Morphological and molecular characterization of *Pungentus sufiyanensis* n. sp. and additional data on *P. engadinensis* (Altherr, 1950) Altherr, 1952 (Dorylaimida: Nordiidae) from northwest of Iran

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The genus *Pungentus* is an interesting dorylaimid genus, often found in forest habitats of the Northern Hemisphere, and with very restricted presence in southern territories. Its taxonomy was updated by Álvarez-Ortega and Peña-Santiago (2014), who listed 16 valid species and other four *inquirendae* or *incertae sedis* and provided a key to their identification as well a compendium of their main morphometrics.

Available information about *Pungentus* species from Iran is very limited. Solouki et al. (2010) recorded *P. engadinensis* (Altherr, 1950) Altherr, 1952 and *P. silvestris* (de Man, 1912) Coomans and Geraert (1962) in [Uremia (West Azarbaijan) and Marand (East Azarbaijan) provinces, respectively], whereas, very recently, Heydari et al. (2019) described a new species, *P. azarbaijanensis*, associated with grass in West Azarbaijan, and *P. engadinensis* in several locations of the country.

Several *Pungentus* populations were collected in the course of a nematological survey conducted in natural and cultivated soils of northwest Iran (East–West Azarbaijan and Kurdistan provinces) to explore the dorylaimid diversity of this region. Their study revealed that they belonged to one new and one known species. The objective of this work was to report *Pungentus sufiyanensis* n. sp. using morphology, morphometric, and molecular methods and provide new data about *P. engadinensis*. 

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**Abstract**

Two species of the genus *Pungentus*, one new and one known, collected in natural vegetation and cultivated soils in northwest of Iran, are studied. *Pungentus sufiyanensis* n. sp. is characterized by its 1.22 to 1.57 mm long body, offset lip region by a constriction and 7 to 9 μm broad, 18 to 21 μm long odontostyle, 304 to 348 μm long neck, 133 to 161 μm long esophageal expansion, mono-opisthodelphic female genital system without anterior uterine sac, slightly backward directed vagina, absence of *pars refringens vaginae*, V = 47–54, rounded-conoid caudal region (17.5–23 μm, c = 65–84, c’ = 0.7–1) with saccate bodies, and the absence of male. Molecular analysis, based on D2-D3 expansion segments of the 28S rDNA (LSU), confirms the monophyly of the family Nordiidae and suggests the monophyly of the genus *Pungentus*, with the new species forming a clade with other Iranian species. New data are presented for six Iranian populations of *P. engadinensis*, and an updated key for the identification of *Pungentus* species is also provided.

**Keywords**

D2-D3, Description, Molecular analysis, Morphology, Morphometrics, Taxonomy.
Pungentus sufiyanensis n. sp. from Iran: Vazifeh et al.

Materials and methods

Extraction and processing of nematodes

Soil samples were collected from the rhizosphere of several crops and orchards of East–West Azerbaijan and Kurdistan provinces, northwest Iran, during the period 2010–2017. Nematodes were extracted following the protocols by Jenkins (1964) and Whitehead and Heming (1965), transferred to anhydrous glycerin according to De Grisse (1969), and mounted on glass slides for handling.

Light microscopy

Mounted specimens were observed under an Olympus BX 41 light microscope equipped with a drawing tube and a DP50 digital camera attached to it. Morphometrics include Demanian indices and the usual measurements and ratios. Line illustrations were prepared using CorelDRAW® software version 12. Microphotographs were edited using Adobe® Photoshop® CS software.

DNA extraction, PCR and sequencing

For the molecular study of the new species, DNA samples were extracted from a live adult nematode, hand-picked, and placed on a clean slide containing a drop of distilled water or worm lysis buffer (WLB) and crushed by a sterilized scalpel. Then, the suspension was transferred to an Eppendorf tube containing 25.65 μl ddH2O, 2.85 μl of DNA template. PCR was carried out using a BIO RAD thermocycler machine in accordance with Archidona-Yuste et al. (2016). PCR cycle conditions were as follows: denaturation at 94°C for 1 min, 35 cycles of denaturation at 94°C for 30 s, annealing of primers at 55°C for 45 s and extension at 72°C for 3 min followed by a final elongation step at 72°C for 10 min. The purified PCR products were sent for sequencing to Bioneer Company, South Korea.

