

Evidence for the Synonymy of *Laimaphelenchus hyrcanus* with *L. belgradiensis*, two species of nematodes belonging to Aphelenchoididae

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INTRODUCTION AND AIM

Laimaphelenchus species are mainly mycophagous and are typically associated with mosses, algae, lichens, conifer wood, or the galleries of bark beetle larvae; they have not been shown to damage conifers, although a possible link to oak decline has been suggested. Species delimitation in this genus relies particularly on tail-terminus morphology and the presence or absence of a vulval flap in females. During a survey to identify members of the superfamily Aphelenchoidea in the Lordegan region, Chaharmahal va Bakhtiari Province, Iran, a population of *Laimaphelenchus* was isolated from the dead bark of a living oak tree, a microhabitat potentially favorable for fungal growth. The aims of the present study are to: (1) compare the studied population with *Laimaphelenchus hyrcanus* and *L. belgradiensis* reported from various regions; and (2) assess its molecular phylogenetic relationships with other populations of these species using the 28S rDNA D2–D3 region.

METHODS

Collection of Biological Material

Dead bark was collected from a living oak tree and cut into pieces (<1 cm). Nematodes were extracted using the tray method to obtain clean biological material for analysis.

DNA Extraction & Amplification

DNA was extracted from a single female specimen. The D2–D3 expansion segments of the LSU rDNA were amplified using the D2A/D3B primer pair. Amplicons were sequenced bidirectionally and checked via BLAST for similarity and confirmation.

Bayesian Phylogenetic Inference

Phylogenetic reconstruction was performed under the GTR+G+I substitution model in MrBayes, running for 6×10⁶ generations. *Aphelenchus avenae* was included as the outgroup to root the tree.

Fixation and Processing

Extracted nematodes were fixed in hot 4% formaldehyde, processed into anhydrous glycerin, and examined using an Olympus BX51 microscope for morphometric measurements, drawings, and photomicrographs.

Sequence Alignment & Cleaning

The sequences were aligned using MAFFT with the Q-INS-i algorithm. Gblocks was applied to refine and retain only high-quality alignment regions.

Tree Visualization & Graphic Editing

Resulting phylogenetic trees were visualized in Dendroscope and finalized in CorelDRAW for publication-quality figures.

RESULTS AND DISCUSSION

This species is characterized by a body length of 621–796 μm, a stylet of 10.5–13 μm, three lateral lines, the presence of a vulval flap, and a post-uterine sac measuring 50–78 μm (2.2–3.6 times the vulval body width). The tail is conoid to nearly cylindrical, ventrally curved, and ends in a single offset terminus bearing four tubercles arranged in a square, followed by 10–12 finger-like projections.

Males show a four-part tail terminus, a (2+2+2) pattern of genital papillae, and spicules measuring 18.5–20 μm. Based on overall morphometric and morphological similarities, this population is most closely related to *L. hyrcanus* and *L. belgradiensis*.

Compared with the original description of *L. belgradiensis*, this population has a shorter stylet (10.5–13 μm vs. 13.6–15.2 μm) but a longer post-uterine sac (50–78 μm vs. 17.6–48 μm). Relative to *L. hyrcanus*, the post-uterine sac is shorter (50–78 μm vs. 97–152 μm). Earlier studies on decaying-oak populations in Iran also reported variability in post-uterine sac length and questioned its reliability for species delimitation.

A later reassessment of species boundaries resulted in a redescription of *L. belgradiensis* and suggested that *L. hyrcanus* may be its junior synonym.

In the present study, phylogenetic analysis of the D2–D3 LSU region placed this population within a strongly supported clade (posterior probability = 1.00), further supporting the synonymy unless future genomic data indicate otherwise.

