

Research Paper

Cite this article: Mirzaie Fouladvand Z, Atighi MR, Pourjam E, Pedram M and Peña-Santiago R (2024). New insights into the taxonomy and the evolutionary relationships of the rare nematode genus *Lindseyus* Ferris & Ferris, 1973 (Nematoda, Dorylaimida, Belonidiridae), after the molecular characterization of an Iranian population of its type species. *Journal of Helminthology*, **98**, e79, 1–10 <https://doi.org/10.1017/S0022149X24000646>.

Received: 17 June 2024

Revised: 02 September 2024

Accepted: 04 September 2024

Keywords:

Morphology; morphometry; phylogeny; taxonomy; 18S; 28S rDNA

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New insights into the taxonomy and the evolutionary relationships of the rare nematode genus *Lindseyus* Ferris & Ferris, 1973 (Nematoda, Dorylaimida, Belonidiridae), after the molecular characterization of an Iranian population of its type species

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Abstract

A new Iranian population of *Lindseyus costatus* is characterized, including morphometrics, microphotographs, and molecular (18S, 28S rDNA) studies. It is compared with previously known populations. The results derived from molecular analyses failed to find close evolutionary relationships with other traditional genera of Belonidiridae and Swangeriinae, adding more uncertainties about the internal phylogeny of the family. The taxonomy of *Lindseyus* is revised, with a new diagnosis, list of species, key to their identification, and a compilation of their main morphometrics.

Introduction

Ferris and Ferris (1973) proposed the new genus *Lindseyus*, with *L. costatus* as its type and only species, collected from freshwater habitats in Indiana, USA. It was characterized by, among other traits, having a long and slender body, faint basket-like cheilostom, very small odontostyle, belonidirid pharyngeal expansion, and sexual dimorphism in tail shape, being regarded as a member of Roqueidae Thorne, 1964 and compared with *Roqueus* Thorne, 1964. Later, three other species were added to the catalogue of the genus. Dhanachand and Jairajpuri (1980) described *L. indicus* from India. Coomans and Kheiri 1986 transferred *Dorylaimellus heterurus* Schuurmans-Stekhoven & Teunissen, 1938 (= *Roqueus africanus* Andrassy, 1970) to *Lindseyus*. And Choi and Khan (1999) described the fourth species, *L. juwangens*, from Korea.

Coomans and Kehiri (1986) provided an excellent morphological study of *L. costatus* and revised the taxonomy of both *Roqueus* and *Lindseyus*, which were classified in the tribe Roqueini Thorne, 1964, subfamily Swangeriinae Jairajpuri, 1964, family Belonidiridae Thorne, 1939. Subsequent contributions raised some controversy about the position of *Lindseyus* in the Dorylaimida system. Thus, Jairajpuri and Ahmad (1992) included it in Swangeriinae, but did not recognize the tribe Roqueini; meanwhile, Andrassy (2009) classified it in Swangeriidae Jairajpuri, 1964, Roqueinae Thorne, 1964. Unfortunately, no molecular study of a *Lindseyus* representative has so far been accomplished.

The presence of *L. costatus* in Iran was previously reported by Coomans and Kheiri (1986) and Shahabi *et al.* (2016). A nematological survey recently conducted to explore the nematode diversity of this country resulted in the finding of a small population of the species, making possible the obtaining of fresh specimens for their molecular study aimed at providing new evidence to elucidate the evolutionary relationships of the genus. The results are presented in the following.

Material and Methods

Sampling, nematodes extraction, mounting, and microscopic studies

Several soil samples were collected near Gazeh village, Sepiddasht rural district, Khorramabad county, Lorestan province, western Iran in spring 2023 (2023-6-6). Nematodes were directly extracted from soil using a series of 20, 60, and 270 mesh sieves (USA standard mesh numbers) having 850-, 250-, and 53- μ m openings size, respectively. A few specimens of interest were handpicked under a Nikon SMZ1000 stereomicroscope, heat-killed by adding boiling 4%

formalin solution, transferred to anhydrous glycerin according to De Grisse (1969), mounted on permanent slides, and examined using a Nikon Eclipse E600 light microscope. Photographs were taken using a Nikon Eclipse 80i light microscope provided with DIC (differential interference contrast) optics and a Nikon Digital Sight DS-U1 camera.

DNA extraction, polymerase chain reaction, sequencing, and phylogenetic analyses

DNA was separately extracted from two specimens by their squashing on a clean slide using a cover slip and TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0). DNA samples were stored at -20°C until being used as polymerase chain reaction templates. The 18S rDNA was amplified using the combination of the following primers: forward 988F (5'-CTCAAAGATTAAGCCATGC-3') and reverse 1912R (5'-TTTACGGTCAGAAGCTAGGG-3') (Holterman *et al.* 2006), forward primer SSU22F (5'-TCCAAGGAAGGCAGCAGGC-3'), and reverse primer SSU13R (5'-GGGCATCACAGACCTGTTA-3') (Dorris *et al.* 2002), forward primer 965F (5'-GGCGATCAGATACCGCCCTAGTT-3') (Mullin *et al.* 2005), and reverse primer 2646R (5'-GCTACCTTGTTACGACTTTT-3') (Holterman *et al.* 2006). The D2–D3 expansion segments of 28S rDNA were amplified using the primer pairs: forward D2A (5'-ACAAGTACCGTGAGGGAAAGT-3') and reverse D3B (5'-TGCGAAGGAACCAGCTACTA-3') (Nunn, 1992). The thermal cycling program for amplification of all aforementioned genomic fragments was as follows: denaturation at 95°C for 4 min, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at $50\text{--}52^{\circ}\text{C}$ for 30–60 s, and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. DNA sequencings were performed using the same primers used in polymerase chain reaction.