Results

Systematics

Pungentus sufiyanensis n. sp. (Figs. 1, 2; Tables 1, 2)

Description

Female: slender (a=40-50) nematodes of medium size, 1.22 to 1.57 mm long. The body cylindrical, tapering toward both ends but more so toward the anterior extreme as the caudal region is short and rounded. Upon fixation, habitus slightly curved ventrad, to an open C-shape. Cuticle three layered, especially distinguishable at caudal region, bearing fine transverse striations, 2 to 3.5 μm thick at anterior region, 3 to 6 μm at mid-body, and 7 to 10 μm at tail. Lateral chords 8 to 11 μm thick or occupying one-fourth to one-third of mid-body diameter. The lip region is somewhat angular, offset by a weak but perceptible constriction, with nearly truncated anterior margin, 2.1 to 2.6 times as wide as height and 21 to 27% of body diameter at neck base; lips mostly amalgamated, with hardly protruding papillae. Amphidial fovea cup-shaped, opening at the level of constriction, with the aperture 4 to 5 μm long or 52 to 60% of lip region diameter. Cheilostom nearly cylindrical, 1.2 to 1.8 times as long as the lip region diameter, with visible sclerotised walls in its anterior half, and bearing four accessions number MN855359 as indicated on the phylogenetic tree of Table 2.

Phylogenetic analyses

The newly generated sequences were aligned with the other segments of 28S rDNA gene sequences available in GenBank using MEGA6 software (Tamura et al., 2013). Paravulvus hartingii (de Man, 1880) Heyns, 1968 (AY593062) as outgroup was chosen. Bayesian analysis (Bi) was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The best fit model of DNA evolution was obtained using MrModeltest 2.3 (Nylander, 2004) with Akaike-supported model in conjunction with PAUP* v4.0b10 (Swofford, 2003). Bi analysis under the general time-reversible model with invariable sites and a gamma-shaped distribution (SYM + I + G) model for the 28S rDNA gene was done. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority rule consensus tree and posterior probabilities (PP) were given on appropriate clades. The tree was visualized using the program Figtree 1.4.3 v.
distinct, sclerotized, circumoral platelets. Odontostyle slightly arcuate dorsally, slender, well sclerotized, 2.0 to 2.5 times as long as the diameter of lip region, 1.1 to 1.4% of total body length, and aperture 2 to 3 μm long or occupying 9 to 17% its length. Guiding ring double. Odontophore rod-like, 0.8 to 1.0 times the odontostyle length. Nerve ring situated at 109 to 121 μm or 30 to 35% of the neck length from the anterior end. Pharynx entirely muscular, consisting of an anterior portion enlarging gradually into the basal expansion that is 8.4 to 13 times as long as width, 4.1 to 5.2 times as long as body diameter at neck base and occupies 40 to 45% of total neck length; gland nuclei located as follows: DN=61–63, S,N₁=68–70, S,N₂=77–80, S,N₃=89–92 according to Loof and Coomans (1970). Cardia hemispherical, almost as long as wide, 8-11×7-10 μm. Genital system monopistodelphic, without anterior uterine sac. Genital branch well developed, 154 to 203 μm long or 9 to 16% of total body length. Ovary reflexed, 61 to 97 μm long, usually not reaching the sphincter level, with oocytes arranged first in several rows and then in a single row. Oviduct joins ovary subterminally, 48 to 62 μm or 1.1 to 2.0 times the corresponding body diameter long, consisting of a slender portion made up of prismatic cells and developed pars dilatata with perceptible lumen. Oviduct-uterus junction marked by a sphincter. Uterus a simple tube-like structure.
Pungentus sufiyanensis n. sp. from Iran: Vazifeh et al.

