

Research Paper

Cite this article: Asgari M, Mohammadlou M, Eskandari A, Abolafia J and Peña-Santiago R (2024). Description and molecular characterization of *Trachactinolaimus persicus* sp. n. from Iran (Nematoda, Dorylaimida, Actinolaimidae), with new insights into the taxonomy and evolutionary relationships of the genus. *Journal of Helminthology*, **98**, e18, 1–11  
<https://doi.org/10.1017/S0022149X24000075>.

Received: 07 December 2023

Revised: 19 January 2024

Accepted: 19 January 2024

Keywords:






Cladistics; morphology; morphometrics; phylogeny; 28S rDNA

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# Description and molecular characterization of *Trachactinolaimus persicus* sp. n. from Iran (Nematoda, Dorylaimida, Actinolaimidae), with new insights into the taxonomy and evolutionary relationships of the genus

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## Abstract

A new species of the free-living nematode genus *Trachactinolaimus*, collected in natural habitats of northern Iran, is described, including morphological and molecular (28S-rDNA) data. *Trachactinolaimus persicus* sp. n. is characterized by its 1.95–2.44 mm long body, lip region weakly offset by depression and 18–20 µm wide, odontostyle 25–27 µm long, neck 540–636 µm long, pharyngeal expansion occupying one-half of the total neck length, bipartite uterus 2.6–3.9 body diameters long, vulva ( $V = 49–53$ ) pore-like, tail long and filiform in both sexes (174–223 µm,  $c = 10.0–13.4$ ,  $c' = 5.9–7.0$  in females, 165–196 µm,  $c = 10.7–13.8$ ,  $c' = 4.6–5.8$  in males), spicules 68–75 µm long, and 12–14 almost contiguous ventromedian supplements with hiatus. Molecular data supports the monophyly of the genus and the hypothesis that Dorylaimidae are the sister group of actinolaims. The taxonomy of *Trachactinolaimus* is updated, including diagnosis, list of species, key to their identification, and a compendium of their main morphometrics. *Dominiactinolaimus* is regarded as its junior synonym.

## Introduction

The genus *Trachactinolaimus* Andrassy, 1963 is an interesting dorylaimid (order Dorylaimida) nematode taxon, which displays low diversity and very vast geographical distribution as, at present, it includes five species, known to occur in four continents. Andrassy (1963) created this genus to accommodate a new (and the type) species, *T. radulatus*, inhabiting moss habitats in Angola. Much later, Vinciguerra (1988) transferred *Paractinolaimus dominicus* Hunt, 1978 from Dominica to this genus; Wu and Liang (1999) described *T. brevicaudatus*, collected in soil samples from China; Eliava and Jgenti (2006) discovered *T. montanus* in a Georgian forest; and, very recently, Zhang *et al.* (2023) found *T. nanjingensis* associated with mosses in China.

Since its original description the concept of the genus was not modified in any substantial aspect. As a member of the family Actinolaimidae, it is characterized and easily recognizable by the long, filiform tail of both sexes and the presence of abundant small denticles in its cheilostom. Nevertheless, its evolutionary relationships were a matter of some controversy (Coomans *et al.* 1990).

A population of *Trachactinolaimus* was collected in the course of a nematological survey conducted in northern Iran. Its study revealed that it belonged to an undiscovered species of the genus. Thus, this contribution aims to present its morphological description, provide its molecular (D2-D3 region of 28S rDNA) characterization, discuss its evolutionary relationships in the context of its family Actinolaimidae, and update its taxonomy.

## Material and Methods

### Sampling, extraction, and morphological identification of nematodes

Several moss samples were collected in the sub-mountain zones of Siahkal forests, located in Gilan province, northern Iran. Moss-dwelling nematodes were extracted using the tray method (Whitehead & Hemming 1965), handpicked under a stereomicroscope, killed by adding hot FPG (4:1:1, formaldehyde: propionic acid: glycerin) solution, transferred to anhydrous glycerine according to De Grisse (1969), and mounted on permanent glass slides to be observed under light microscopy (LM). Then, nematodes were measured and photographed using an Eclipse 80i microscope (Nikon, Tokyo, Japan) equipped with differential interference contrast optics, a

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drawing tube (camera lucida), and a DS digital camera. Ink drawings were made from sketches taken with the camera lucida and/or from photomicrographs processed with Adobe Photoshop CS8. For scanning electron microscopy (SEM), specimens preserved in glycerine were selected and prepared for observation with SEM according to Abolafia (2015). They were cleaned in distilled water, dehydrated in a graded ethanol-acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope (5 kV) (Zeiss, Oberkochen, Germany). Morphological comparisons were performed with the descriptions of other characterized species of *Trachactinolaimus*. Morphometrics include Demanian indices and other measurements and ratios, some of them presented in a separate table; meanwhile, others form part of the literal description of species. All measurements were recorded in  $\mu\text{m}$ , except body length in mm.

### DNA extraction, PCR, and sequencing

Following morphological confirmation, some fresh individuals of *Trachactinolaimus* were selected for DNA extraction. DNA was extracted using the modified Chelex method (Rashidifard *et al.* 2019). Each nematode was transferred to an Eppendorf tube containing 20  $\mu\text{l}$  of nuclease-free water, 25  $\mu\text{l}$  Chelex (5%, w/v), and 5  $\mu\text{l}$  of proteinase K (20 mg/ml). The microtubes were incubated at 56°C for 2 h, then at 95°C for 10 min, and the obtained solutions were used as DNA template. Five  $\mu\text{l}$  of each extracted DNA was added to the polymerase chain reaction (PCR) mixture in a 0.2 ml Eppendorf tube containing 15  $\mu\text{l}$  2X Master mix (Ampliqon, Odense, Denmark), 1  $\mu\text{l}$  of each primer (10 pmol/ $\mu\text{l}$ ), and 8  $\mu\text{l}$  ddH<sub>2</sub>O, to a final volume of 30  $\mu\text{l}$ . The D2-D3 region of 28S rDNA (LSU) was amplified using forward D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and reverse D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (Nunn 1992; De Ley *et al.* 1999). PCR reactions were carried out in a DNA thermal cycler (Hybaid, Ashford, Middlesex, UK). The PCR cycle conditions were as follows: initial denaturation cycle at 94°C for 15 min., followed by 35 cycles of denaturation at 94°C for 45 sec; annealing cycle at 56°C for 45 sec; extension cycle at 72°C for 1 min, and finally elongation cycle at 72°C for 5 min. After DNA amplification, the quality of PCR was checked by electrophoresis of 4  $\mu\text{l}$  of the PCR reactions in 1% agarose gel containing SYBR Green I. Products were visualised and photographed under ultraviolet light. The length and concentration of each PCR product were measured by comparison with a low DNA mass ladder (Invitrogen, Carlsbad, CA). The PCR products were purified and sequenced directly for both strands using the same primers with an ABI 3730XL sequencer (Bioneer, Seoul, South Korea). The newly obtained sequences of the D2-D3 region of 28S rDNA were submitted to the GenBank database under accession number PP187312.