For phylogenetic analyses, newly generated 18S and 28S sequences were compared with those other nematode species available in GenBank using the BLAST homology search program (<https://www.ncbi.nlm.nih.gov/>). The selected 18S sequences including the new sequence were aligned using the Q-INS-i algorithm of the online version of MAFFT v.7 (<https://mafft.cbrc.jp/alignment/server/index.html>) (Kato & Standley 2013) and post-edited manually. The selected 28S sequences including the newly generated sequence were aligned similar to SSU dataset, and the alignment was post-edited with Gblocks program (version 0.91b), with all three less stringent parameters (http://phylogeny.lirmm.fr/phylo.cgi/one_task.cgi?task_type=gblocks). The model of base substitution for each dataset was selected using MrModeltest 2 (Nylander 2004). The Akaike supported model, a general time reversible model, including among-site rate heterogeneity and estimates of invariant sites (GTR + G + I) was used in both 18S and 28S analyses. Bayesian analysis was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) running the chains for 10×10^6 generations. After discarding burn-in samples, the remaining samples were retained for further analyses. The Markov chain Monte Carlo method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon 1999) using the 50% majority rule. Convergence of model parameters and topology were assessed based on average standard deviation of split frequencies and potential scale reduction factor values. Adequacy of the posterior sample size was evaluated using autocorrelation statistics as implemented in Tracer v.1.6 (Rambaut & Drummond 2009). Sequences of *Clavicaudoides clavicaudatus* (Altherr, 1953) Heyns, 1968 and *Aquatides aquaticus* (Thorne,

1930) Heyns, 1968 species were used as outgroups for the 18S tree (species and accession numbers in the tree) and sequences of *Mononchus truncatus* Bastian, 1865 and *Prionchulus punctatus* Cobb, 1917 were used as outgroups for the 28S tree (species and accession numbers in the tree). The output files of the trees were visualized using Dendroscope V.3.2.8 (Huson & Scornavacca 2012) and digitally drawn in CorelDRAW software version 17. The Bayesian posterior probability values exceeding 0.50%, are given on appropriate clades.

Results

Identification of the material examined and comparison with previously reported populations

Lindseyus costatus was very well characterized by Coomans and Kehiri (1986), who provided precise details of its morphology, a comprehensive table of measurements and ratios, and myriad excellent line illustrations, giving nice information for comparative purposes. The general morphology (Figs 1 & 2) of the Iranian specimens examined here perfectly fit those provided by these authors. Regarding their morphometrics (Table 1), they are in general concordance with those previously known, especially with those of another Iranian population studied by Coomans and Kheiri (*op. cit.*). Nevertheless, some differences in some measurements and ratios are noted too. First, the general size of the specimens here studied is larger, particularly appreciable in female body length (5.87–6.90 vs 4.30–5.67 mm in a total of 20 specimens of three different populations) and less so in the case of males (4.58–5.96 vs 4.10–4.81 mm in a total of seven specimens). Second, and more interestingly, two male secondary sexual traits apparently differ in a significant way as the nematodes here studied bear longer spicules (66–73 vs 44–61 μm long) and higher number of ventromedian supplements (10–13 vs 5–8). These differences are provisionally regarded as intraspecific variability of the species, and a possible consequence of the low number of specimens studied so far, especially in the case of males.

Molecular characterization of the material examined

After sequencing and editing, two sequences were obtained for phylogenetic analyses. One 18S rDNA sequence 1481-bp long (acc. PP868171) showed 99.12% identity to a SSU sequence (KM092519) assigned to *Amblydorylaimus isokaryon* (Loof, 1975) Andrassy, 1998, 99.12% to a sequence (DQ141212) assigned to *Aporcelaimellus obtusicaudatus* (Bastian, 1965) Altherr, 1968, and 99.05% to another *A. obtusicaudatus* sequence (AY284811). Surprisingly, it was only 98.24% identical to a sequence (AY284824) assigned to *Oxydirus oxycephalus* (de Man, 1885) Thorne, 1939 and 97.97% to a sequence (AY284823) assigned to *O. oxycephaloides* (de Man, 1921) Thorne, 1939, these corresponding to two members of the family Belondiridae and the subfamily Swangeriinae.

One 28S rDNA sequence 604-bp long (acc. PP868173) was 86.74% identical to a sequence (MK920111) assigned to *Makatinus aquaticus* Jiménez-Guirado, 1994, and less than 86% to several sequences of the genus *Aporcelaimellus* Heyns, 1965.