37 to 51 μm long or 0.9 to 1.5 times the corresponding body diameter long. Sperm in genital tract absent. Vagina slightly directed backward, extending 15 to 20 μm inwards and occupying 39 to 45% of the corresponding body diameter; pars proximalis vaginae 9-12 × 11-15 μm, with nearly sigmoid walls and surrounded by moderately developed, circular musculature and pars distalis 2 to 3.5 μm and pars refringens vaginae obscure in specimens examined. Vulva a nearly equatorial, transverse slit, preceded by a V-shaped depression of body surface. Prerectum 2.1 to 3.2 and rectum 0.6 to 1.0 times as long as the anal body diameter. The caudal region short, rounded-conoid, slightly more straight at the ventral side, where it bears saccate bodies; two pairs of caudal pores are present.
**Table 1. Morphometric data for *Pungentus sufiyanensis* n. sp.**

| Characters                     | Sufiyan population | Bokan population | Marand population |
|--------------------------------|--------------------|------------------|-------------------|
|                                | Holotype           | Paratypes        | Female            | Female            |
| n                              | –                  | 7                | 8                 | 2                 |
| L                              | 1.49               | 1.53 ± 0.08      | 1.41 ± 0.09       | 1.27 ± 0.07       |
|                                | (1.40–1.57)        | (1.33–1.54)      | (1.22–1.33)       |                   |
| a                              | 45                 | 42.0 ± 3.3       | 47.0 ± 2.8        | 46.0 ± 2.3        |
|                                | (40.0–47.0)        | (42.0–50.0)      | (43.0–49.0)       |                   |
| b                              | 4.4                | 4.5 ± 0.1        | 4.4 ± 0.3         | 3.9 ± 0.7         |
|                                | (4.3–4.8)          | (4.0–5.0)        | (3.9–4.0)         |                   |
| c                              | 68                 | 75.0 ± 6.1       | 70.0 ± 4.7        | 68.0 ± 1.4        |
|                                | (65.0–84.0)        | (66.0–75.0)      | (67.0–70.0)       |                   |
| c´                             | 1                  | 0.8 ± 0.01       | 0.9 ± 0.07        | 0.9 ± 0.01        |
|                                | (0.7–1.0)          | (0.8–1.0)        | (0.8–1.0)         |                   |
| V                              | 50                 | 49.0 ± 1.0       | 49.0 ± 1.0        | 51.0 ± 2.3        |
|                                | (47.0–50.0)        | (48.0–51.0)      | (49.0–54.0)       |                   |
| Lip region diam.               | 8                  | 8.4 ± 0.3        | 8.2 ± 0.6         | 8.0 ± 0.5         |
|                                | (8.0–9.0)          | (7.5–9.0)        | (7.0–9.0)         |                   |
| Odontostyle length             | 20                 | 19.0 ± 0.7       | 19.0 ± 1.4        | 20.0 ± 0.4        |
|                                | (18.0–20.0)        | (18.0–20.5)      | (19.0–21.0)       |                   |
| Odontophore length             | 18                 | 17.0 ± 0.8       | 16.5 ± 0.2        | 17.0 ± 1.8        |
|                                | (15.0–18.0)        | (16.0–17.0)      | (16.0–19.0)       |                   |
| Guiding ring from ant. end     | 13                 | 13.0 ± 0.5       | 13.5 ± 0.0        | 14.0 ± 0.0        |
|                                | (12.0–14.0)        | (13.5)           | (14.0)            |                   |
| Neck length                    | 325                | 335 ± 7          | 340 ± 15          | 315 ± 10          |
|                                | (325–348)          | (304–368)        | (309–328)         |                   |
| Phar. expansion length         | 148                | 150.0 ± 2.8      | 149.0 ± 5.2       | 138.0 ± 4.7       |
|                                | (147.0–155.0)      | (138.0–161.0)    | (133.0–144.0)     |                   |
| Body diam. at neck base        | 30                 | 33.0 ± 1.9       | 29.0 ± 1.8        | 26.0 ± 0.0        |
| mid-body anus                  | (30.0–38.0)        | (27.0–31.0)      | (26.0)            |                   |
|                                | 32                 | 35.0 ± 1.6       | 29.0 ± 0.4        | 27.5 ± 0.3        |
|                                | (32.0–38.0)        | (28.0–30.0)      | (27.0–28.0)       |                   |
|                                | 20                 | 23.0 ± 2.6       | 19.5 ± 1.1        | 20.0 ± 0.6        |
|                                | (20.0–26.0)        | (18.0–21.0)      | (19.0–21.0)       |                   |
| Prerectum length               | 75                 | 71 ± 11          | 67.0 ± 3.1        | 83.0 ± 2.0        |
|                                | (57–90)            | (62.0–73.0)      | (82.0–85.0)       |                   |
| Rectum length                  | 18                 | 19.0 ± 1.8       | 20.0 ± 1.2        | 20.5 ± 0.4        |
|                                | (18.0–23.0)        | (19.0–22.0)      | (20.0–21.0)       |                   |
| Tail length                    | 21                 | 20.0 ± 1.8       | 19.0 ± 2.0        | 18.5 ± 0.2        |
|                                | (18.0–23.0)        | (17.5–23.0)      | (18.0–19.0)       |                   |

