

# On the identity of the genus *Epacrolaimus* Andrassy, 2000 (Nematoda, Dorylaimida), with new insights into its phylogeny

## Research Paper

**Cite this article:** Peña-Santiago R, Castillo P (2022). On the identity of the genus *Epacrolaimus* Andrassy, (Nematoda, Dorylaimida), with new insights into its phylogeny. *Journal of Helminthology* **96**, e72, 1–14. <https://doi.org/10.1017/S0022149X2200058X>

Received: 22 July 2022

Revised: 30 August 2022

Accepted: 30 August 2022

### Key Words:

Axial odontostyle; molecular analysis; morphology; mural tooth; *Sectonema*

### Author for correspondence:

R. Peña-Santiago, E-mail: [rpena@ujaen.es](mailto:rpena@ujaen.es)

R. Peña-Santiago<sup>1</sup>  and P. Castillo<sup>2</sup> 

<sup>1</sup>Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Jaén, Spain and <sup>2</sup>Instituto de Agricultura Sostenible (IAS), CSIC, Córdoba, Spain

### Abstract

The type species of the genus *Epacrolaimus*, *Epacrolaimus declinatoaculeatus*, is studied from the re-examination of type material of *Aporcelaimus vorax*, its junior synonym, and the observation of several Iberian populations and a few Iranian specimens. Morphologically, it displays a recognizable morphological pattern characterized by, among other features, the incurved nature of its odontostyle aperture, presence of perioral liplets or lobes, lip region 24–31 µm wide, odontostyle 21–25 µm long and comparatively anterior location of S<sub>2</sub>N pharyngeal gland nuclei. Nevertheless, variations in some morphological traits (vagina shape and tail shape) and in several morphometrics (body length, uterus length, vulva position, tail length and spicule length) are also noted. Sequences of D2–D3 domains of the 28S rDNA, 18S rDNA and COI mtDNA were obtained from several Iberian populations. Their analyses, in particular those from D2–D3 sequences, revealed the existence of a highly supported clade ((*Epacrolaimus* + *Sectonema*) + *Metaporcelaimus*), with a closer relationship between *Epacrolaimus* and Palearctic populations of *Sectonema*, whereas the remaining aporcelaimid genera occupied placements in other clades. These results are discussed, with especial emphasis on the intricate separation of *Epacrolaimus* and *Sectonema*, which display significantly different protruding stomatal structure in spite of their close evolutionary relationship as derived from molecular trees.

## Introduction

The genus *Epacrolaimus* was proposed by Andrassy (2000) to accommodate two species. One of them, *Epacrolaimus declinatoaculeatus* (Kreis, 1924), transferred from *Aporcelaimus* Thorne & Swanger, 1936, was designated as its type species whereas the second one, *Epacrolaimus imperator*, was described for the first time. Besides, Andrassy regarded *Aporcelaimus vorax* Thorne & Swanger, 1936, a well-known taxon, as the new junior synonym of the type species. Later, Pedram *et al.* (2012) added a third species, *Epacrolaimus reyesi*, from Iran.

Being a member of the family Aporcelaimidae Heyns, 1965, *Epacrolaimus* was regarded as very close to *Aporcelaimus*, differing from this by its more offset lip region (strongly vs. hardly differentiated or almost amalgamate, respectively), conspicuous (vs. absent) inner perioral liplets, strongly wrinkled (vs. rather smooth) cuticle at vulval lips and the position of pharyngeal gland nuclei. The identity of the genus has not been a matter of further discussion or analysis, and its evolutionary relationships remain unexplored.

The development of molecular methods using different fragments of nuclear ribosomal and mitochondrial gene sequences to be used in DNA barcoding during the last years has led to improved species diagnosis and delimitation, and to clarify some aspects of the phylogeny of dorylaimid (order Dorylaimida) taxa (Mullin *et al.*, 2005; Pedram, 2017; Álvarez-Ortega *et al.*, 2018; Álvarez-Ortega & Peña-Santiago, 2019; Varela-Benavides & Peña-Santiago, 2019; Cai *et al.*, 2020; Heydari *et al.*, 2020).