### Phylogenetic analysis

For phylogenetic relationships, analysis was based on 28S rDNA. The newly obtained sequence was manually edited using Chromas 2.6.6 (Technelysium, Queensland, Australia) and aligned with other 28S rDNA sequences available in GenBank using the ClustalW alignment tool implemented in MEGA7 (Kumar *et al.* 2016). Poorly aligned regions at extremes were removed from the alignments using MEGA7. The best-fit model of nucleotide substitution used for the phylogenetic analysis was statistically selected using jModelTest 2.1.10 (Darriba *et al.* 2012). The phylogenetic tree was

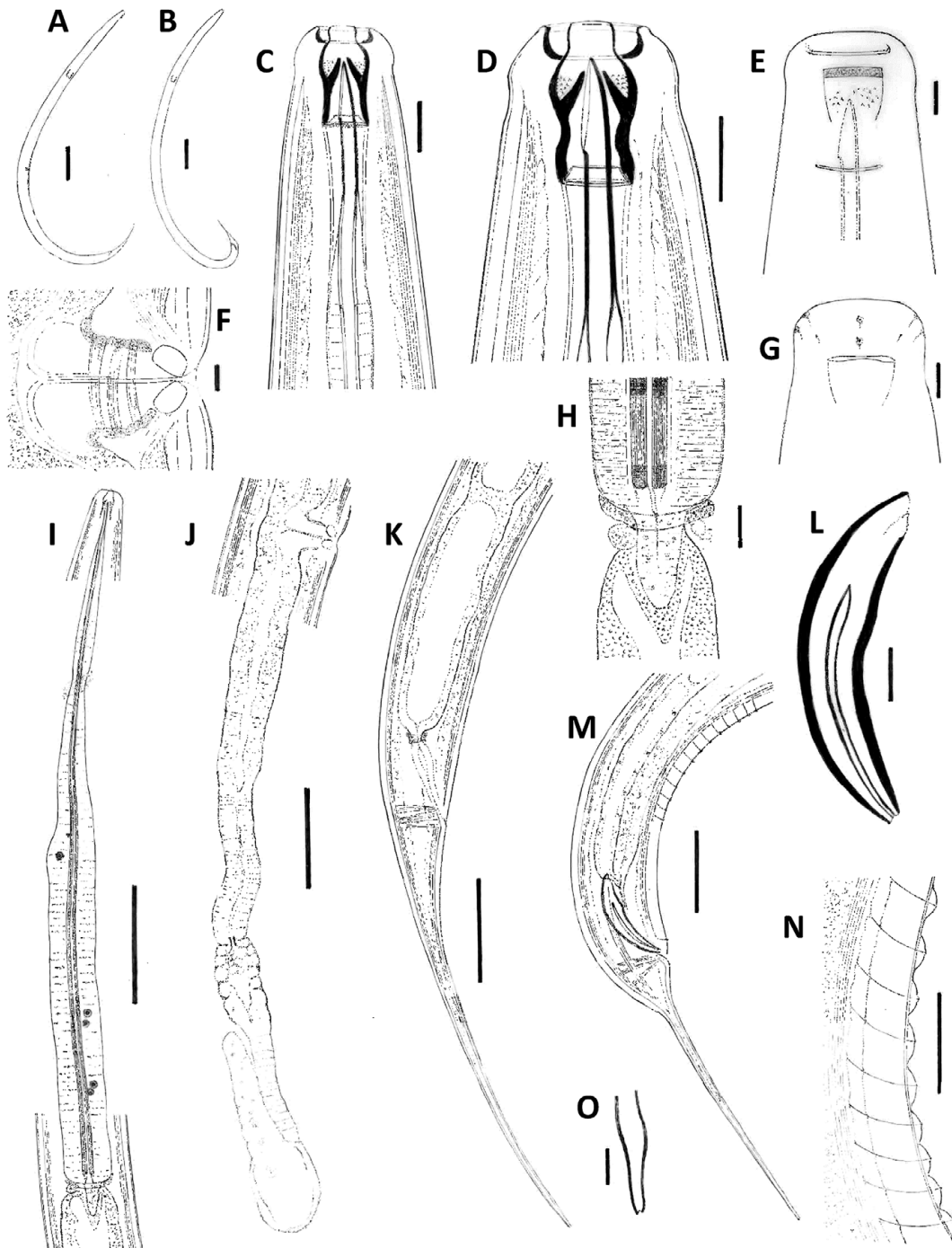
generated with the Bayesian inference method using MrBayes 3.2.6 (Ronquist *et al.* 2012). Two mononchid and five nygolaimid sequences were chosen as outgroups. The analysis under the generalized time reversible and invariant sites and gamma distribution (GTR + I + G) model was initiated with a random starting tree and run with Markov Chain Monte Carlo (Larget & Simon, 1999) simulations for  $1 \times 10^6$  generations. A total of 25% of samples were discarded as burn-in. The tree was visualized and saved with FigTree 1.4.4 (Rambaut 2018).

## Results

***Trachactinolaimus persicus* sp. n.** (Figures 1–4, morphometrics in Table 1).

**Material examined:** Fifteen females and nine males from one location, in excellent state of preservation.

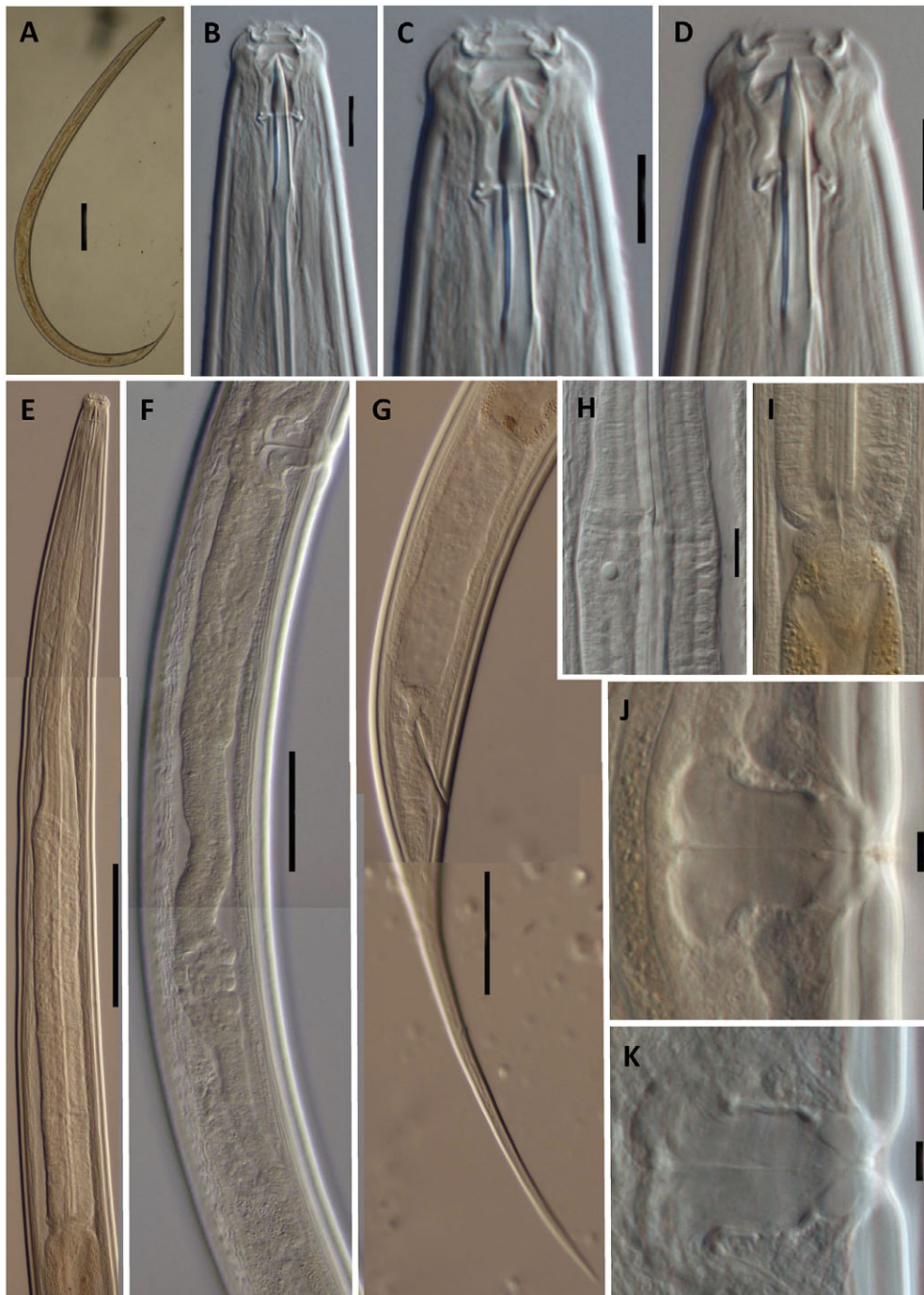
**Adult:** Medium-sized nematodes, 1.95–2.44 mm long. Body cylindrical, appreciably tapering towards the anterior end and much more strongly towards the posterior end as the tail is long and filiform in both sexes. Upon fixation, habitus curved ventrad, C-shaped in females, G-shaped in males. Cuticle smooth, 2–2.5  $\mu\text{m}$  thick at anterior region, 4–5  $\mu\text{m}$  in mid-body and 4.5–6  $\mu\text{m}$  on tail, two-layered, with a thin outer layer and a thicker inner layer. Lip region slightly expanded and hardly offset by a weak depression, 2.0–2.5 times as wide as high and *ca* one-third (28–36%) of body diameter at neck base, with amalgamated lips and low papillae, anterior margin visibly sunken, in lateral view appearing as a 11–12  $\mu\text{m}$  wide depression 2.5–3.5  $\mu\text{m}$  deep, bearing an elevated perioral part that occupies about one-half of depression width; SEM observations (Figure 4b, c): oral field wide, delimited by a thick, ring-like structure, offset from the adjoining part of lip region, with inner labial papillae close to it but out the field, oral aperture surrounded by a circular projection bearing abundant radial prongs, labial and cephalic papillae small and weakly prominent on lip region surface, amphid aperture a comparatively wide transverse slit. Cheilostom typical actinolaimid, 17–20  $\mu\text{m}$  long (from fixed guiding ring to anterior end), consisting of a labial chamber 9–10  $\mu\text{m}$  wide bearing four onchia and scattered denticles, and postlabial portion somewhat narrower than labial chamber and with thick walls. Odontostyle strong, 8.2–10.9 times as long as wide, longer (1.3–1.4 times) than lip region diameter, and 1.06–1.31% of body length, with large aperture occupying 10–11  $\mu\text{m}$  or two-fifths (39–42%) of total length. Guiding ring double, distinct, especially the fix ring. Odontophore rod-like, lacking any differentiation, slightly shorter (0.8–0.9 times) than odontostyle. Pharynx entirely muscular, very gradually enlarging into the basal expansion that is 7.1–10.1 times as long as wide, 4.2–5.9 times as long as body diameter at neck base, and occupies one-half (49–52%) of the total neck length; gland nuclei located as follows: DO = 49–52, DN = 52–55, S<sub>1</sub>N<sub>1</sub> = 75–77, S<sub>1</sub>N<sub>2</sub> = 76–78, S<sub>2</sub>N = 85–88. Nerve ring located at 155–177  $\mu\text{m}$  or 27–30% of the total neck length from the anterior end. Pharyngo-intestinal junction consisting of a 23–30 x 13–17  $\mu\text{m}$  conoid cardia enveloped by intestinal tissue, a ring-like structure surrounding the junction between pharyngeal base and cardia, and a dorsal lobe. Tail long and filiform, often almost straight, first appreciably tapering, then much more gradually until the very finely rounded tip, its inner core extending to 55–71% of tail length, thus with a appreciably hyaline portion; caudal pores two pairs, one lateral, another subdorsal, situated *ca* one body diameter behind the anal/cloacal aperture.