Note: All measurements are in μm (except L, in mm) and in the form: mean±SD (range).
Table 2. Nematode species, locality, associated host and sequences used in this study.

| Species                        | Locality                                           | Host-plant                   | Accession number |
|--------------------------------|----------------------------------------------------|------------------------------|------------------|
| *Enchodelus cf longispiculus*  | Gorgan province, Iran                              | –                            | KP190119         |
| *Enchodelus sp*                | Hamedan province, Iran                             | –                            | KP190120         |
| *Enchodelus sp*                | –                                                  | –                            | EF207240         |
| *Enchodelus macrodorus*        | –                                                  | –                            | AY593054         |
| *Enchodeloides signyensis*     | –                                                  | –                            | KY881719         |
| *Enchodorus dolichurus*        | –                                                  | –                            | KR184124         |
| *Enchodorus dolichurus*        | –                                                  | –                            | KR184125         |
| *Enchodorus yeatsi*            | Andimeshk, Khuzestan province, Iran                | Mosses in a natural region   | KX691911         |
| *Heterodorus youbertghostai*   | Sabalan mountains, Iran                            | Grasslands                   | KR184127         |
| *Heterodorus youbertghostai*   | Arasbaran forests, Kaleybar, East-Azarbayjan province, Iran | Grasses                    | KR184126         |
| *Heterodorus brevidentatus*    | Kerman, Iran                                       | –                            | KP963962         |
| *Longidorella penetrans*       | –                                                  | –                            | HM235515         |
| *Longidorella cf macramphis*   | –                                                  | –                            | AY593042         |
| *Paravulvus hartingii*         | –                                                  | –                            | AY593062         |
| *Pungentus silvestris*         | –                                                  | –                            | AY593052         |
| *Pungentus silvestris*         | –                                                  | –                            | AY593053         |
| *Pungentus engadinensis*       | –                                                  | –                            | AY593050         |
| *Pungentus engadinensis*       | Damghan, Semnan province, Iran                      | Fruit trees                  | MH346473         |
| *Pungentus engadinensis*       | Noshahr, Mazandaran province, Iran                 | Forest trees                 | MH346474         |
| *Pungentus monohystera*        | Germany                                            | Sediment                     | MF325343         |
| *Pungentus monohystera*        | Germany                                            | Sediment                     | MF325344         |
| *Pungentus azarbaijanensis*    | West-Azarbaijan province, Iran                     | Grasses                      | MH346476         |
| *Pungentus azarbaijanensis*    | West-Azarbaijan province, Iran                     | Grasses                      | MH346477         |
| *Pungentus sufiyanensis n. sp.*| Sufiyan, East-Azarbaijan province, Iran            | Black cherry trees           | MN855359         |
| *Rhyssocolpus vinciguerrae*    | Astara forests, north-western Iran                 | Forest trees                 | KP204547         |

Male: unknown.

Molecular characterization: one sequence of the D2-D3 segment of 28S rDNA nearly 800 bp long from the new species was obtained. The results of its analysis are represented in the molecular tree of Figure 3.

