Nayak (Available online: <http://genetics.wustl.edu/tslab/protocols/genomic-stuff/worm-genomic-dna-prep/>). The method was scaled down to accommodate a single *S. lupi* nematode.

1. Add 500 µl worm lysis buffer (0.1M Tris-Cl pH 8.5, 0.1M NaCl, 50 mM EDTA pH 8.0, 1% SDS) to approximately 1/2 an *S. lupi* worm in a 2 ml microcentrifuge tube, duplicate.
2. Add 30 µl Proteinase K (20 mg/ml) and vortex.
3. Incubate at 56°C for at least an hour or until the worm has disintegrated. Vortex at regular intervals.
4. Add 200 µl of 5M NaCl and mix thoroughly by inverting the tube.
5. Add 200 µl CTAB solution (10 % CTAB in 0.7M NaCl) and incubate for 10 min at 37°C.
6. Add 1 ml chloroform, mix and centrifuge at 14 000 rpm for 5 minutes. Recover aqueous phase.
7. Add 1 ml Phenol/Chloroform/isoamyl alcohol (25:24:1), mix and centrifuge at 14 000 rpm for 5 minutes. Recover aqueous phase.
8. Add approximately 500 µl (0.6 volume) of -20°C isopropanol invert and mix. Centrifuge at 14 000 rpm for 5 min.
9. Wash twice with 250 µl of 70% Ethanol.
10. Dry at 56°C for 3-5 min and resuspend in 40 µl of 1X TE buffer.
11. Add 1 µl of RNase A (4mg/ml, Promega) and incubate at 37°C for 1 hour.
12. Add 1 µl of 20% SDS, 0.5 µl of 0.5M EDTA pH 8.0, 1 µl of Proteinase K and incubate for 1 hours at 56°C.
13. Add 1 µl of 10M Ammonium Acetate.
14. Extract twice with 500 µl Phenol/Chloroform/isoamyl alcohol (25:24:1) and once with 500 µl chloroform.
15. Add 1 ml ice cold absolute ethanol and centrifuge at 14 000 rpm for 10 min.
16. Wash twice with 250 µl of 70% ethanol.
17. Dry at 56°C for 3-5 min and resuspend DNA in 200 µl UHQ water or 1x TE buffer.

**Supplementary table 1:** Mitochondrial tRNA of *Spirocerca lupi* South Africa compared to *Spirocerca lupi* China. Insertions relative to *S. lupi* South Africa colored green, deletions in red. Nucleotide changes depicted in yellow. Basic models were interpreted from predictions modeled on the MITOS web server (Bernt et al., 2013).