**Figure 1.** *Trachactinolaimus persicus* sp. n. from Iran (drawings) (a) Female, entire; (b) Male, entire; (c, d) Anterior body region, lateral median view; (e) Anterior body region, lateral submedian view; (f) Vagina; (g) Anterior body region, lateral surface view; (h) Pharyngo-intestinal junction; (i) Neck region; (j) Female, posterior genital branch; (k) Female, posterior body region; (l) Spicule; (m) Male, posterior body region; (n) Ventromedian supplements, in part; (o) Lateral guiding piece. Scale bars: a, b = 200  $\mu$ m; c, d, h, l = 10  $\mu$ m; e–g, o = 5  $\mu$ m; i = 100  $\mu$ m; j, k, m = 50  $\mu$ m; n = 20  $\mu$ m.

**Female:** Genital system diovarian, with equally and well-developed genital branches that occupies 282–400  $\mu$ m or 13–17% of body length. Ovaries reflexed, variably sized, 89–136  $\mu$ m long, often not surpassing the sphincter level, with oocytes first in several rows and then in a single row. Oviduct subterminally joining the ovary, 70–164  $\mu$ m long or 1.6–2.3 times the body diameter, consisting of a long distal part made of prismatic cells and an appreciable proximal *pars dilatata* with wide lumen and

usually bearing sperm cells inside. A shallow sphincter separates oviduct and uterus. Uterus a tube-like structure 160–243  $\mu$ m long or 2.6–3.9 times the body diameter, with two regions, almost equally long, the proximal one barely wider and ampler lumen inside, the distal one with narrower lumen. Vagina extending inwards 24–31  $\mu$ m to reach up to one-half (40–50%) of body diameter: *pars proximalis* 14–20  $\times$  15–19  $\mu$ m, with almost straight or slightly sigmoid walls surrounded by weak circular muscles;

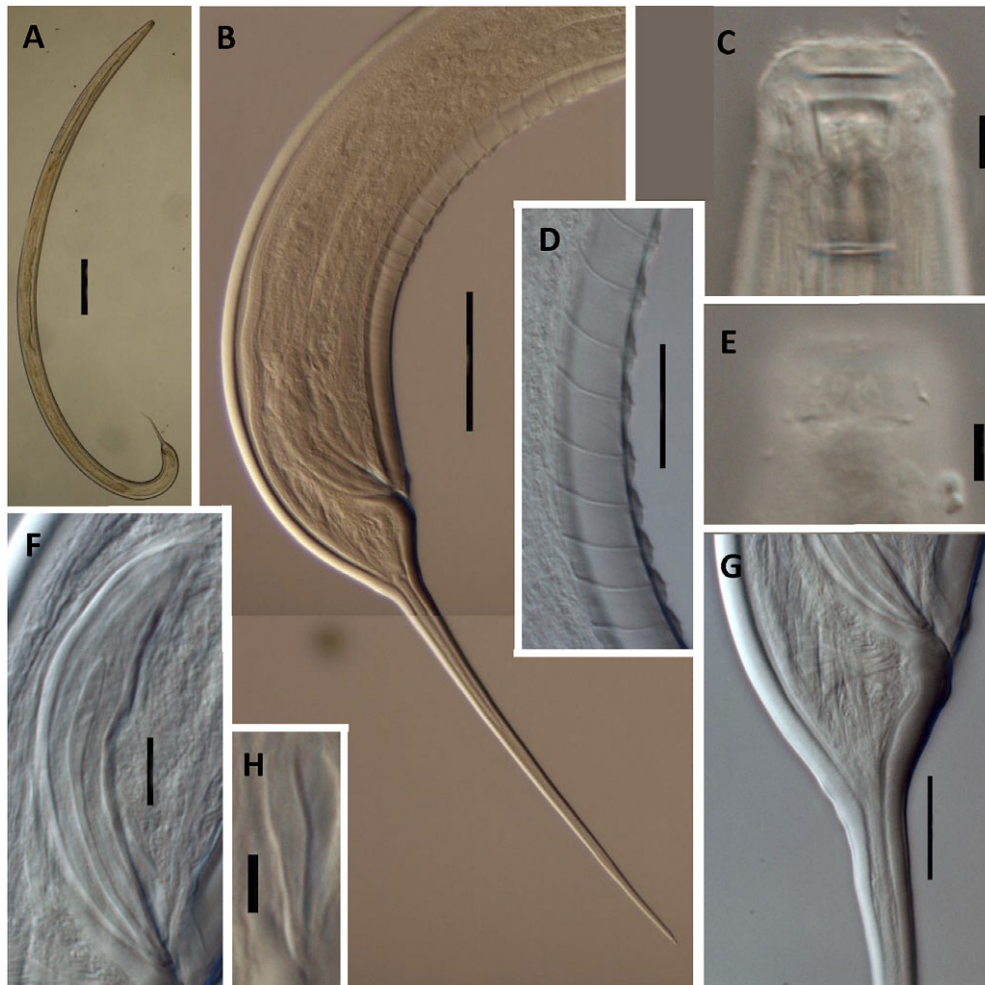


**Figure 2.** *Trachactinolaimus persicus* sp. n. (female). (a) Entire; (b–d) Anterior body region, lateral median view; (e) Neck region; (f) Posterior genital branch; (g) Posterior body region; (h) Pharyngeal enlargement; (i) Pharyngo-intestinal junction; (j, k) Vagina. Scale bars: a = 200  $\mu\text{m}$ ; b–d, h, i = 10  $\mu\text{m}$ ; e = 100  $\mu\text{m}$ ; f, g = 50  $\mu\text{m}$ ; j, k = 5  $\mu\text{m}$ .