This contribution aims to elucidate the phylogeny of the genus *Epacrolaimus* by means of an integrative approach combining morphological and molecular data, and to update the taxonomy of the genus.

## Material and methods

### Nematodes

Type material of *A. vorax*, consisting of nine females of two locations, belonging to Thorne's collection and deposited in the United States Department of Agriculture Nematode Collection (Beltsville, MD, USA), were available by courtesy of Dr Z. Handoo. Twenty-eight females and two males of *E. declinatoaculeatus*, collected in several locations of the southern Iberian

© The Author(s), 2022. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.



Peninsula, including the specimens described by Martínez-Olías *et al.* (2005), deposited with nematode collection of Nematology laboratory at the University of Jaén, Spain, were studied for comparative purposes. Five Iranian females of *E. declinatoaculeatus* were loaned by Dr G. Niknam (University of Tabriz, Iran).

### Morphological and morphometrical study

Specimens preserved in anhydrous glycerine and mounted on either glass or Cobb's permanent slides were observed, measured and photographed using an Eclipse 80i microscope (Nikon, Tokyo, Japan) equipped with differential interference contrast optics, a drawing tube (camera lucida) and a DS digital camera. Morphological study was mainly focused on the most relevant traits, those referred to cuticle, lip region, odontostyle, pharynx, female genital system, male genital system and its accessory elements, and caudal region of both sexes. Morphometrics included Demanian indices and other measurements and ratios, some of them presented in separate tables, and others form part of the literal description of species.

### Molecular characterization

For molecular analyses, and in order to avoid mistakes in case of mixed populations in the sample, single specimens were temporarily mounted in a drop of 1 M sodium chloride containing glass beads to ensure that specimens conformed with the target population. This was followed by DNA extraction from single individuals as described by Archidona-Yuste *et al.* (2016). The D2–D3 domains were amplified using the D2A (5'-ACAAGTACCCTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAAACAGCTACTA-3') primers (De Ley *et al.*, 1999). The internal transcribed spacer (ITS) region was amplified using forward primer 18S (5'-TTGATTACGTCCCTGCCCTTT-3') and reverse primer 26S (5'-TTTCACTCGCCGTTACTAAGG-3') (Vrain *et al.*, 1992). The portion of 18S rRNA was amplified using primers 988F (5'-CTCAAAGATTAAGCCATGC-3'), 1912R (5'-TTTACGGTCAGAACTAGGG-3'), 1813F (5'-CTGCGTGA GAGGTGAAAT-3') and 2646R (5'-GCTACCTTGTTACGACTTTT-3') (Holterman *et al.*, 2006). Finally, the portion of the cytochrome c oxidase I (COI) gene was amplified as described by Lazarova *et al.* (2006) using the primers COIF (5'-GATTTTGGKCATCCWGARG-3') and COIR (5'-CWACATAA TAAGTATCATG-3').

All polymerase chain reaction (PCR) assays were done according to the conditions described by Archidona-Yuste *et al.* (2016). The amplified PCR products were purified using ExoSAP-IT (Affimetrix, USB products) and used for direct sequencing on a DNA multicapillary sequencer (Model 3130XL genetic analyser; Applied Biosystems, Foster City, CA, USA), using the BigDye Terminator Sequencing Kit V.3.1 (Applied Biosystems, Foster City, CA, USA), at the StabVida sequencing facilities (Caparica, Portugal). The newly obtained sequences were submitted to the GenBank database under the accession numbers indicated on the phylogenetic trees.