Diagnosis and relationships: the new species is characterized by its slender (a=40-50) and 1.22 to 1.57 mm long body, lip region offset by constriction and 7 to 9 μm broad, odontostyle 18 to 21 μm long, neck 304 to 348 μm long, pharynx expansion 133 to 161 μm long or 40 to 45% of total neck length, female genital system mono-opisthodelphic, without anterior uterine sac, vagina slightly directed backward, pars refringens vaginae absent, V=47-54 and caudal region rounded-conoid (17.5-23 μm, c=65-84, c’=0.7-1) with saccate bodies. Male absent.
Figure 3: Phylogenetic tree of the *Pungentus sufiyanensis* n. sp. using D2-D3 expansion segments of the 28S rDNA gene inferred from a Bayesian analysis under SYM + I + G model (−lnL = 3,311.6086; AIC = 6,637.2173; freqA = 0.2474; freqC = 0.2381; freqG = 0.2699; freqT = 0.2446; R(a) = 1.0335; R(b) = 5.3584; R(c) = 1.8784; R(d) = 0.7817; R(e) = 8.4264; R(f) = 1.0000). Posterior probability values exceeding 50% are given on appropriate clades. Newly obtained sequence is in bold letters.
The new species resembles *P. angulatus* Jairajpuri and Baqri, 1966 and *P. longidens* (Thorne and Swanger, 1936) Andrásy, 1986 in its monoposthodelphic female genital system, with the absence of prevulval sac and comparatively short odontostyle (less than 30 μm long) and caudal region (c-ratio more than 60). Nevertheless, it differs from *P. angulatus*, an Indian species also known to occur in Hungary (Andrásy, 2009), by having larger general size (1.22-1.57 vs 0.8-1 mm long, n=22), lip region offset by a weak (vs strong) constriction, longer odontostyle (18-21 vs 14-16 μm) and neck (304-348 vs 225 μm), and relatively shorter female tail (c’=0.7-1 vs 1.3) with (vs without) saccate bodies. It differs from *P. longidens*, a poorly known (but apparently close) species originally described from Spain, by its shorter odontostyle (18-21 vs 26 μm long, 2.0-2.5 times vs hardly more than thrice the lip region diameter), more posterior location of guiding ring (at appreciably more vs less than lip region diameter from the anterior end), and rounded conoid (vs short rounded to hemispheric) female tail with (vs without) saccate bodies.

*P. sufiyanensis* n. sp. is phylogenetically related to *P. azarbaijanensis* but can be differentiated by the female genital system (mono-posthodelphic vs didelphic-amphidelphic).

A Nblast search of the D2-D3 sequence of *P. sufiyanensis* n. sp. showed 96, 96, 99, 96, and 95% of similarity with *P. azarbaijanensis* (MH346476), *P. engadinensis* (AY593050), *P. monohystera* (MF325343), *P. silvestris* (AY593052), and *Enchodelus macrondonus* (AY593054), respectively, with 27, 26, 2, 26, and 26 different nucleotides, respectively, too. As derived from the analysis of the new sequence herein obtained, the evolutionary relationships of the new species with other representatives of the order Dorylaimida are shown in Figure 3. The most remarkable achievement is that the new species comes close to *P. azarbaijanensis*, another Iranian species. These both species form a clade together with other *Pungentus* species, suggesting a low supported monophyly of this genus based upon currently available sequences. All the sequences of Nordiidae representatives constitute a highly supported (100%) clade, a fact that confirms the monophyly of this taxon. Leaving aside *Pungentus* sequences, the remaining ones form together a second clade, which is not well supported, within the family Nordiidae.

**Type habitat and locality:** the habitat and locality type was Northwest Iran, East-Azerbaijan province, Sufiyan, Roodghat area, Zeinabad village (GPS coordinates: N 38°17’30”, E 46°07’53”, altitude 1527 m a.s.l.), where the specimens were collected from the rhizosphere of black cherry trees (*Prunus cerasus* L.).

**Other localities and habitats:** samples were collected from two locations in Northwest Iran: East-Azerbaijan province, Marand district, Kondolaj village, from the rhizosphere of almond and walnut trees; West Azerbaijan province, Bokan district, Khorasaneh area (GPS coordinates: N 36°35’68”, E 46°00’00’’90”) from the rhizosphere of natural vegetation.