Evolutionary relationships of the genus *Lindseyus*

Morphologically, *Lindseyus* displays a peculiar combination of key traits: very short odontostyle for large to very large nematodes,

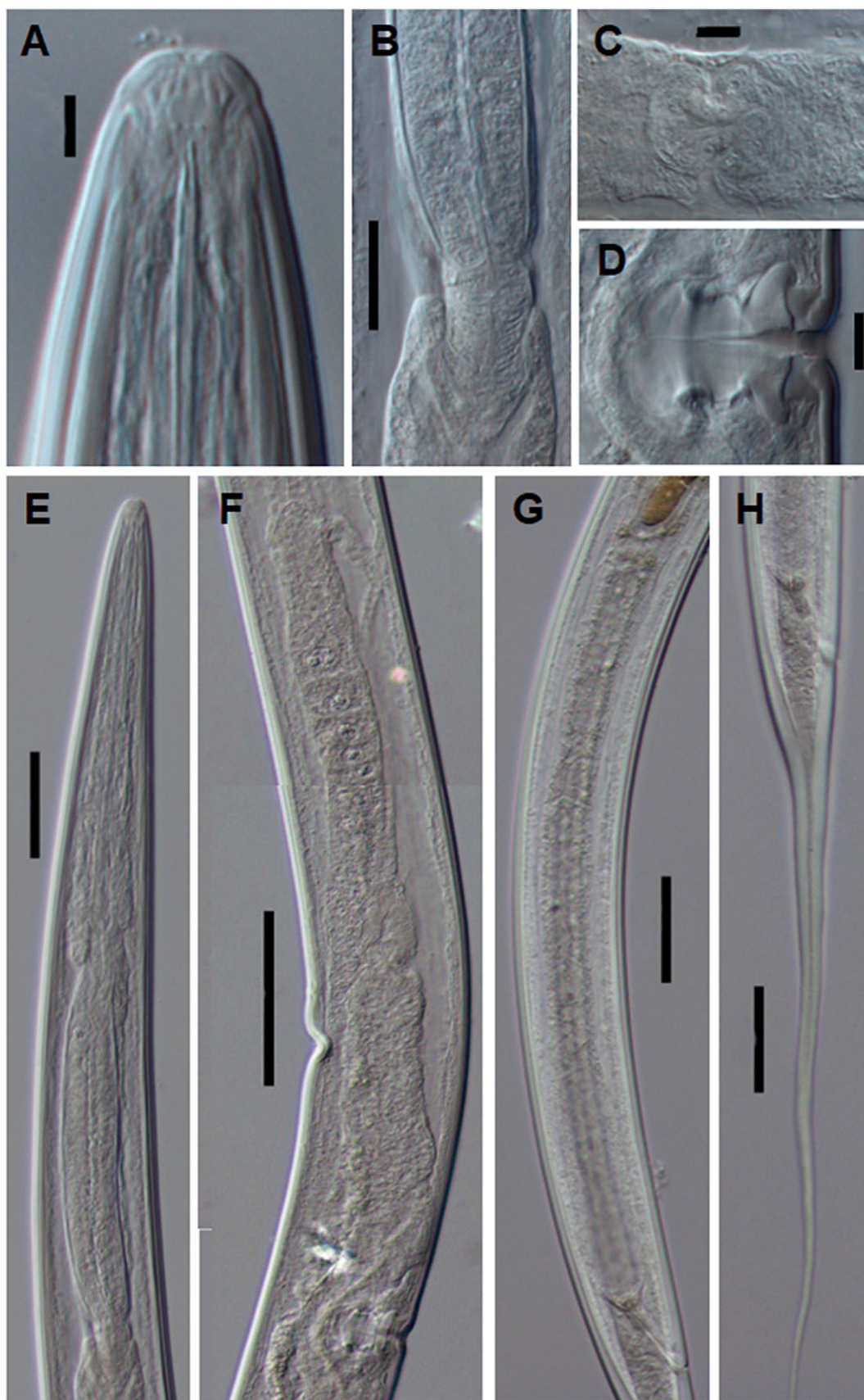


Figure 1. Light microphotographs of *Lindseyus costatus* Ferris & Ferris, 1973 from Lorestan province, Iran (female). A: Anterior body region, latero-medial view. B: Pharyngo-intestinal junction. C: Oviduct-uterus junction. D: Vagina. E: Neck region. F: Anterior genital branch. G: Prerectum. H: Caudal region. Scale bars: A = 5 µm; B = 20 µm; C, D = 10 µm; E, G, H = 50 µm; F = 100 µm.