### Phylogenetic analyses

The D2–D3 domains of the 28S rDNA, 18S rDNA, and COI mtDNA sequences of the recently recovered *Epacrolaimus* populations were obtained in this study. These sequences, together with other sequences belonging to species of the family

Aporcelaimidae from GenBank, were used for phylogenetic analyses. Outgroup taxa for each dataset were chosen following previously published studies (Álvarez-Ortega & Peña-Santiago, 2019; Álvarez-Ortega *et al.*, 2021). Multiple sequence alignments of the different genes were made using the FFT-NS-2 algorithm of MAFFT V.7.450 (Katoh *et al.*, 2019). Sequence alignments were manually visualized using BioEdit (Hall, 1999) and edited by Gblocks ver. 0.91b (Castresana, 2000) using options for a less stringent selection (minimum number of sequences for a conserved or a flanking position: 50% of the number of sequences +1; maximum number of contiguous non-conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half). Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The best-fit model of DNA evolution was obtained using JModelTest V.2.1.7 (Darriba *et al.*, 2012) with the Akaike information criterion (AIC). The best-fit model, the base frequency, the proportion of invariable sites, the gamma distribution shape parameters and substitution rates in the AIC were then used in MrBayes for the phylogenetic analyses. BI analyses were performed under a general time-reversible model and a gamma-shaped distribution (GTR + G) for the D2–D3 domains of the 28S rDNA and invariable sites and a gamma-shaped distribution (GTR + I + G) under a general time-reversible model with 18S rDNA, and a transition model with invariable sites and a gamma-shaped distribution (TIM1 + I + G) for the partial COI gene. All Bayesian analyses were run separately per dataset with four chains for  $4 \times 10^6$  generations. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples of 30% and evaluating convergence, the remaining samples were retained for in-depth analyses. The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) were given on appropriate clades. Trees from all analyses were visualized using FigTree software version v.1.42 (Rambaut, 2014). A combined analysis of the three ribosomal genes was not undertaken due to some sequences not being available for all species.

## Results

### Morphological study of type material of *A. vorax*

#### New York population (online supplementary fig. S1, morphometrics in table 1)

Very slender ( $a = 54\text{--}57$ ) and very large-sized nematodes, body 6.20–6.63 mm long. Body cylindrical, visibly tapering towards the anterior end, less so towards the posterior end as the tail is short and rounded. Upon fixation, habitus regularly curved ventrad, C- or J-shaped. Cuticle three-layered, more appreciable at caudal region, 6–7  $\mu\text{m}$  thick at the anterior region, 7.5–14.0  $\mu\text{m}$  in midbody, and 16–19  $\mu\text{m}$  on tail, consisting of two thinner outer and inner layers and a much thicker intermediate layer with perceptible radial striation. Lateral chord 16.5–25.0  $\mu\text{m}$  broad, occupying up to one-fifth (14–20%) of midbody diameter. Lip region offset by deep constriction, 3.1–3.3 times as broad as high and about one-quarter (24–28%) of body diameter at neck base, lips separate, rounded, with low papillae, but bearing a projecting, perioral lobe (not offset liplet) each. Amphid fovea funnel-like or cup-like, apparently duplex, its aperture 12  $\mu\text{m}$  long or slightly less than one-half of the lip region diameter. Cheilostome 8.5–10.5  $\mu\text{m}$  long, as long as wide, with thick walls. Odontostyle 3.7–4.2 times as long as wide, shorter (0.7–0.8 times) than lip