**Type material:** female holotype and paratypes were deposited with the Nematode Collection of the Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran. The new species binomial has been registered in the Zoobank database (zoobank.org) under the identifier B1F2B3F6-558F-4688-BFFC-0F90BD101357.

**Etymology:** the species name refers to the type locality of the new species, Sufiyan, East-Azerbaijan province, northwest of Iran.

**Pungentus engadinensis** (Altherr, 1950) Altherr, 1952. (Fig. 4; Table 3)

**Remarks:** the six populations of this species herein examined are, morphologically and morphometrically, very similar to each other, but some minor differences have also been noted, which are regarded as intraspecific variations. Anterior uterine sac according to Andrásy (2009) and Peña-Santiago et al. (2013), varying from absent (as in our population) to present with different sizes. Thus, anterior uterine sac in Sufiyan population varied from absent to 8.5 μm long, but in all the remaining populations it was of different sizes. Saccate bodies were occasionally present (Peña-Santiago et al., 2013), and according to Heydari et al. (2019) saccate bodies were not present in their own Belgian populations and not seen in Sufiyan and Urmia populations but they were present in Divandarreh, Bokan, Maragheh, and Basmenj populations. *Pars refringens vaginae*, consisting of two small sclerotized pieces, were distinguishable in Divandarreh and Maragheh populations, but they were more inconspicuous in other populations. Vagina orientation also displays some differences: backwards directed in Bokan and Sufiyan populations and near perpendicular to body axis in other populations. Present Iranian populations of *P. engadinensis* fit very well with those previously studied by other authors (for comparative purposes, see Coomans and Geraert, 1962; Andrásy, 2009; Peña-Santiago et al., 2013; Álvarez-Ortega and Peña-Santiago, 2014; Heydari et al., 2019).

**Pungentus engadinensis** is a widely distributed species, having been recorded in Asia, Europe, and North America, where it mostly inhabits moist soils (Andrásy, 2009). In Iran, it has previously been reported (Kazemi, 2016) from the rhizosphere...
of vineyards in Uremia, West-Azarbaijan province; rangelands in Divandarreh, Kurdistan province; natural vegetation in Bokan, West-Azarbaijan province and Maragheh, East-Azarbaijan province; common wheat from Sufiyan, East-Azarbaijan province and Basmenj, East-Azarbaijan province, but in the form of taxonomic papers from three locations of the country reported by Heydari et al. (2019) and Solouki et al. (2010).

**Key to species of the genus Pungentus**
(Modified after Álvarez-Ortega and Peña-Santiago, 2014)

1. Female genital system didelphic-amphidelphic ..... 2
   Female genital system mono-opisthodelphic ..... 8

2. Odontostyle exceptionally long, 67 to 70 μm or more than three times lip region diam. ....... macrostylus
   Odontostyle distinctly shorter, up to 40 μm or about twice the lip region diam. .......................... 3

3. Odontostyle 18 to 28 μm long .......................... 4
   Odontostyle 30 μm long ............................... 6

4. Female tail rounded conoid and longer (44 μm, c = 35-38, c’ = 1.2).............................. parapungens
   Female tail rounded and shorter (<35 μm, c > 40, c’ up to 1.1).............................................. 5

5. Tail bearing abundant saccate bodies; male present .................................................. marietani
   Tail lacking saccate bodies; male absent ..........................
Table 3. Morphometric data for six Iranian populations of *Pungentus engadinensis*.