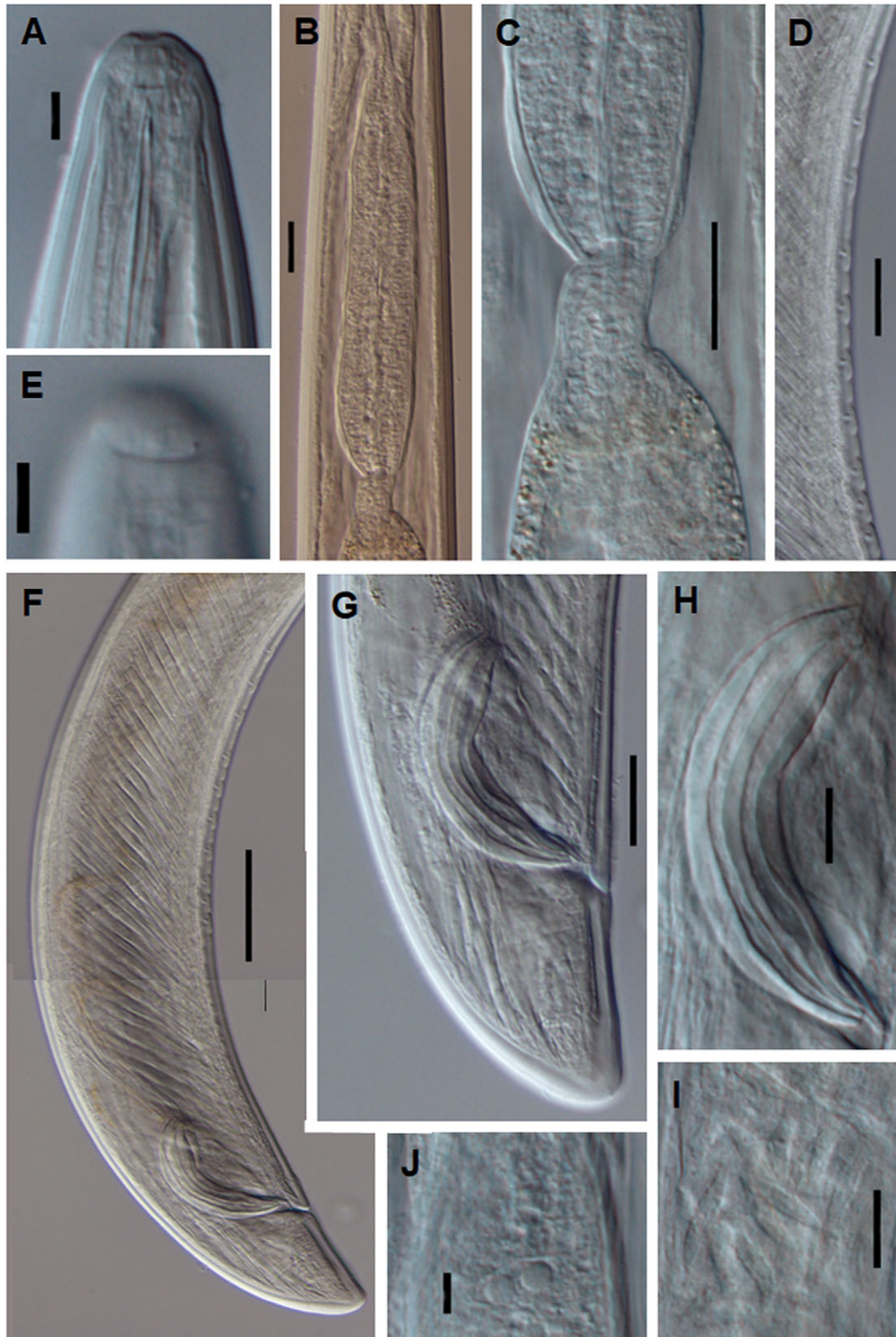


Figure 2. Light microphotographs of *Lindseyus costatus* Ferris & Ferris, 1973 from Lorestan province, Iran (male). A: Anterior body region, latero-medial view. B: Pharyngeal expansion. C: Pharyngo-intestinal junction. D: Ventromedian supplements. E: Anterior region, lateral surface view, showing amphid aperture. F: Posterior body region. G: Caudal region and spicule. H: Spicule. I: Sperm cells. J: DN with two nucleoli. Scale bars: A, E, J = 5 μ m; B-D, G = 20 μ m; F = 50 μ m; H, I = 10 μ m.

extremely short neck ($b > 10$), pharyngeal expansion enveloped by a strong sinistral spiral muscular sheath, cardia embraced by intestine only at its posterior part, and tail with sexual dimorphism. These features should be regarded at least as relevant

synapomorphies of the genus, which conform a very recognizable pattern and support the monophyly of the taxon. *Lindseyus* is very close to *Roqueus*, both mainly differing in the nature of the spiral sheath surrounding their pharyngeal expansion: with strongly

Table 1. Morphometrics of a new Iranian population of *Lindseyus costatus* Ferris & Ferris, 1973 (measurements in μm , except L in mm, and in the form average \pm standard deviation [range])

Country	Iran		USA (type)		Canada		Iran	
Habitat	Freshwater		Freshwater lake				Rice field	
Reference	Present study		(1, 2)		(2, 3)		(2)	
n	5♀♀	4♂♂	6♀♀	♂	3♀♀	♂	11♀♀	5♂♂
Character								
L	5.87–6.90	4.58–5.96	4.30–5.67	4.10	4.90–5.40	4.17	4.45–5.50	4.42–4.81
a	79–97	67–101	67–86	77	77–88	83	80–95	75–96
b	15–21	13–17	13–17	14	16–17	15	13–17	14–15
c	11–20	109–161	10–14	87	13–14	116	11–15	94–100
c'	7–14	0.9–1.1	?	?	11–13	1.0	10–15	1.1–1.3
V	37–41	–	34–41	–	38–40		37–43	–
Lip region diameter	10–12	10–12	12–13.5	?	10–10.5	11	10–11.5	10–11
Odontostyle length	4	4	4.5–5	?	4–5.5	4.5	4–4.5	4–4.5
Odontophore length	11	10	15	?	11.5–12	13	14–17	13
Neck length	330–432	350–379	290–390	290	?	286	306–333	300–325
Pharyngeal expansion length	133–185	137–163	112–172	?	?	119	126–148	112–140
Body diameter – neck base	51–65	50–65	?	?	?	?	?	?
mid-body	64–82	59–75	60–80	?	?	?	55–60	50–60
anus/cloaca	38–53	40–46	?	?	?	?	30–35	35–38
Prerectum length	195–400	251	190–370	320	256	?	225–365	365–528
Rectum/cloaca length	30–50	?	?	?			30–40	?
Tail length	312–535	37–49	350–570	47	352–425	36	310–460	44–50
Spicules length	–	66–73	–	56	–	57	–	44–50
Ventromedian supplements	–	10–13	–	7	–	?	–	5–8