tRNA	Anticodon	China	South Africa
Ala	TGC		
Arg	ACG		
Asn	GTT		

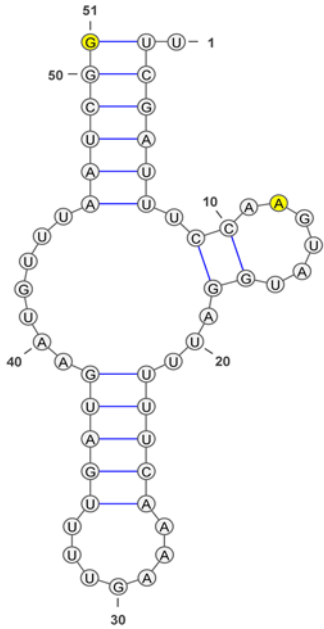
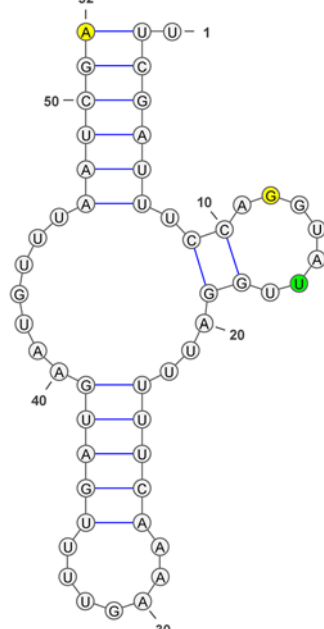
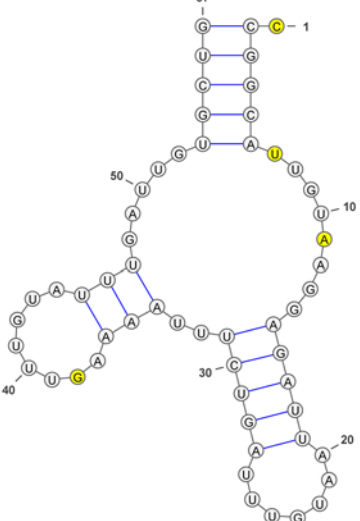
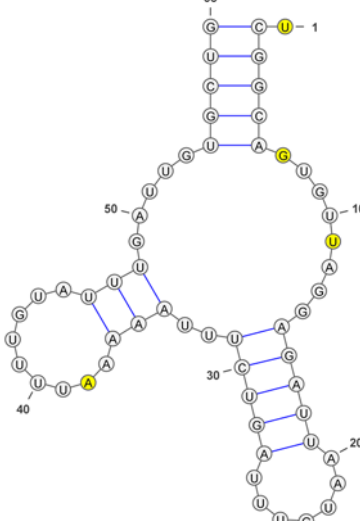
tRNA	Anticodon	China	South Africa
Asp	GTC		
Cys	GCA		
Gln	TTG		

tRNA	Anticodon	China	South Africa
Glu	TTC		
Gly	TCC		

His	GTG		
tRNA	Anticodon	<b>China</b>	<b>South Africa</b>
Ile	GAT		
Leu1	TAG		

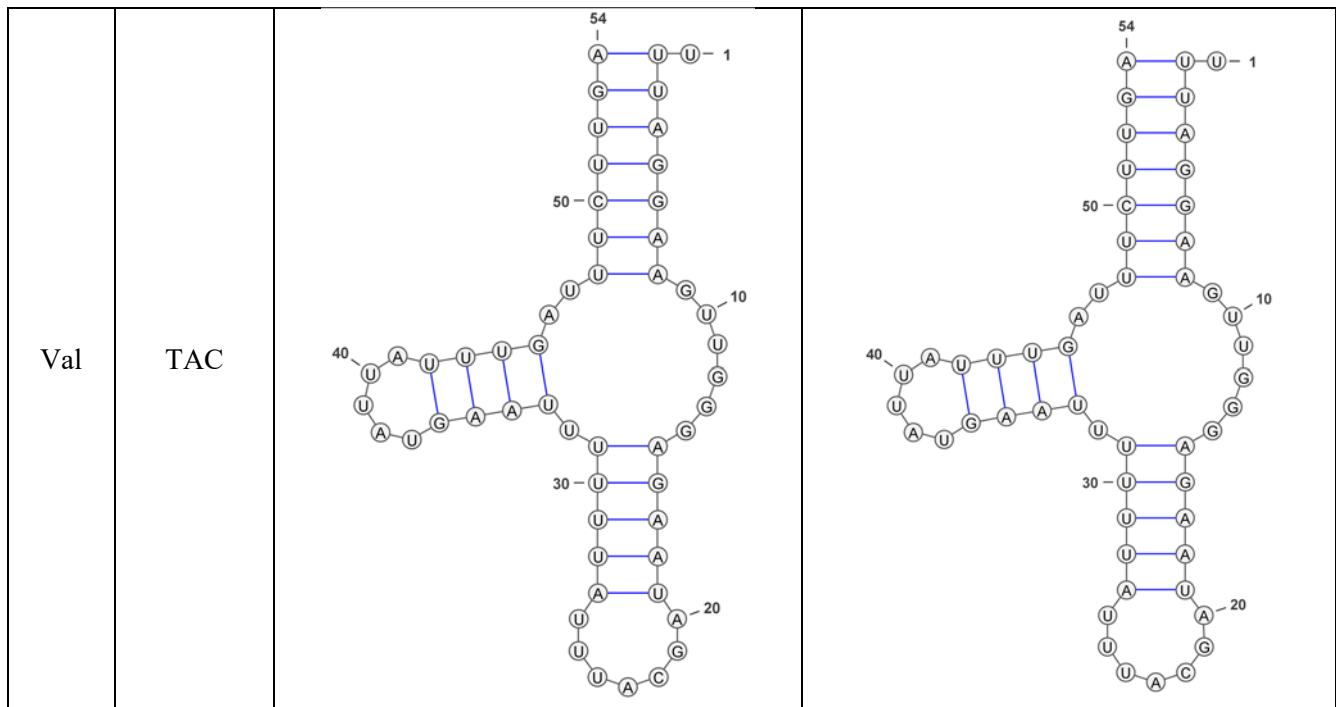
Leu2	TAA		
<b>tRNA</b>	<b>Anticodon</b>	<b>China</b>	<b>South Africa</b>
Lys	TTT		
Met	CAT		