*pars refringens* with (in lateral view) two close together trapezoidal or drop-shaped sclerotized pieces 6–7  $\times$  3.5–4  $\mu\text{m}$  and with a combined width of 12–15  $\mu\text{m}$ ; *pars distalis* 4–7  $\mu\text{m}$  long. Vulva an almost pore-like opening preceded of a weak depression. Pre-rectum 3.8–5.2, rectum 1.4–1.6 anal body diameters long.

**Male:** Genital system diorchic, with opposite testes. Pre-rectum 5.4–6.3, cloaca 1.7–1.9 times as long as body diameter at level of cloacal aperture. Cloacal aperture a transverse arched opening. In addition to the ad-cloacal pair, located at 7–10  $\mu\text{m}$  from the cloacal aperture, there is a series of 12–14, almost contiguous, 4–13  $\mu\text{m}$

apart, ventromedian supplements located on a low ventral longitudinal ridge that is separated from the adjoining body by two parallel longitudinal grooves, with the most posterior supplement situated at 75–86  $\mu\text{m}$  from the ad-cloacal part, thus with an appreciable hiatus. Spicules dorylaimid, 5.2–6.0 times as long as wide and 1.9–2.1 times longer than body diameter at cloacal aperture: head 12–13  $\mu\text{m}$  long or 26–30% of spicule length, 1.7–2.1 times as long as wide, with its dorsal side conspicuously longer than the ventral one; median piece occupying less than one-fourth (21–24%) of spicule diameter; posterior tip 2–2.5  $\mu\text{m}$  wide; curvature 130–136°. Lateral



**Figure 3.** *Trachactinolaimus persicus* sp. n. (male). (a) Entire; (b) Posterior body region; (c) Anterior body region, lateral median view; (d) Ventromedian supplements, in part; (e) Anterior body region, lateral surface view; (f) Spicule; (g) Anterior part of caudal region; (h) Lateral guiding piece. Scale bars: a = 200  $\mu$ m; b = 50  $\mu$ m; c, e, h = 5  $\mu$ m; d, g = 20  $\mu$ m; f = 10  $\mu$ m.

guiding pieces 17–23  $\mu$ m long, 4.9–6.7 times as long as wide, gradually tapering until a rather narrow posterior tip.

### Molecular characterization

One sequence of the D2-D3 region of 28S rDNA with 739 bp was obtained for *Trachactinolaimus persicus* sp. n. The sequence was analysed and compared with three sequences of *Trachactinolaimus nanjingensis* (ON054911, ON054912, ON054913) and other rDNA sequences of dorylaims available in the GenBank database. In a segment in common with 708 bp, both *Trachactinolaimus* species show 38 bp differences (substitution, insertions, or deletions) or 95% similarity.

### Diagnosis

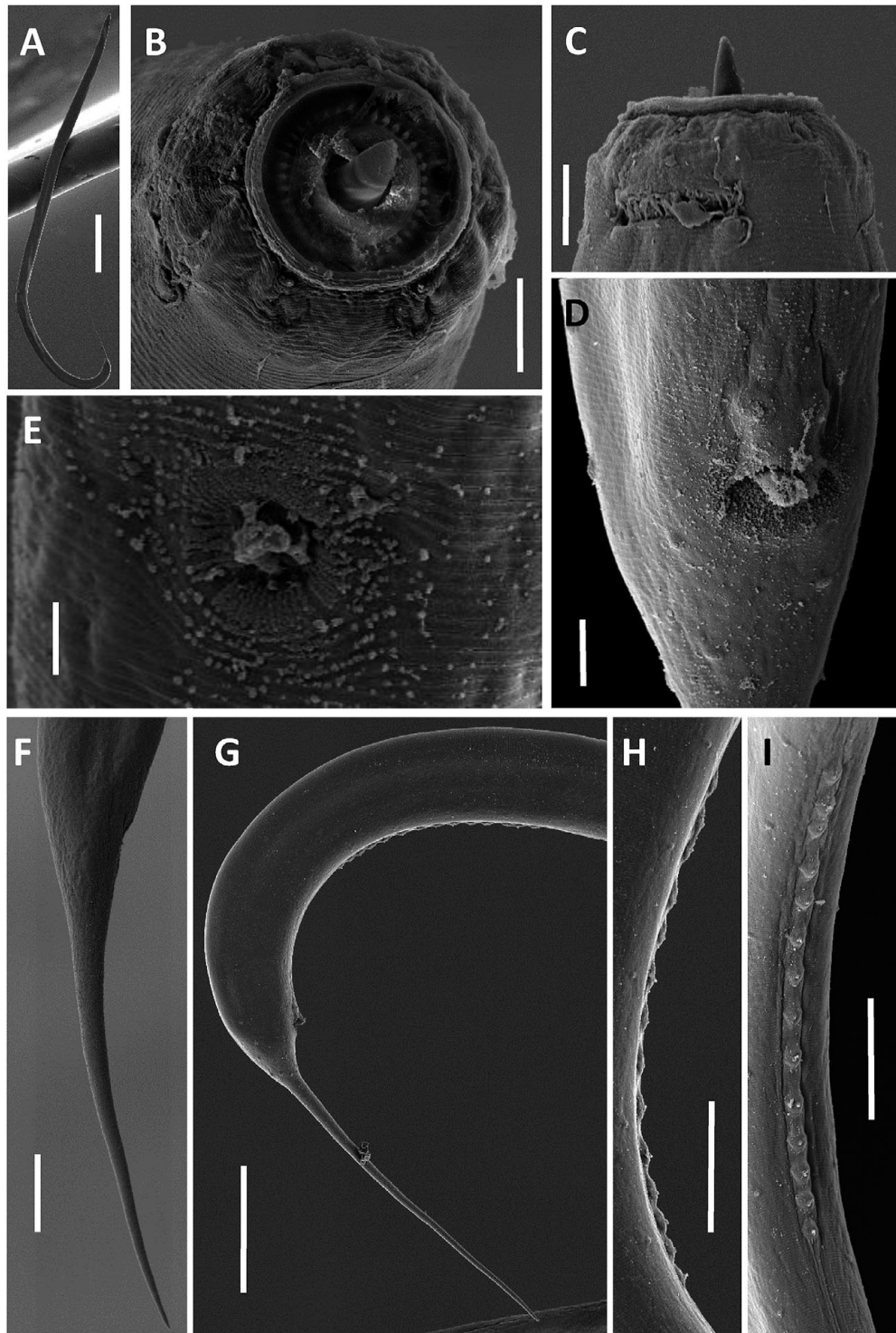
The new species is characterized by its 1.95–2.44 mm long body, lip region weakly offset by depression and 18–20  $\mu$ m wide, odontostyle 25–27  $\mu$ m long with aperture occupying *ca* two-fifths of its length, double guiding ring, neck 540–636  $\mu$ m long, pharyngeal expansion occupying *ca* one-half of the total neck length, female genital system diovarian with bipartite uterus 2.6–3.9 body diameters long, vulva ( $V = 49$ –53) a transverse slit, tail long and filiform in both sexes (174–223  $\mu$ m,  $c = 10.0$ –13.4,  $c' = 5.9$ –7.0 in females,

165–196  $\mu$ m,  $c = 10.7$ –13.8,  $c' = 4.6$ –5.8 in males), spicules 68–75  $\mu$ m long, and 12–14 almost contiguous ventromedian supplements with hiatus.