References: 1 – Ferris & Ferris (1973); 2 – Coomans & Kehiri (1986); 3 – Ebsary (1984).

sinistral vs almost straight muscular bands, respectively. Coomans and Kheiri (1986) emphasized the importance of this difference, regarded the sinistral sheath observed in *Lindseyus*, a very unusual trait in Belondiridae, as an apomorphic condition, and proposed the tribe Roqueini to accommodate both genera.

Present molecular analysis is the first one provided for a *Lindseyus* representative and its results are presented in the corresponding trees of Fig. 3 (18S rDNA) and Fig. 4 (28S rDNA). In both cases, the evolutionary relationships of *L. costatus* are difficult to elucidate for several reasons. First, branching of the trees is poorly resolved. Second, *L. costatus* sequences form part of weakly supported (87% and 58%, respectively) clades. Third, there is not a close relationship of these sequences with other belondirid ones, even appearing separated from *Oxydirus* sequences, tentatively their closest taxon. Fourth, 18S tree shows *L. costatus* sequence forming part of a maximally supported clade together with a sequence identified as *cf. Oxydirus* sp, from Costa Rica, which probably belongs to a *Lindseyus* population.

Present results provide additional data about the identity of *L. costatus*, but add more doubts than certainties about the evolutionary relationships of belondirid taxa. Thus, the polyphyly of Belondiridae is once more reasserted, with *Oxydirus* sequences situated far from other representatives of the family, but now the

monophyly of Swangeriinae is also questioned as *Lindseyus* and *Oxydirus* sequences neither appear close in the molecular trees, an issue that should be cleared up in the future with further studies.

Taxonomy of the genus *Lindseyus*

Diagnosis

Belondiridae, Swangeriinae, Roqueini. Large- to very large-sized nematodes, 3.46–7.25-mm long, with very slender body. Cuticle dorylaimid. Lip region rounded, continuous with the adjoining body, with amalgamated lips. Amphid fovea goblet-like, with aperture occupying ca two-thirds of the lip region diameter. Cephalic framework, if present, is weakly sclerotized. Cheilostom is a truncate cone with thin walls. Odontostyle short and attenuate but showing visible lumen and aperture. Guiding ring simple. Odontophore rod-like, lacking any differentiation. Pharynx extremely short (*b*-ratio 12–28), entirely muscular, abruptly enlarging into the basal expansion that occupies less than one-half of the total neck length and is surrounded by a conspicuous sinistrally spiral sheath of musculature. Cardia tongue-like, only partially enveloped by intestinal tissue. Female genital system diovarian, with very well-developed *pars refringens vaginae* and transverse vulva. Tail dissimilar in sexes, long and filiform in females, short and rounded