Phe	TTG		
tRNA	Anticodon	China	South Africa
Pro	AGG		
Ser1	TCT		

Ser2	TGA		
<b>tRNA</b>	<b>Anticodon</b>	<b>China</b>	<b>South Africa</b>
Thr	TGT		

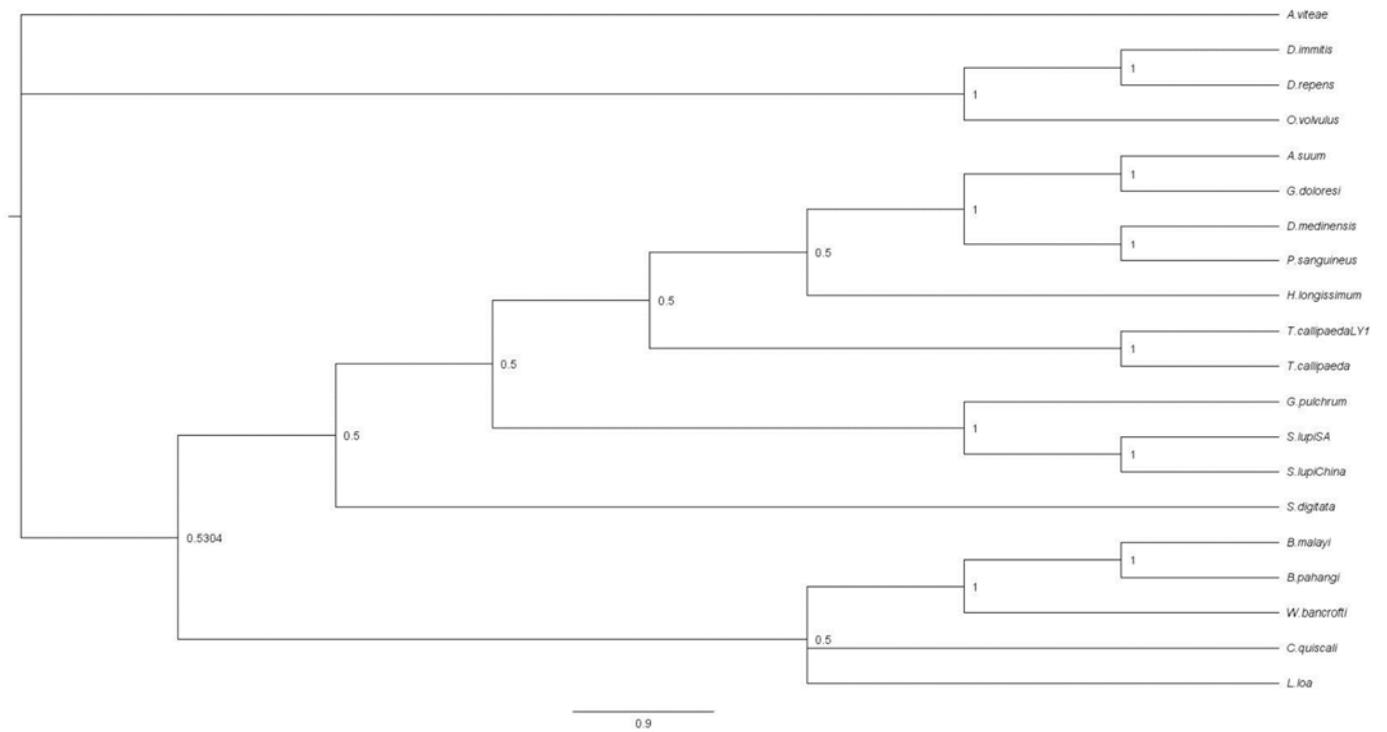


Trp	TCA		
Tyr	GTA		
<b>tRNA</b>	<b>Anticodon</b>	<b>China</b>	<b>South Africa</b>



**Supplementary table 2:** Pairwise distance of 12 protein coding genes 20 nematode species as calculated by MEGA.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 <i>A. Virteae</i>	1.088	0.250	0.266	0.275	0.233	0.212	1.029	1.252	0.392	0.597	0.263	0.208	1.476	0.240	0.344	0.382	0.421	0.431	0.237	
2 <i>A. suum</i>		1.081	1.121	1.075	1.030	0.974	0.691	0.499	1.086	1.118	1.023	1.029	0.972	1.009	1.053	1.116	1.107	1.125	1.007	
3 <i>B. malayi</i>			0.120	0.270	0.260	0.223	1.076	1.315	0.408	0.588	0.257	0.225	1.539	0.257	0.360	0.399	0.428	0.441	0.147	
4 <i>B. pahangi</i>				0.281	0.264	0.238	1.109	1.319	0.413	0.619	0.269	0.236	1.549	0.262	0.369	0.408	0.442	0.452	0.154	
5 <i>C. quisquali</i>					0.272	0.232	1.005	1.262	0.403	0.561	0.272	0.244	1.392	0.263	0.359	0.399	0.437	0.449	0.255	
6 <i>D. immitis</i>						0.147	1.026	1.232	0.371	0.584	0.251	0.181	1.472	0.225	0.327	0.365	0.421	0.434	0.232	
7 <i>D. repens</i>							0.934	1.212	0.325	0.530	0.224	0.155	1.414	0.188	0.300	0.333	0.392	0.405	0.196	
8 <i>D. medinensis</i>								0.813	1.069	1.120	0.991	0.997	0.654	0.950	1.037	1.108	1.068	1.064	0.998	
