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Canine *Dracunculus* Nematode Infection, Toledo, Spain

Appendix

PCRs and Sequencing

After morphological characterization, the worm was sent to Berlin for molecular analyses. PCRs targeting two nuclear loci (18S and 28S) and one mitochondrial locus (cytochrome oxidase subunit I, COI) were conducted. For the PCR each reaction contained 200 µM dNTPs, 0.5 µM of each primer pair (Appendix Table), 0.4 U Phusion Hot Start II High-fidelity DNA polymerase (Thermo Scientific) and 2 µl template DNA in 20 µl 1 × HF buffer. The PCR protocol included initial denaturation at 98°C for 30 s, 40 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 30 s and elongation at 72°C for 30 s, followed by a final extension at 72°C for 5 min. PCR products were cloned and submitted for Sanger sequencing to LGC Genomics (Berlin).

Phylogenetic Analyses

The CO1 DNA sequences were aligned codon-wise using MUSCLE (*1*) as implemented in Mega7 (*2*). The alignment of rRNA sequences was performed with MAFFT using the command line command line: mafft -qinsi–maxiterate 2–reorder input. The optimal substitution models and the phylogenetic tree were determined on the IQTREE web server (*3*) applying the Modelfinder (*4*) and IQTREE (*5*) softwares, respectively. Modelfinder was run using autoselect of the optimal substitution model also considering FreeRate heterogeneity models. Both, ultrafast bootstrapping (*6*) and Shimodaira–Hasegawa approximate likelihood ratio tests (SH-aLRTs) (*7*) were conducted with 1000 replicates. The final command line was: path_to_iqtree -s iqtree_Alignment.phy -st DNA -m TESTNEW -bb 1000 -alrt 1000. Trees were visualized using FigTree v1.4.4.

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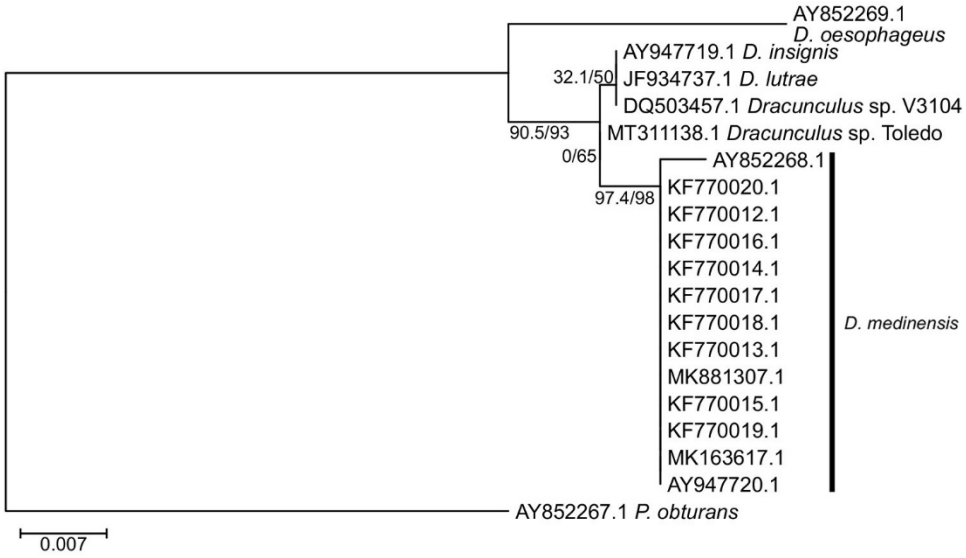
Appendix Table. PCR primers used to amplify *Dracunculus* spp. DNA fragments

Primer name	Target	Sequence	Reference
COI_Nema_Fw	Cytochrome c oxidase subunit 1	GAAAGTTCTAATCATAARGATATTGG	(*8*)
COI_Nema_Rv	Cytochrome c oxidase subunit 1	ACCTCAGGATGACCAAAAAAYCAA	(*8*)
Nematode-18sfor	18S rDNA	AACTGCGAACGGCTCATTAGAGCAGATGT	(*9*)
SSU_R26	18S rDNA	CATTCTTGGCAAATGCTTTCG	(*10*)
Nematode-28sfor	28S rDNA	GGCGAGTGAACGGGGAGAAGCCCAGCGCTGA	(*9*)
Nematode-28srev	28S rDNA	TTTCCTTCACAGTACTTGTTTGCTATCGAAT	(*9*)



Appendix Figure. Maximum-likelihood phylogenetic tree of *Dracunculus* spp. sequences available from GenBank. The tree was rooted using a sequence from the nematode *Philometra obturans* (Spirurida, Dracunculoidea, Philometridae) as outgroup. The scale bar represents 0.007 substitutions per site. Node support values before and behind the slash provide the results of ultrafast bootstrapping and the Shimodaira-Hasegawa likelihood ratio test. GenBank accession numbers were used an end node labels.