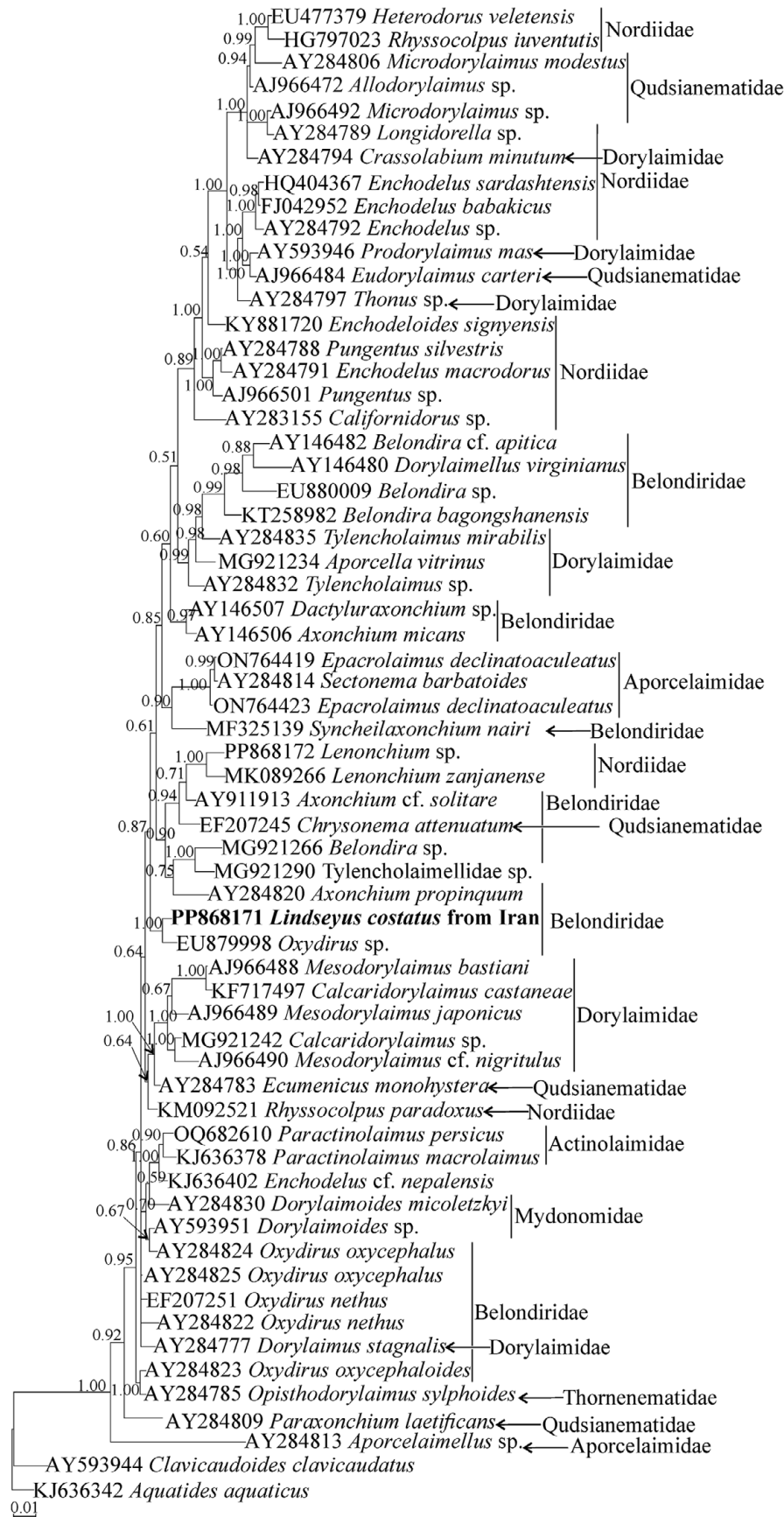


Figure 3. Bayesian 50% majority rule consensus tree inferred using 18S rDNA sequences of *Lindseyus costatus* Ferris & Ferris, 1973 from Lorestan province, Iran, under GTR+I+G model. Bayesian posterior probabilities values exceeding 0.50 are given for appropriate clades. Newly obtained sequence is in bold font. Scale bar = expected changes per site.