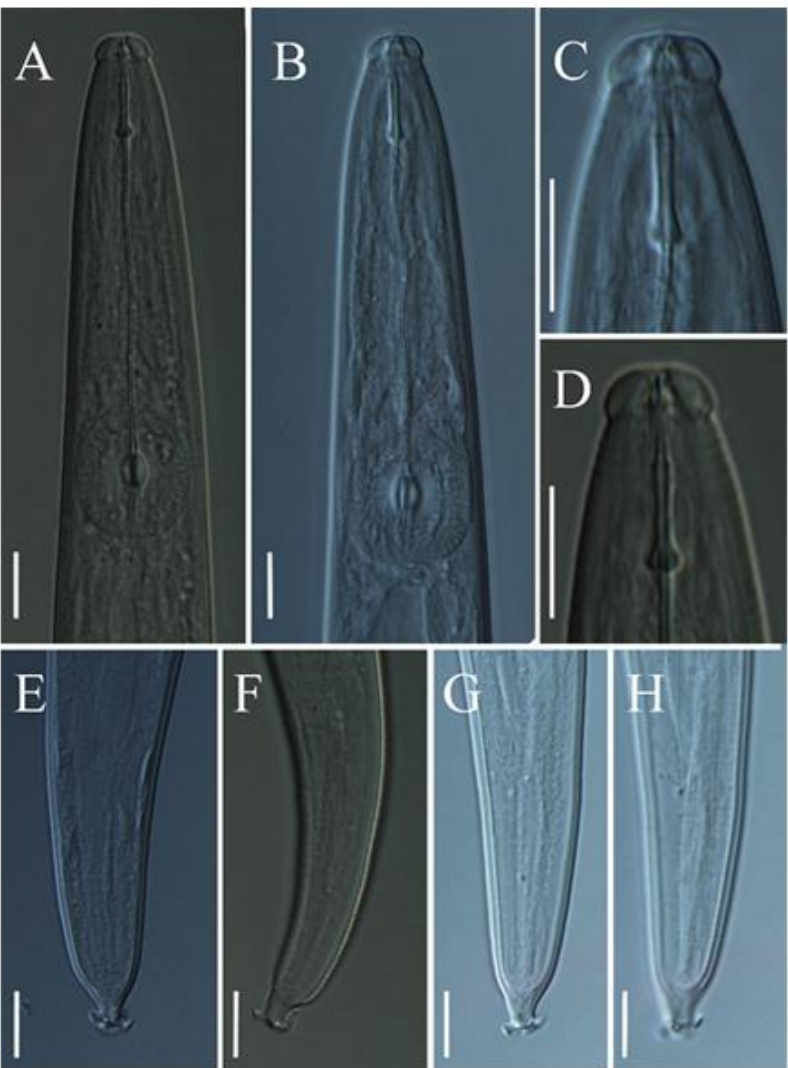


Figure 1. Population of *Laimaphelenchus belgradiensis* from Lordegan region, Iran. A, B: Anterior region (stylet and median bulb); C, D: Anterior region (stylet); E-H: tail. all scale bars = 10 μm.