| Locality          | Urmia population | Divandarreh population | Bokan population | Maragheh population | Sufiyan population | Basmenj population |
|-------------------|------------------|-------------------------|------------------|---------------------|--------------------|--------------------|
|                   | Female           | Female                  | Female           | Female              | Female             | Female             |
| n                 | 5                | 6                       | 7                | 7                   | 5                  | 6                  |
| L                 | 0.90±0.06        | 1.10±0.01               | 0.99±0.07        | 1.00±0.05           | 0.95±0.03          | 0.99±0.03          |
|                   | (0.83–1.00)      | (0.91–1.22)             | (0.90–1.10)      | (0.90–1.10)         | (0.90–0.99)        | (0.96–1.06)        |
| a                 | 39.0±2.4         | 40.5±0.5                | 39.0±2.3         | 35.5±1.4            | 38.0±1.9           | 34.8±2.0           |
|                   | (36.5–42.0)      | (36.5–50.5)             | (34.5–41.5)      | (34.0–37.0)         | (36.0–41.0)        | (35.0–37.0)        |
| b                 | 3.7±0.2          | 4.0±0.6                 | 3.9±0.2          | 4.0±0.2             | 4.0±1.0            | 3.8±0.2            |
|                   | (3.5–4.5)        | (3.8–5.0)               | (3.5–4.0)        | (4.0–4.5)           | (3.9–4.2)          | (3.5–4.2)          |
| c                 | 47.0±4.6         | 60.5±5.5                | 52.0±0.5         | 60.5±6.4            | 54.0±3.4           | 58.6±7.7           |
|                   | (42.0–52.5)      | (55.0–68.0)             | (44.5–59.0)      | (52.0–72.0)         | (50.0–59.0)        | (46.0–68.0)        |
| c’                | 1.1±0.08         | 0.9±0.09                | 1.0±0.01         | 0.0±0.06            | 0.94±0.05          | 0.81±0.07          |
|                   | (1.0–1.2)        | (0.7–1.1)               | (0.9–1.1)        | (0.8–0.9)           | (0.9–1.0)          | (0.7–0.9)          |
| V                 | 48.0±2.5         | 45.5±0.8                | 46.0±1.4         | 46.0±1.3            | 47.0±1.6           | 44.3±3.2           |
|                   | (44.0–52.0)      | (44.5–47.0)              | (44.0–48.0)       | (44.0–47.0)         | (45.0–49.0)        | (41.0–49.0)        |
| Lip region diam.  | 8.3±0.5          | 10.6±0.2                | 10.0±0.6         | 10.0±0.5            | 8.6±0.4            | 8.8±0.4            |
|                   | (8.0–9.0)        | (10.0–11.0)              | (8.0–11.0)        | (9.0–11.0)          | (8.0–9.0)          | (8.0–9.0)          |
| Odontostyle length| 15.0±0.5         | 18.0±0.5                | 15.5±0.1         | 17.5±0.2            | 16.0±0.0           | 15.9±0.9           |
|                   | (14.5–16.0)      | (17.5–18.5)              | (14.0–17.0)       | (16.0–18.0)         | (16.0)             | (15–17.5)          |
| Odontophore length| 19.5±0.6         | 15.6±2.1                | 16.6±1.8         | 20.8±1.3            | 14.2±0.6           | 19.6±0.9           |
|                   | (19.0–20.5)      | (13.0–18.5)              | (14.1–19.3)       | (19.0–22.0)         | (13.0–15.0)        | (17.0–21.0)        |
| Guiding ring from ant. end | 10.3±0.5 | 11.2±0.4 | 10.6±0.8 | 10.3±0.4 | 10.2±0.4 | 11.3±0.4 |
|                   | (10.0–11.4)      | (11.0–12.0)              | (9.0–12.0)        | (10.0–11.0)         | (9.0–11.0)         | (9.6–12.0)          |
| Neck length       | 238±13           | 265±14                  | 259±21           | 302±13              | 236±11             | 254±10             |
|                   | (223–255)        | (233–295)               | (228–293)        | (228–329)           | (219–248)          | (243–266)          |
| Phar. expansion length | 91.2±8.3  | 104.2±3.1  | 89.6±9.7  | 108.2±4.4  | 96.0±9.1  | 101.0±5.2  |
|                   | (84.0–105.0)     | (102.0–108.0)            | (81.0–106.0)      | (101.0–116.0)       | (85.0–108.0)       | (95.0–109.0)        |
| Body diam. at neck base | 26.2±0.4  | 29.6±2.7  | 22.2±1.5  | 26.0±0.7  | 23.0±0.7  | 26.0±0.9  |
|                   | (25.0–27.0)     | (25.0–34.0)              | (21.0–24.1)       | (25.0–27.0)         | (22.0–24.0)        | (25.0–27.0)         |
| mid-body          | 27.4±0.3         | 32.2±2.0                | 23.3±0.8         | 28.3±0.4            | 24.8±1.0           | 28.2±1.3           |
|                   | (27.0–28.2)     | (28.0–37.0)              | (22.0–25.0)       | (27.0–29.1)         | (24.0–25.0)        | (27.0–30.0)         |
| anus              | 18.3±0.5         | 22.3±2.3                | 18.1±0.6         | 20.0±0.7            | 18.0±0.7           | 20.0±1.7           |
|                   | (17.0–19.0)     | (20.0–25.0)              | (17.0–19.0)       | (19.0–21.0)         | (17.0–19.0)        | (19.0–22.0)         |
| Prerectum length  | 72.6±13.2        | 85.1±12.4               | 55.2±7.7         | 82.2±2.2            | 40.0±6.8           | 64.3±5.1           |
|                   | (52.0–94.0)     | (72.0–101.0)             | (50.3–64.0)       | (77.0–88.0)         | (30.2–50.0)        | (59.0–69.0)         |
| Rectum length     | 17.6±0.2         | 20.3±1.5                | 14.3±0.5         | 18.1±1.2            | 16.0±2.4           | 22.0±2.2           |
|                   | (16.0–18.0)     | (18.0–22.0)              | (13.0–16.0)       | (16.0–20.0)         | (14.0–20.0)        | (19.0–25.0)         |
| Tail length       | 18.5±0.8         | 17.5±0.9                | 19.0±0.7         | 16.7±1.2            | 18.0±0.8           | 17.1±2.4           |
|                   | (17.5–22.5)     | (16.5–21.0)              | (18.0–20.0)       | (14.0–18.0)         | (17.0–19.0)        | (15.0–21.0)         |