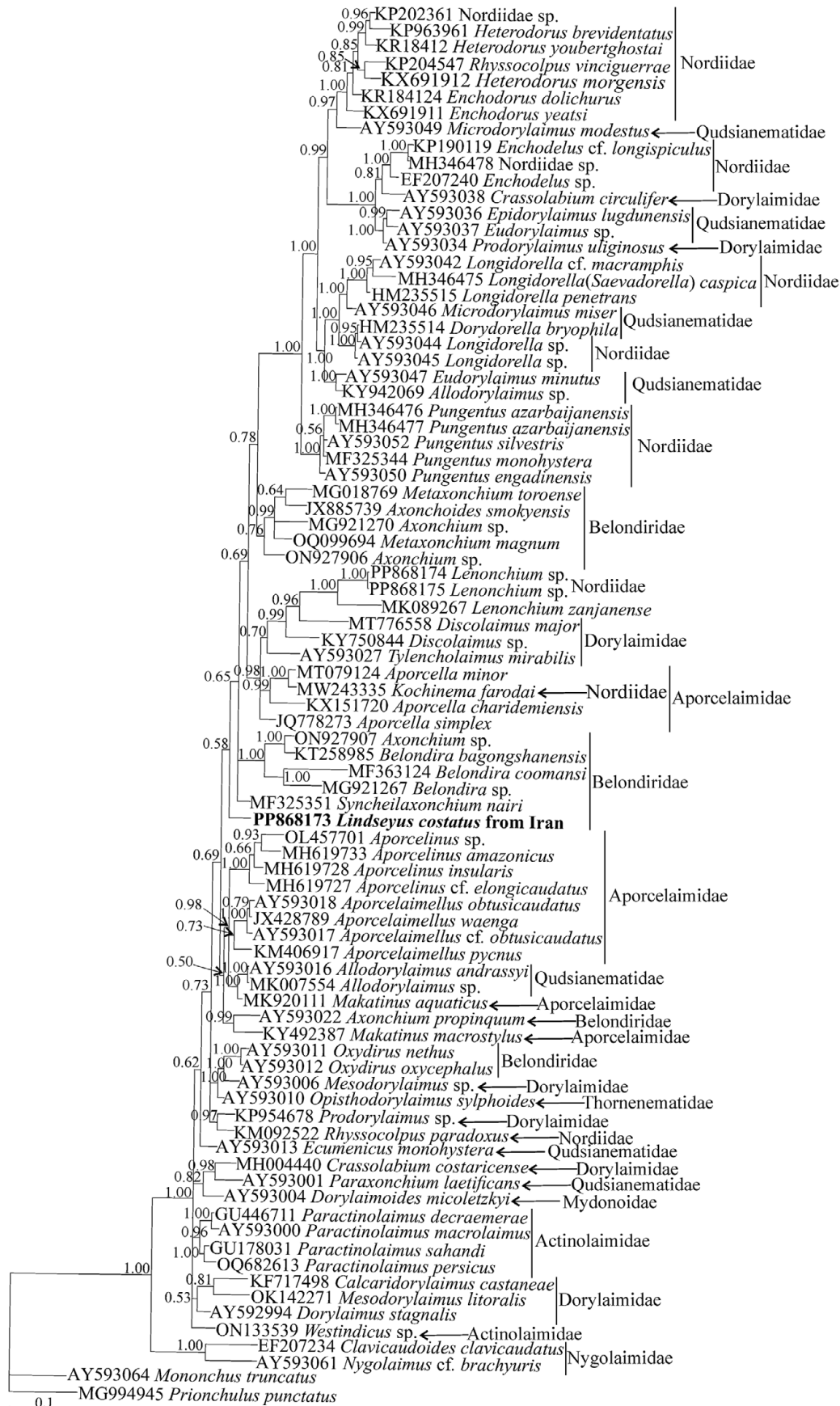


Figure 4. Bayesian 50% majority rule consensus tree inferred using 28S rDNA D2-D3 sequences of *Lindseyus costatus* Ferris & Ferris, 1973 from Lorestan province, Iran, under GTR+I +G model. Bayesian posterior probabilities values exceeding 0.50 are given for appropriate clades. Newly obtained sequence is in bold font. Scale bar = expected changes per site.

Table 2. Compendium of morphometrics of species belonging to the genus *Lindseyus* Ferris & Ferris, 1973 (measurements in μm except L in mm)

Species	n	Feature														Country	Reference ²
		L	a	b	c	c'	V	Lrd ¹	Odont.	Neck	Ph.exp.	Prerect.	Tail	Spicul.	Ve.sup.		
<i>costatus</i>	6♀♀	4.30–5.67	67–86	13–17	10–14	?	34–41	12–13.5	4.5–5	290–390	112–172	190–370	350–570	–	–	USA-Indiana	1, 2
	♂	4.10	77	14	87	?	–	?	?	290	?	320	47	56	7		
	3♀♀	4.90–5.40	77–78	16–17	13–14	11–13	38–40	10–10.5	4.5–5	?	?	256	352–425	–	–	Canada-Quebec	2, 3
	♂	4.17	83	15	116	1.0	–	11	4.5	286	119	?	36	57	?		
	11♀♀	4.45–5.50	80–95	13–17	11–15	10–15	37–43	10–11.5	4–4.5	306–333	126–148	225–365	310–460	–	–	Iran	2
	5♂♂	4.43–4.81	75–96	14–15	94–100	1.1–1.3	–	10–11	4–4.5	300–325	112–140	365–528	44–50	55–61	5–8		
	6♀♀	5.87–7.25	79–99	15–21	12–20	7–14	38–41	10–12	4–4.5	330–432	133–185	251	312–346	–	–	Iran	4
	5♂♂	4.57–6.26	67–106	13–18	109–169	0.9–1.1	–	10–12.5	4	350–375	137–163	360	37–43	66–79	10–13		
<i>heterurus</i>	3♀♀	5.57	68	14	11	?	37	?	?	398 ⁴	?	?	506 ⁴	–	–	Former Zaire	5, 6
	♂	4.47	81	12	124	?	–	?	?	372 ⁴	?	?	36 ⁴	?	?		
	4♀♀	5.50–6.08	83–91	13–17	11–14	14–16	35–38	?	5–6	342–415	124–152	573–799	437–509	–	–	Ivory Coast	6
	15♂♂	4.67–5.72	78–96	13–16	115–160	0.9–1.3	–	10.5–12	4.5–6	340–392	122–149	683–860	33–45	50–63	9–14		
	?♀♀ ³	5.67–6.73	82–101	15–17	9–14	13–22	33–36	11–12	5.5–6.5	356–404	?	286–550	440–607	–	–	Spain	7, 8, 9
	?♂♂ ³	4.97–5.71	84–99	14–16	138–160	0.9–1.0	–	11–12	5.5–6.5	352–372	?	637–774	35–39	51–56	12–13		
<i>As L. africanus</i>	?♀♀	4.2–5.1	98–106	13–15	9–10	16–20	36–37	9–10	6	?	?	?	?	–	–	Ivory Coast	10
	?♂♂	3.2	100	12	9	?	–	9–10	6	?	?	?	?	50	13		
	♀	6.5	100	18	9	?	34	12	7	361 ⁴	134 ⁴	?	?	–	–	South Africa	11
	<i>indicus</i>	7♀♀ ³	3.66–5.22	59–104	11–17	10–13	11–12	33–40	9–11	8–11	304 ⁴	?	230–370	348–400	–	–	India
	6♂♂ ³	3.46–3.83	54–82	12–15	81–101	1.0–1.3	–	9–11	9–11	?	?	270–340	37–46	53–56	8–10		
	<i>juwangens</i>	2♀♀	3.46–4.08	84–94	13–15	12	10–11	40	12	6.5–7.5	259–281	?	255–261	297–349	–	–	South Korea
	♂	5.13	77	13	82	0.9	–	?	?	400	151 ⁴	592	63	76	8		

¹Abbreviations: Lrd: Lip region diameter. Odont.: Odontostyle length. Ph. exp.: Pharyngeal expansion length. Prerect.: Prerectum length. Spicul.: Spicule length. Ve. sup.: Number of ventromedian supplements.