### Separation from its relatives

Morphometrically, the new species is similar to *T. dominicus*, *T. montanus*, and *T. radulatus*, three poorly characterized species (see also general discussion). It differs from *T. dominicus*, a Neotropical (Dominica) endemism (Hunt 1978), in its smaller general size (body length 1.95–2.44 vs. 2.31–2.64 mm, respectively), comparatively less slender body ( $a = 32$ –44 vs.  $a = 48$ –56), relatively shorter tail ( $c' = 5.9$ –7.0 vs. 9.6–12.7 in females, 4.6–5.8 vs. 6.2–8.0 in males), longer spicules (68–75 vs. 58  $\mu$ m), and higher number of ventromedian supplements (12–14 vs. 9). Besides, Coomans *et al.* (1990), who studied type specimens, illustrated the lip region of *T. dominicus* lacking an elevated perioral region at the centre of labial depression (see their Figure 4C), a relevant feature that might represent an additional significant difference between both species.

It differs from *T. montanus*, at present an European (Georgian) endemism (Eliava & Jgenti 2006), in its longer odontostyle (25–27 vs. 23–24  $\mu$ m), much shorter odontophore (0.8–0.9 vs. 1.5 times the odontostyle), absence of a uterine Z-like differentiation (vs. Z-like



**Figure 4.** *Trachactinolaimus persicus* sp. n. (SEM). (a) Male, entire; (b) Lip region, in face view; (c) Lip region, sublateral view; (d) Cloacal aperture, ventral view; (e) Vulva, ventral view; (f) Female, caudal region; (g) Male, caudal region; (h) Ventromedian supplements, lateral view; (i) Ventromedian supplements, ventral view. Scale bars: a = 200  $\mu$ m; b–d = 5  $\mu$ m; e = 2  $\mu$ m; f, h, i = 20  $\mu$ m; g = 50  $\mu$ m.

differentiation elongated), relatively shorter female tail ( $c = 10.0$ – $13.4$  vs.  $9.8$ – $10.3$ ), and larger spicules ( $68$ – $75$  vs.  $61$ – $65$   $\mu$ m). The original description of *T. montanus* is a bit laconic, and the corresponding illustrations lack details for comparative purposes, but available data about this species support its separation from the new one herein described.

It differs from *T. radulatus*, an Afrotropical (Angola) endemism, in its comparatively narrower lip region ( $18$ – $20$  vs.  $22$ – $24$   $\mu$ m, calculated from literal description), longer spicules ( $68$ – $75$  vs.  $56$ – $61$   $\mu$ m) and lower number of ventromedian supplements ( $12$ – $14$  vs.  $14$ – $17$ ). Besides, Coomans *et al.* (1990), who studied type material, described a well-developed Z-like differentiation at both

**Table 1.** Main morphometrics of *Trachactinolaimus persicus* sp. n. from Iran. Measurements in  $\mu\text{m}$  except L in mm, and in the form: average  $\pm$  SD (range)

| Character n                 | Holotype | Paratypes                   | Paratypes                   |
|-----------------------------|----------|-----------------------------|-----------------------------|
|                             | ♀        | 14♀♀                        | 9♂♂                         |
| L                           | 2.34     | 2.19 $\pm$ 0.17 (1.95–2.41) | 2.26 $\pm$ 0.15 (2.08–2.44) |
| a                           | 32       | 35.4 $\pm$ 3.1 (32–42)      | 36.8 $\pm$ 0.2 (34–44)      |
| b                           | 3.8      | 3.8 $\pm$ 0.2 (3.5–4.2)     | 3.8 $\pm$ 0.2 (3.6–4.1)     |
| c                           | 11.6     | 11.3 $\pm$ 0.9 (10.0–13.4)  | 12.3 $\pm$ 1.2 (10.7–13.8)  |
| c'                          | 6.5      | 6.5 $\pm$ 0.3 (5.9–7.0)     | 5.1 $\pm$ 0.4 (4.6–5.8)     |
| V                           | 53       | 50.9 $\pm$ 1.3 (49–53)      | –                           |
| Lip region diameter         | 19.5     | 19.2 $\pm$ 0.3 (19–20)      | 19.3 $\pm$ 0.6 (18–20)      |
| Odontostyle length          | 26       | 26.5 $\pm$ 0.3 (25–27)      | 25.9 $\pm$ 0.8 (25–27)      |
| Odontophore length          | 23       | 22.3 $\pm$ 0.9 (21–24)      | 22.6 $\pm$ 0.1 (21–24)      |
| Neck length                 | 618      | 575 $\pm$ 25 (540–618)      | 587 $\pm$ 26 (544–636)      |
| Pharyngeal expansion length | 312      | 289 $\pm$ 15 (269–313)      | 296 $\pm$ 17 (270–324)      |
| Body diameter at neck base  | 67       | 58.5 $\pm$ 6.1 (48–68)      | 59.4 $\pm$ 6.8 (48–68)      |
| midbody                     | 72       | 62.3 $\pm$ 6.6 (52–72)      | 61.8 $\pm$ 7.6 (53–72)      |
| anus/cloaca                 | 31       | 30.1 $\pm$ 1.7 (26–32)      | 35.8 $\pm$ 1.1 (34–38)      |
| Vulva – anterior end        | 1229     | 1115 $\pm$ 86 (979–1246)    | –                           |
| Prerectum length            | 162      | 137 $\pm$ 12 (124–162)      | 209 $\pm$ 10 (195–227)      |
| Rectum/cloaca length        | 48       | 44.8 $\pm$ 2.8 (40–50)      | 65.6 $\pm$ (60–70)          |
| Tail length                 | 202      | 194 $\pm$ 16 (174–223)      | 183 $\pm$ 10 (165–196)      |
| Spicule length              | –        | –                           | 71.1 $\pm$ 2.7 (68–75)      |
| Ventromedian supplements    | –        | –                           | 12–14                       |

uteri (see their Figure 4G–H), indeed a significant morphological difference between both species.

### Type locality and habitat

Northern Iran, Gilan Province, Siyahkal County, Gilbam Village (GPS coordinates: 37° 4' 26" N, 49° 46' 33" E; altitude: 84 m above sea level), where the new species was recovered from mosses growing on forest trees, collected on 20 October 2022.

### Type material

Female holotype, five female paratypes, and five male paratypes have been deposited at the Nematode Collection of the Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Spain. Seven female paratypes and three male paratypes have been deposited at the Nematode Collection of the Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

### Etymology

The species epithet refers to 'Persia', the Latin former name of Iran.

### Discussion

#### Taxonomy and evolutionary relationships of *Trachactinolaimus*

**Historical outline:** Thorne (1967) proposed the family Trachypleurosididae (in Actinolaimoidea) to include the genera *Trachypleurosum*

Andrássy, 1959 (= *Trachypleura* Thorne, 1939, nec Jackel (1900)) and *Actinolaimoides* Meyl, 1957 and distinguished them from other actinolaims by the long and filiform tail of both sexes (vs. rounded-tailed males in other genera). Andrássy (1976) also included *Trachactinolaimus* in Trachypleurosidae, whereas Siddiqi (1982) stated that *Actinolaimoides* was not a true actinolaimid taxon but a member of Nordiidae. Several authors (Baqri *et al.* 1975; Vinciguerra 1988; Coomans 1990) recommend re-lowering the actinolaims to family rank, and, consequently, their families to subfamilies. In this system, Vinciguerra (1988), who carried out a fine cladistic analysis of actinolaims, classified *Trachypleurosum* as the only genus of Trachypleurosinae and characterised this subfamily by lacking onchia. Moreover, *Trachactinolaimus* was considered to be a member of Actinolaiminae Thorne, 1939. Coomans *et al.* (1990), who studied type material of *Trachypleurosum* and *Trachactinolaimus*, questioned the evolutionary relationships of both genera as they confirmed the presence of onchia in *Trachypleurosum* and regarded the long tail in both sexes, a remarkable trait shared by the two genera, as a plesiomorphic condition. Jairajpuri and Ahmad (1992; see also Khan & Jairajpuri 1994) proposed the subfamily Paractinolaiminae in Actinolaimidae to group five genera, including *Trachactinolaimus* and the new genus *Dominiactinolaimus*, characterised by having cheilostomal denticles among other distinctive features, whereas *Trachypleurosum* was maintained as the only member of the family Trachypleurosidae. Vinciguerra (2006) did not recognize any subfamily within Actinolaimidae, and Andrássy (2009) classified *Trachactinolaimus* and *Trachypleurosum* under Trachypleurosinae and regarded *Dominiactinolaimus* as identical to and a junior synonym of the latter.