Note: All measurements are in μm (except L, in mm) and in the form: mean ± SD (range).
6. Less slender body ($a = 30-33$); tail longer (40-44 $\mu$m, $c = 45-50$) .......................................................... crassus

More slender body ($a > 40$); tail shorter (up to 30 $\mu$m) .......................................................... 7

7. Less slender body ($a = 40-43$); ($c = 67$ and $c' = 0.8$); male present................................................. angulosus

More slender body ($a = 47-59$); ($c = 71-87$ and $c' = 1.0-1.1$); male absent ......................... azarbaijanensis

8. Tail conoid and longer ($c' = 1.3$) ...................... sparsus

Tail conoid and shorter ($c' \text{ very rarely exceeding } 1.0$) .......................................................... 9

9. Prevulval sac well developed, one body diam. long .......................................................... monohystera

Prevulval sac very short or absent .......................... 10

10. Odontostyle more than 30 $\mu$m long ......................

Odontostyle up to 30 $\mu$m long ...................... 12

11. Tail distinctly clavate .............................................. clavatus

Tail not clavate .............................................. silvestris

12. Female tail shorter ($c > 63$) .................................. 13

Female tail longer ($c < 63$) .................................. 15

13. Body 0.8 to 1.0 mm long; odontostyle 14 to 16 $\mu$m long .......................................................... angulatus

Body more than 1.0 mm long; odontostyle longer ($> 18 \mu$m) .......................................................... 14

14. Lip region angular; more slender body ($a = 56$); odontostyle 26 $\mu$m long; tail lacking saccate bodies .......................................................... longidens

Lip region rounded; less slender body ($a = 40-50$); odontostyle 18 to 21 $\mu$m long; tail bearing saccate bodies .......................................................... sufiyanensis sp. n.

15. Less slender body ($a = 26-30$) ...................... fuglensi

More slender body ($a > 30$) ............................. 16

16. Odontostyle 12 to 13 $\mu$m long ......................... minor

Odontostyle 14 to 17 $\mu$m long ......................... 17

17. Pharyngeal expansion occupying ca two-fifths of total neck length ........................................ engadinensis

Pharyngeal expansion occupying ca three-fifths of total neck length ........................................ fagi

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