9 <i>G. doloresi</i>									1.264	1.293	1.258	1.224	1.021	1.223	1.211	1.260	1.256	1.264	1.245	
10 <i>G. pulchrum</i>										0.585	0.385	0.355	1.460	0.346	0.332	0.367	0.444	0.461	0.381	
11 <i>H. longissimum</i>											0.418	0.573	1.459	0.548	0.582	0.623	0.599	0.616	0.574	
12 <i>L. loa</i>												0.204	1.417	0.257	0.351	0.389	0.423	0.437	0.234	
13 <i>O. volvulus</i>													1.479	0.205	0.305	0.339	0.402	0.413	0.200	
14 <i>P. sanguineus</i>														1.410	1.441	1.522	1.459	1.460	1.431	
15 <i>S. digitata</i>															0.306	0.341	0.392	0.407	0.231	
16 <i>S. lupi SA</i>																	0.066	0.374	0.418	0.332
17 <i>S. lupi China</i>																		0.401	0.453	0.365
18 <i>T. callipaeda LY1</i>																			0.037	0.414
19 <i>T. callipaeda</i>																				0.421
20 <i>W. bancrofti</i>																				



**Supplementary figure 1:** Bayesian phylogenetic tree of 20 nematode species based on 12 mitochondrial protein coding genes. The number above the branches represents the Bayesian posterior probabilities.





## References

Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritsch, G., Pütz, J., Middendorf, M., Stadler, P.F., 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.* 69, 313–319.

Mello, C.C., Fire, A., 1995. DNA transformation., in: *Methods in Cell Biology. Caenorhabditis Elegans Modern Biological Analysis of an Organism*. Academic Press, pp. 451–482.