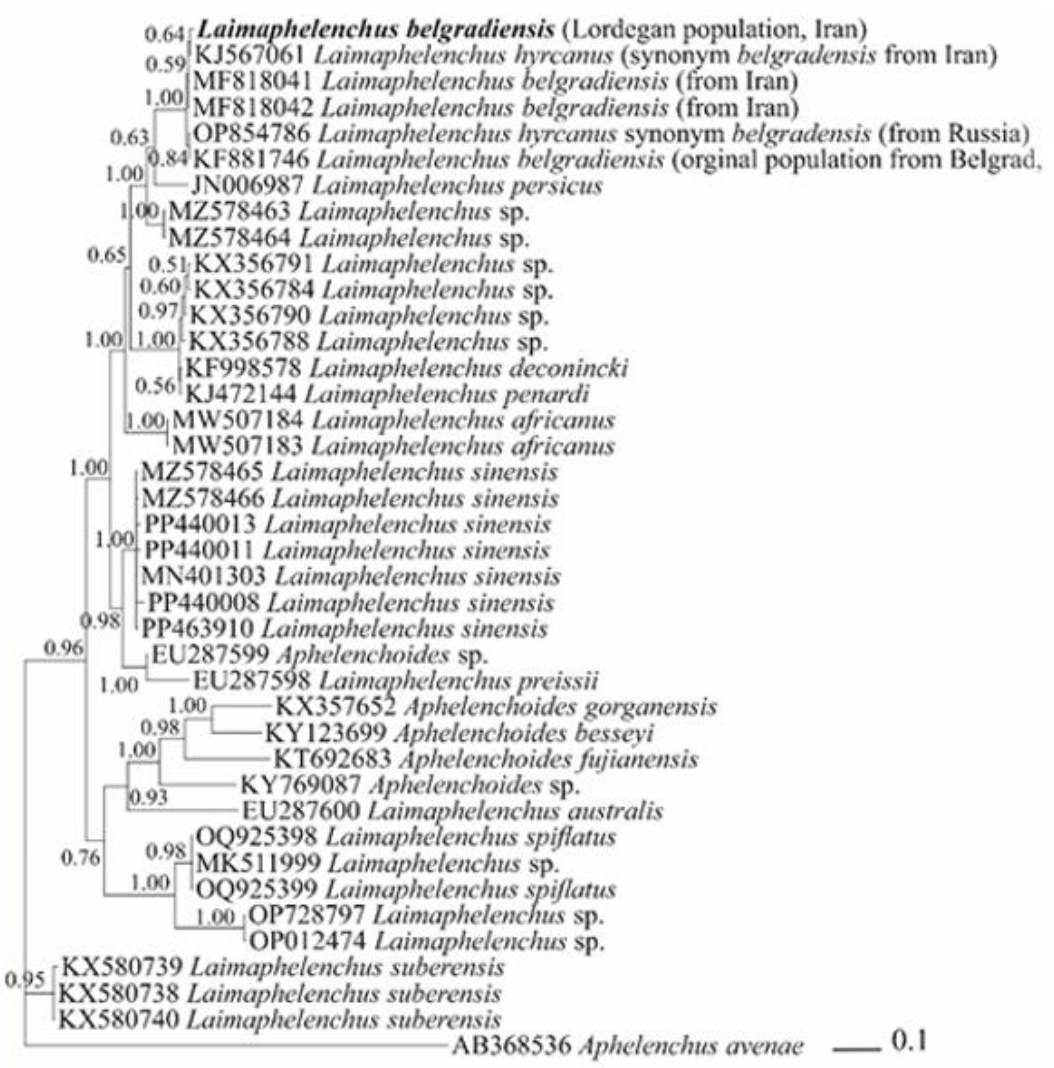


Figure 2. Bayesian 50% majority rule consensus tree of *Laimaphelenchus belgradiensis* based on 28S rDNA D2-D3 segment sequences under GTR + I + G model. Bayesian posterior probability values more than 0.50 are given for appropriate clades. The new sequence is indicated in bold.

Characters	Lordegan region		Pedram <i>et al.</i> , 2018		Miraeiz <i>et al.</i> , 2015		Oro, 2014
	Females	Males	Females	Males	Females	Males	Females
n	5	2	8	3	10	5	30
L	621.5-796	731.5, 759	862-998	916-1001	671-878	809-874	737-941
a	28.4-35.2	38.0, 40.6	35.6-44.4	45.8-50.0	30-36	38.5-45.2	32.9-42.3
c	18.2-22.1	16.3, 16.5	17.8-23.0	16.0-17.5	16.4-21.4	17.6-18.5	20.0-29.7
c'	2.7-3.2	3.2, 3.3	2.9-3.9	3.2-3.9	3.1-3.5	2.8-3.1	2.4-3.1
T or V	65.5-68.0	55.6, 57.7	64.6-66.3	-	63.2-67.8	63-79	61.9-67.5
Stylet	10.5-13.0	12, 12	11-12	10-12	11-12	11-12	13.6-15.2
Excretory pore	97-109	111, 117.5	88.0-126	118, 131	86-112	98-113	-
Post-vulvar uterine sac	50-78	-	165-173	-	97-152	-	17.6-48.0
Tail length	30-38	45, 46	38-53	53-63	36-48	46-49	30-40
Spicule length (arc line)	-	18.5, 20.0	-	23-26	-	22-24	-

Table 1: Morphometric data of *Laimaphelenchus belgradiensis* collected from Lordegan city, Iran and their comparison with the original description and other populations (measurements in micrometers, except for indices).

CONCLUSION

It is noteworthy that a prior study by Pedram *et al.* (2018) proposed the synonymy of *Laimaphelenchus hyrcanus* with *L. belgradiensis*, based on a convergence of molecular, morphological, and nomenclatural evidence. Molecular analysis of the D2-D3 expansion segments of the 28S rDNA gene revealed minimal nucleotide divergence (a maximum of five differences), a level consistent with intraspecific variation rather than interspecific differentiation. This genetic similarity was corroborated by morphological data. The two species are nearly indistinguishable, and the discovery of a previously overlooked shared characteristic—a third vestigial pair of caudal papillae in males—further reinforced their affinity. Ultimately, in accordance with the Principle of Priority in zoological nomenclature, *L. belgradiensis* was designated as the senior synonym due to its earlier publication date (January 15, 2015) compared to *L. hyrcanus* (February 5, 2015). In the present study, molecular data from the 28S region of a sample population from Lordegan, isolated from the dry bark of a living oak tree, also placed it within the same clade. Furthermore, its morphometric and morphological characteristics overlap with those of various populations previously identified as *L. hyrcanus* and *L. belgradensis*. Therefore, it can be confidently concluded that *L. hyrcanus* is a junior synonym of *L. belgradensis*.

REFERENCES

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