*New evidence based on morphological data:* As mentioned, one of the most recognizable traits of *Trachactinolaimus* is its long-tailed males, an unusual feature of actinolaims, only shared with *Trachypleurosum*. Their long-tailed males easily separate these two genera from the remaining genera of Actinolaimidae (16 of 18 valid, cf. Andrassy 2009) that display sexual dimorphism in tail shape as all of them have rounded-tailed males. This represents a major morphological difference that was originally used by Thorne (1967) to create the family Trachypleurosidae and later by Andrassy (2009) to keep the subfamily Trachyplerosinae. Nevertheless, and as pointed out by Coomans et al. (1990), the long and filiform tail, in this case in males, should be interpreted as a primitive (plesiomorphic) condition that does not warrant that *Trachactinolaimus* and *Trachypleurosum* form a monophyletic taxon, therefore assuming that reduction of tail length might have occurred more than one time throughout the evolutionary history of actinolaims. In this sense, Coomans et al. (1990) stated that (p. 153) “*Trachactinolaimus* is very close to *Paractinolaimus*, differing from it only in shape of male tail.” This matter is probably one of the reasons why Vinciguerra (2006) did not recognize any subfamilial division in Actinolaimidae.

On the other hand, *Trachactinolaimus* and *Trachypleurosum* are very similar taxa, differing in the presence vs. absence of cheilostomal denticles, respectively. According to Coomans et al. (1990), the former is more plesiomorphic as the absence of denticles should be regarded as a derived (apomorphic) condition in actinolaims (cf. Vinciguerra 1988).

*Molecular approach:* Molecular analysis, whose results are presented in the tree of Figure 5, shows some relevant findings. First, and in spite of the limited number of available sequences of actinolaimid taxa, the monophyly of Actinolaimidae is confirmed as their sequences form a totally (100%) supported clade. Second, the genus *Trachactinolaimus* should be regarded as a monophyletic taxon too because its four sequences (of two species) constitute a totally (100%) supported subclade too, separated from the remaining actinolaimid sequences, which seem to be a more heterogeneous group. Third, a close evolutionary relationship between actinolaims and members of the family Dorylaimidae (genera *Dorylaimus*, *Labronema*, *Nevadanema*, and *Prodorylaimus*) is observable, thus confirming that Dorylaimidae might be the outer sister group of actinolaims (Holterman et al. 2008; Andrassy 2009).

### Updated taxonomy of *Trachactinolaimus*

*Diagnosis:* Actinolaimidae. Medium- to large-sized nematodes, 1.95–4.41 mm long. Cuticle dorylaimid. Lip region hardly offset by a shallow depression, with totally fused lips; oral field sunken, delimited by a ring-like margin; oral aperture often protruding, surrounded by a crown-like structure consisting of numerous, contiguous and radial prongs. Amphid fovea cup-like, its aperture occupying ca one-half of lip region diameter. Cheilostom consisting of a wide labial chamber bearing four large onchia and abundant denticles, and a postlabial section with thick walls. Odontostyle strong, with wide aperture. Guiding ring double. Odontophore rod-like, lacking any differentiation. Pharynx entirely muscular, gradually enlarging into the basal expansion that occupies ca one-half of the total neck length. Female genital system diovarian, with uterus including or not a Z-like differentiation, *pars refringens vaginae*, and longitudinal or pore-like vulva. Tail similar in sexes, long and filiform. Spicules dorylaimid. Ventromedian supplements 12–23 in number, almost contiguous, with large hiatus.

### Type species

*T. radulatus* Andrassy, 1963

### Other species

*T. brevicaudatus* Wu & Liang, 1999

*T. dominicus* (Hunt, 1978) Vinciguerra, 1988

= *Paractinolaimus dominicus* Hunt, 1978

= *Dominiactinolaimus dominicus* (Hunt, 1978) Jairajpuri & Ahmad, 1992

= *Trachypleurosum dominicum* (Hunt, 1978) Andrassy, 2009

*T. montanus* Eliava & Jgenti, 2006

*T. najingensis* Zhang, Ji, Guo, Qing & Li, 2023

*T. persicus* sp. n.

### Key to species identification

- 1– Larger general size, body length 2.64–4.41 mm in females. Male tail appreciably shorter ( $c = 22\text{--}39$ ,  $c' = 1.9\text{--}3.5$ ) .....
  - 2– Smaller general size, body length 1.95–2.70 mm in females. Mail tail appreciably longer ( $c = 7.8\text{--}16.6$ ,  $c' = 4.0\text{--}8.0$ ) ..... **3**
    - 2– Females 4.02–4.41 mm long. Spicules 67–76  $\mu\text{m}$  long. Ventromedian supplements 20–23 in number ..... ***brevicaudatus***
      - Females 2.64–3.42 mm long. Spicules 55–64  $\mu\text{m}$  long. Ventromedian supplements 15–19 in number ..... ***najingensis***
    - 3– Uterus bearing a Z-like differentiation ..... **4**
      - Uterus lacking a Z-like differentiation ..... **5**
    - 4 – Odontostyle 23–24  $\mu\text{m}$  long. Shorter female tail ( $c' = 4.0\text{--}6.5$ ) ..... ***montanus***
      - Odontostyle 27  $\mu\text{m}$  long. Longer female tail ( $c' = 8\text{--}9$ ) ..... ***radulatus***
    - 5 – Less slender body ( $a = 32\text{--}44$ ). Longer tail ( $c' = 9.6\text{--}12.7$  in females, 6.2–8.0 in males). Spicules 58  $\mu\text{m}$  long. 9 ventromedian supplements ..... ***dominicus***
      - More slender body ( $a = 48\text{--}56$ ). Shorter tail ( $c' = 5.9\text{--}7.0$  in females, 4.6–5.8 in males). Spicules 68–75  $\mu\text{m}$  long. 12–14 ventromedian supplements ..... ***persicus* sp. n.**

Tables 1 & 2 compile the main morphometrics of *Trachactinolaimus* species.

### Other concluding remarks

Actinolaims are an easily recognizable dorylaimid taxon characterised by the presence of four large teeth (onchia) at their heavily sclerotized cheilostom, which represents a very remarkable autapomorphy of the group. Molecular analysis herein presented results in the confirmation that they form part of a robustly supported clade, then confirming their monophyly, with Dorylaimidae as their sister (and more plesiomorphic) taxon.