²References. 1 – Ferris and Ferris (1973). 2 – Coomans and Kheiri (1986). 3 – Ebsary (1984). 4 – Present paper. 5 – Schuurmans-Stekhoven and Teunissen (1938). 6 – Mulk *et al.* (1978). 7 – Jiménez-Guirado (1989). 8 – Jiménez-Guirado and Murillo-Navarro (2004). 9 – Jiménez-Guirado *et al.* (2007). 10 – Andrassy (1970a). 11 – Andrassy (1970b). 12 – Dhanachand & Jairajpuri (1980). 13 – Choi & Khan (1999).

³Specimens from two or more locations.

⁴Calculated from illustrations and/or other morphometrics.

conoid in male. Spicules dorylaimid. Ventromedian supplements 5–14 in number, shortly spaced in general, with large hiatus.

Etymology

Originally named after Prof. A.A. Lindsey, ecologist at Purdue University, Indiana, USA.

Type species:

L. costatus Ferris & Ferris, 1973

Other species:

L. heterurus (Schuurmans-Stekhoven & Teunissen 1938) Coomans & Kheiri, 1986

= *Dorylaimellus heterurus* Schuurmans-Stekhoven & Teunissen, 1938

= *Dorylaimus heterurus* (Schuurmans-Stekhoven & Teunissen 1938) Heyns, 1963

= *Paradorylaimus heterurus* (Schuurmans-Stekhoven & Teunissen 1938) Andrassy, 1969

= *Roqueus heterurus* (Schuurmans-Stekhoven & Teunissen 1938) Mulk, Coomans & Baqri, 1978

= *Roqueus africanus* Andrassy, 1970

= *Lindseyus africanus* (Andrassy, 1970) Coomans & Kheiri, 1986

L. indicus Dhanachand & Jairajpuri, 1980

L. juwangens Choi & Khan, 1999

Key to species identification

1a	–	Odontostyle 8–11 µm long (but see remarks)	<i>indicus</i>
1b	–	Odontostyle up to 7 µm long	2
2a	–	Distinct cuticular differentiations (paravulvae) present at both sides of vulva	<i>heterurus</i>
2b	–	No cuticular differentiation present at both sides of vulva	3
3a	–	Smaller females, 3.46–4.08-mm long. Odontostyle 6.5–7.5-µm long	<i>juwangens</i>
3b	–	Larger females, 4.30–7.25-mm long. Odontostyle 4–5-µm long	<i>costatus</i>

Table 2 provides a compendium of main morphometrics of species.

Remarks

Lindseyus is a well-characterized, homogeneous genus. Morphologically, it displays a very recognizable pattern, with only minor interspecific differences. Morphometrically, however, it shows wide variations in the main measurements and ratios, at least in its oldest species, namely *L. costatus* and *L. heterurus*. These two species, which were described in great details by Coomans and Kheiri (1986) and Mulk *et al.* (1978; see also Jiménez-Guirado and Murillo-Navarro 2004), respectively, significantly differ in the absence/presence of strong cuticular differentiations at both sides of the vulva, a remarkable trait that was observed in the three contributions (Andrassy, 1970a; Mulk *et al.* 1978; Jiménez-Guirado & Murillo-Navarro 2004) in which *L. heterurus* populations were studied. Besides, *L. costatus* bears somewhat smaller odontostyle than *L. heterurus* (4–5 vs 5–7 µm, respectively). It would be

interesting to confirm such differences by means of molecular analyses. Another different question is the identity of the two youngest species, namely *L. indicus* and *L. juwangens*, whose original (and only available) descriptions are not so well-detailed.

Dhanachand and Jairajpuri (1980) distinguished *L. indicus* from type population of *L. costatus* in (p. 163) “the shape of lip region, in having a longer odontostyle but smaller odontophore and cardia, in the shape and size of spicules and lateral guiding pieces and in the number of ventromedian supplements (odontostyle = 5–6 µm, odontophore 22 µm, lateral guiding pieces tapered and ventromedian supplements 7 in *L. costatus*”. Nevertheless, these differences are fewer when *L. indicus* is compared with other *L. costatus* populations later recorded (for instance, see data provided in Table 2), being reduced to slightly smaller males (3.46–3.83, n = 6 vs 4.10–6.26 mm, n = 12, respectively) and, most importantly, significantly longer odontostyle (8–11 vs 4–5 µm). Nevertheless, Dhanachand and Jairajpuri’s original illustrations 4B, C suggest that odontostyle might be shorter than indicated by the authors, perhaps comparable to that of *L. costatus*. (See also Coomans and Kheiri’s (1986) discussion about the difficulties to measure the odontostyle in *Lindseyus* populations.)

Choi and Khan (1999) described *L. juwangens* on the basis of only two females and one male and distinguished it from type population of *L. costatus* in having (p. 33) “shorter body length (3.4–4.0 mm vs 4.3–5.6 mm); more slender body (a = 83.6–94.4 vs a=67.2–86.3); shorter tail length (0.29–0.34 mm vs 0.35–0.57 mm) and in the number of ventromedian supplements 7 in *L. costatus*”. Notwithstanding, these morphometrical differences are minimum when all the available information about *L. costatus* is taken into account (see Table 2).

Acknowledgements. The kind assistance of the late father of the first author during our samplings is appreciated. This research was financially supported by Tarbiat Modares University. The Spanish author (R.P.S.) is thankful for the financial support of the University of Jaén, Spain, through the research program ‘PAIUA 2023/2024: EI_RNM2_2023’.

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