Available information also suggests (supports) the idea that *Trachactinolaimus* is a natural (monophyletic) genus, certainly primitive within actinolaims when its tail shape (long and filiform in both sexes, a plesiomorphic condition) is compared to that observed in other genera (rounded-tailed males, an apomorphic state) with the



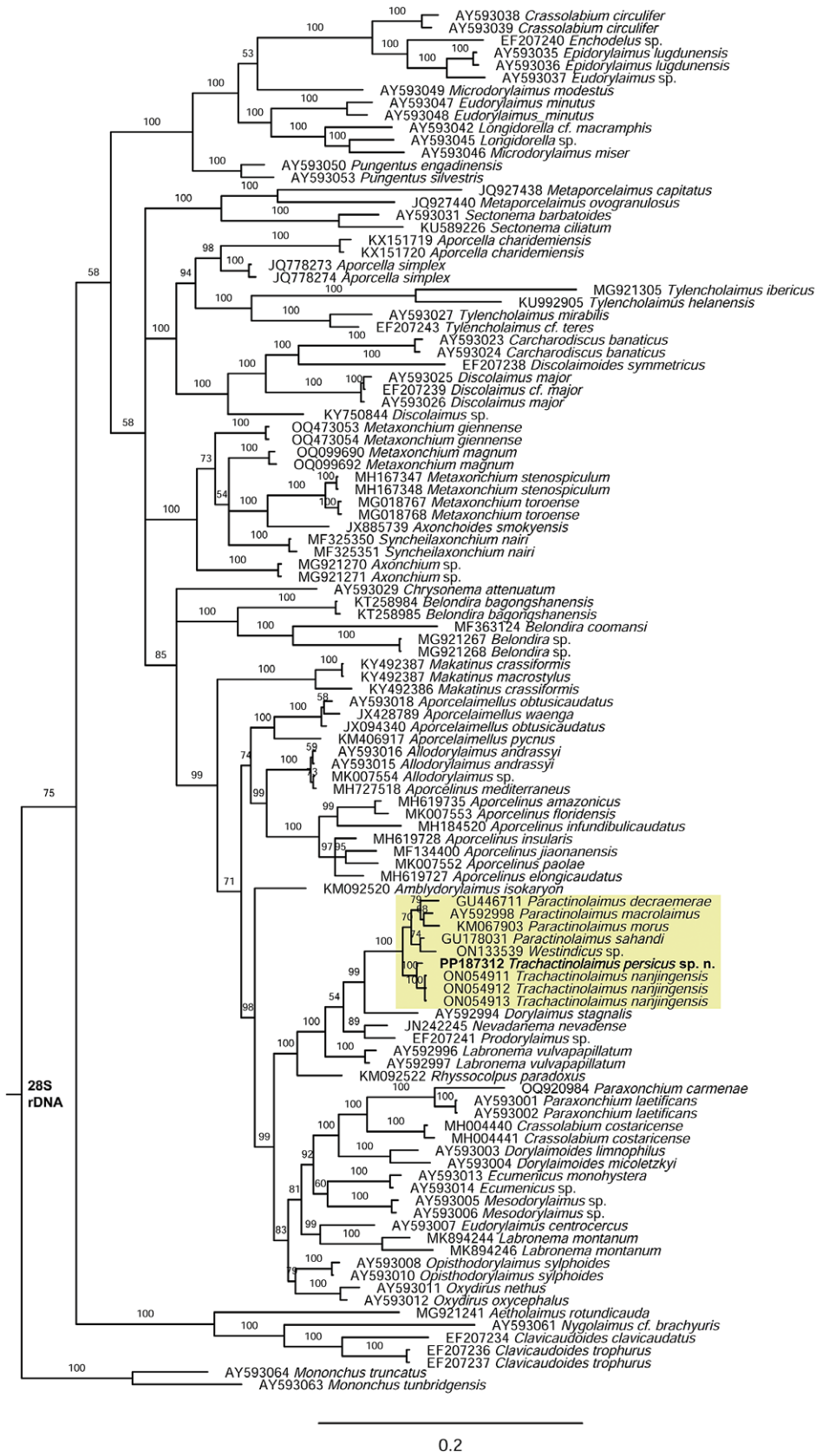


Figure 5. Bayesian Inference tree from the newly sequenced *Trachactinolaimus persicus* sp. n. based on sequences of the 28S rDNA region. Bayesian posterior probabilities (%) are given for each clade. Scale bar shows the number of substitutions per site.

**Table 2.** Main morphometrics (ranges) of species of the genus *Trachactinolaimus*. Measurements in  $\mu\text{m}$  except L in mm

| Species                     | brevicaudatus |           | dominicus        |                  | montanus |          | nanjingensis |           | radulatus        |                  |
|-----------------------------|---------------|-----------|------------------|------------------|----------|----------|--------------|-----------|------------------|------------------|
| Population                  | type          |           | type             |                  | type     |          | type         |           | type             |                  |
| Country                     | China         |           | Dominica         |                  | Georgia  |          | China        |           | Angola           |                  |
| Reference                   | 1             |           | 2                |                  | 3        |          | 4            |           | 5                |                  |
| Character n                 | 2♀♀           | 5♂♂       | 4♀♀ <sup>1</sup> | 7♂♂ <sup>1</sup> | 6♀♀      | 6♂♂      | 11♀♀         | 11♂♂      | 8♀♀ <sup>1</sup> | 4♂♂ <sup>1</sup> |
| L                           | 4.02, 4.41    | 3.36–3.50 | 2.52–2.64        | 2.31–2.57        | 2.2–2.7  | 2.0–2.6  | 2.64–3.42    | 2.50–3.21 | 2.37–2.47        | 2.13–2.35        |
| a                           | 50, 46        | 40–60     | 48–53            | 49–56            | 37–43    | 38–44    | 45–60        | 45–62     | 40–42            | 41–45            |
| b                           | 4.9, 5.2      | 3.5–4.4   | 3.9–4.3          | 4.0–4.3          | 3.7–4.0  | 3.5–3.8  | 4.0–4.8      | 3.8–5.0   | 3.8–4.1          | 3.6–4.1          |
| c                           | 21, 20        | 34–39     | 6.9–9.1          | 7.8–11.5         | 9.8–10.3 | 9.2–12.5 | 13–18        | 22–39     | 9.6–10.9         | 12.8–16.6        |
| c'                          | 4.6, 4.9      | 1.9–2.5   | 9.6–12.7         | 6.2–8.0          | 4.0–6.5  | 4–6      | 5.0–9.2      | 2.3–3.5   | 8–9              | 4.5–6.2          |
| V                           | 52, 56        | –         | 49–53            | –                | 47–52    | –        | 49–53        | –         | 50–53            | –                |
| Lip region diameter         | ?             | ?         |                  |                  | ?        | ?        | 18–23        | 18–23     | 22–24*           | 22–24*           |
| Odontostyle length          | 29            | 30–33     | 25–29            | 25–29            | 23–24    | 23–24    | 26–31        | 27–31     | 27               | 27               |
| Odontophore length          | 26, 27        | 24–28     | 21–27            | 21–27            | 34*      | 34*      | 25–32        | 23–30     | ?                | ?                |
| Neck length                 | 822, 848      | 763–960   | 600*             | 567*             | 605*     | 667*     | 578–753      | 541–739   | ?                | ?                |
| Pharyngeal expansion length | ?             | ?         | ?                | ?                | ?        | ?        | ?            | ?         | ?                | ?                |
| Body diameter at neck base  | ?             | ?         | ?                | ?                | ?        | ?        | ?            | ?         | ?                | ?                |
| midbody                     | 80, 95        | 59–86     | 48*              | 45*              | 63*      | 68*      | 48–63        | 42–62     | ?                | ?                |
| anus/cloaca                 | 41, 45        | 40–49     | 29*              | 34*              | ?        | 56*      | 27–33        | 28–32     | ?                | ?                |
| Vulva – anterior end        | 2092, 2461*   |           | 1320*            | –                | 1171*    | –        |              |           | ?                | –                |
| Prerectum                   | 241, 257      | 186–244   | ?                | ?                | ?        | ?        | 115–152      | 121–178   | ?                | ?                |
| Rectum                      | 63, 67        | 58–74     | ?                | ?                | ?        | ?        | 37–48        | 43–48     | ?                | ?                |
| Tail length                 | 190, 222      | 90–110    | 277*             | 212*             | 168–252  | 282*     | 149–248      | 67–112    | ?                | ?                |
| Spicule length              | –             | 67–76     | –                | 58               | –        | 61–65    | –            | 55–64     | –                | 56–61            |
| Ventromedian supplements    | –             | 20–23     | –                | 9                | –        | 12–15    | –            | 15–19     | –                | 14–17            |

References: 1, Wu and Liang (1999); 2, Hunt (1978); 3, Eliava and Jgenti (2006); 4, Zhang et al. (2023); 5, Andrassy (1963).

\*Calculated from description and/or other morphometrics.

<sup>1</sup>Specimens from two or more locations.

exception of *Trachypleurosum*. Thus, it cannot be discarded that *Trachactinolaimus* forms part (together with *Trachypleurosum*, another member of Trachypleurosinae) of the sister group of the remaining actinolaims, a hypothesis that deserves further study (cf. Coomans et al. 1990).

The two *Trachactinolaimus* species (only) known to occur in China, namely *T. brevicaudatus* and *T. nanjingensis*, differ from the remaining components of the genus in several features. First, their general size is appreciably larger (body length 2.50–4.41 vs. 1.95–2.70 mm long). Second, and more interestingly, male tail is tapering less regularly than in females and even the males of other species (first abruptly and then very gradually vs. gradually throughout its length), and its length is significantly shorter than that of their respective females (vs. both females and males with comparable length in other species). Thus, these two species might form part of a geographical subclade of the genus.

Andrassy (2009) regarded *Dominiactinolaimus* as a junior synonym of *Trachypleurosum*. Nevertheless, it was probably a mistake by the author as, having denticles in its cheilostom (Hunt 1978;

Coomans et al. 1990; Jairajpuri & Ahmad 1992), it is identical to *Trachactinolaimus*, not to *Trachypleurosum*.

**Acknowledgements.** The Spanish authors thank the staff (Amparo Martínez-Morales) and equipment of “Centro de Instrumentación Científico-Técnica (CICT)”, University of Jaén, for assistance in obtaining SEM pictures of the new species herein described.

**Financial support.** Funding was provided by the University of Jaén, Spain, through the research program ‘PAIUIJA 2023/2024: EI\_RNM2\_2023’.

**Competing interest.** None.

**Ethical standard.** The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

## References

Abolafia J (2015). A low-cost technique to manufacture a container to process meiofauna for scanning electron microscopy. *Microscopy Research and Technique* **78**, 9, 771–776. <https://doi.org/10.1002/jemt.22538>.

- Andrássy I** (1959). Neubenennungen einiger Homonymen Nematoden-Gattungen. *Nematologica* **4**, 3, 223–226. <https://doi.org/10.1163/187529259X00255>.
- Andrássy I** (1963). Freilebende Nematoden aus Angola, I. Einige moosewohnende Nematoden. *Publicações Culturais da Companhia de Diamantes de Angola* **66**, 55–80.
- Andrássy I** (1976). *Evolution as a basis for the systematization of nematodes*. London: Pitman Publishing.
- Andrássy I** (2009). *Free-living nematodes of Hungary. III. Pedozoologica Hungarica n° 5*. Budapest, Hungary: Hungarian Natural History Museum.
- Coomans A, Vinciguerra MT, Loof PAA** (1990). Status of the genera *Paractinolaimus* Meyl, 1957, *Trachypleurosum* Andrássy, 1959 and *Trachactinolaimus* Andrássy, 1963 (Nematoda: Actinolaimidae) with description of *Trachypleurosum venezolanum* n. sp. *Revue de Nématologie* **13**, 143–154.
- Darriba D, Taboada GL, Doallo R, Posada D** (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772. <https://doi.org/10.1038/nmeth.2109>.
- De Grisse A** (1969). Redescription ou modification de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. *Mededelingen Rijks-faculteit Landbouwwetenschappen Gent* **34**, 351–369.
- De Ley P, Felix AM, Frisse LM, Nadler SA, Sternberg PW, Thomas WK** (1999). Molecular and morphological characterization of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* **1**, 6, 591–612. <https://doi.org/10.1163/156854199508559>.
- Eliava I, Jgenti L** (2006). *Trachactinolaimus montanus* n. sp. (Nematoda: Actinolaimidae) from Adzhara (Western Georgia). *Bulletin of the Georgian National Academy of Sciences* **173**, 596–597.
- Holterman M, Rybarczyk K, Van den Essen S, van Megen H, Mooyman P, Peña-Santiago R, Bongers T, Bakker J, Helder J** (2008). A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources* **8**, 1, 23–34. <https://doi.org/10.1111/j.1471-8286.2007.01963.x>.
- Hunt D.** (1978). Bionomics of *Paractinolaimus vigor* Thorne, 1967 (Dorylaimida: Paractinolaimidae) with a description of *P. dominicus* n. sp. and *Nygalaimuim haguei* n. sp. (Dorylaimida: Aporcelaimidae). *Nematologica* **23** (1977), 4, 452–462. <https://doi.org/10.1163/187529277X00381>.
- Jaekel O** (1900). Einen neuen Chitoniden, *Trachypleura* n. g., aus dem Muschelkalk von Rüdersdorf. *Zeitschrift der Deutschen Geologischen Gesellschaft* **52**, 9–16.
- Jairajpuri MS, Ahmad W** (1992). *Dorylaimida. Free-living, predaceous and plant-parasitic Nematodes*. New Delhi, India: Oxford & IBH Publishing Co.
- Khan Z, Jairajpuri MS** (1994). *The Actinolaims. Predatory soil nematodes from India*. Aligarh, India: Aligarh Muslim University.
- Kumar S, Stecher G, Tamura K** (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 7, 1870–1874. <https://doi.org/10.1093/molbev/msw054>.
- Larget B, Simon DL** (1999). Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* **16**, 6, 750–759. <https://doi.org/10.1093/oxfordjournals.molbev.a026160>.
- Meyl AH** (1957). Free-living nematodes. Exploration hydrobiologique du Lac Tanganika (1946–1947). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique* **3**, 25–51.
- Nunn GB** (1992). *Nematode molecular evolution: an investigation of evolutionary patterns among nematodes based upon DNA sequences*. Ph.D. Thesis, University of Nottingham, Nottingham, UK.
- Rambaut A** (2018). Figtree, a graphical viewer of phylogenetic trees. Available at: <https://github.com/rambaut/figtree/releases/tag/v1.4.4> (accessed 18 September 2023).
- Rashidifard M, Marais M, Daneel MS, Mienie CMS, Fourie H** (2019). Molecular characterisation of *Meloidogyne enterolobii* and other *Meloidogyne* spp. from South Africa. *Tropical Plant Pathology* **44**, 213–224. <https://doi.org/10.1007/s40858-019-00281-4>.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP** (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 3, 539–542. <https://doi.org/10.1093/sysbio/sys029>.
- Siddiqi MR** (1982). Six new genera of dorylaimid nematodes. *Nematologica* **27**, 1981, 397–421.
- Thorne G** (1939). A monograph of the nematodes of the superfamily Dorylaimoidea. *Capita Zoologica* **8**, 1–261.
- Thorne G** (1967). Nematodes of Puerto Rico: Actinolaimoidea, new superfamily with a revision of its genera and species with addenda to Belondiroidea (Nematoda, Adenophorea, Dorylaimida). *University of Puerto Rico Agricultural Experiment Station, Technical Paper* **43**, 1–48.
- Vinciguerra MT** (1988). A new classification of Actinolaimoidea Thorne, 1939 using a cladistic approach. *Nematologica* **33**, 1987, 251–277. <https://doi.org/10.1163/187529287X00399>.
- Vinciguerra MT** (2006). Dorylaimida Part II: Superfamily Dorylaimoidea. In Eyualem-Abebe, Traunspurger W, Andrássy I (eds), *Freshwater Nematodes: Ecology and Taxonomy*. Wallingford, UK: CABI Publishing, 392–467.
- Whitehead AF, Hemning JR** (1965). A comparison of some quantitative methods of extracting semi veriform nematodes from soil. *Annals of Applied Biology* **55**, 1, 25–28. <https://doi.org/10.1111/j.1744-7348.1965.tb07864.x>.
- Wu J, Liang Y** (1999). Two new species of Actinolaimidae Thorne, 1939 (Nematoda: Dorylaimida) from China. *Journal of Nematology* **31**, 4, 475–481.
- Zhang Q, Ji H, Guo F, Qing X, Li H** (2023). Morphological and molecular characterization of *Trachactinolaimus nanjingensis* n. sp. (Dorylaimida: Actinolaimidae) from Nanjing, China. *Nematology* **25**, 3, 307–320. <https://doi.org/10.1163/15685411-bja10222>.