**Table 1.** Morphometrics of *Epacrolaimus declinatooculeatus* (Kreis, 1924) Andrassy, 2000.

Locality	Babylon	Salem	Cazorla	Cazorla	Torres	Valdepeñas	Alcaudete	?	Capileira	?	?
State/Province	New York	Utah	Jaén	Jaén	Jaén	Jaén	Jaén	Málaga	Granada	?	?
Country	United States	United States	Spain	Spain	Spain	Spain	Spain	Spain	Spain	Spain	Iran
<i>n</i>	4♀♀	5♀♀	19♀♀ <sup>a</sup>	♂	♂	♀	♀	3♀♀	2♀♀	2♀♀	5♀♀ <sup>a</sup>
Character											
L	6.46 ± 0.16(6.20–6.63)	7.74 ± 0.87(6.87–8.81)	5.78–7–72	6.35	6.79	7.87	7.04	6.34–6.90	6.62, 6.18	6.51, 6.90	5.83–7.50
a	55.5 ± 1.6(54–57)	58.2 ± 6.3(52–66)	42–69 <sup>b</sup>	50	61	51	42 <sup>b</sup>	47–57	48, 41	49, 54	42–55
b	5.1 ± 0.2(5.0–5.4)	6.0 ± 0.5(5.3–6.6)	4.5–6.4	5.6	6.1	6.4	?	5.5–6–0	6.0, 5.9	5.9, 5.6	4.8–5.7
c	113 ± 4.2(107–118)	119 ± 10(104–130)	100–164	102	154	146	117	119–131	107, 119	105, 108	84–156
c'	0.8 ± 0.0(0.8)	0.9 ± 0.1(0.8–0.9)	0.6–0.8	0.8	0.6	0.7	0.8	0.7–0.8	0.8, 0.6	0.9, 0.9	0.6–1.0
V	53.6 ± 1.4(52–55)	53.2 ± 2.4(50–56)	50–55	–	–	57	52	48–49	44, 50	48, 51	49–54
lip region diameter	27.0 ± 0.7(26–28)	27 ± 2.1(24–30)	24–29	28	28	30	28	28–29	27, 25	28, 28	27–31
odontostyle length	21.9 ± 0.5(21–22.5)	23.3 ± 1.0(22.5–25)	22–25	23	24	23	22	23–24	21, 22	23, 24	23–24
odontophore length	43.8 ± 2.8(39–46)	45.8 ± 3.0(41–49)	42–48	46	?	53	49	45–50	47, 48	49, ?	44–52
neck length	1266 ± 48(1198–1330)	1289 ± 70(1171–1350)	1008–1376	1141	1120	1233	?	1143–1150	1105, 1053	1106, 1238	1226–1372
pharyngeal expansion length	888 ± 49(812–948)	905 ± 53(817–956)	771–1006	765	?	847	?	741–838	748, 697	759, 849	811–941
body diameter at neck base	102 ± 5.8(98–112)	113 ± 23(86–141)	78–140 <sup>b</sup>	101	109	124	140 <sup>b</sup>	105–110	122, ?	102, 118	105–141
midbody	116 ± 3.8(113–123)	135 ± 24(109–164)	88–163 <sup>b</sup>	163	111	153	166 <sup>b</sup>	120–135	139, 151	133, 127	120–154
anus/cloaca	71 ± 2.9(69–76)	76.8 ± 7.8(68–85)	61–91	78	69	74	76	71–76	73, 86	68, 70	79–83
distance vulva–anterior end	3465 ± 167(3212–3673)	4110 ± 404(3783–4682)	3084–3994	–	–	4475	3645	3118–3388	2891, 3060	3130, 3487	3153–3802
prerectum length	239 ± 15(214–250)	258 ± 41(229–287)	183–377	318	?	371	349	213–317	209, ?	250, 251	243–306
rectum/cloaca length	75.0 ± 1.9(72–77)	72.8 ± 3.1(68–76)	61–91	117	?	73	88	70–87	74, 0	70–77	71–86
tail length	57.0 ± 2.9(55–62)	65.2 ± 2.8(62–69)	44–63	62	44	54	60	50–58	62, 52	62, 64	46–77
spicules length	–	–	–	148	175	–	–	–	–	–	–
ventromedian supplements	–	–	–	6	10	–	–	–	–	–	–

Material examined in the present study. Measurements in  $\mu\text{m}$  except L in mm, and in the form average  $\pm$  standard deviation (range).

<sup>a</sup>Specimens collected from two or more locations.

<sup>b</sup>Including specimens visibly flattened.

region diameter, and 0.31–0.34% of body length, its dorsal side hardly 3.5–4.5  $\mu\text{m}$  long, with aperture 17.5–18.5  $\mu\text{m}$  long, occupying 83–86% of the odontostyle length, and showing a very peculiar profile as it is incurved, forming a characteristic straight angle. Odontophore rod-like, 1.9–2.2 times longer than the odontostyle, with thin walls and unusually wide lumen. Pharynx entirely muscular, gradually enlarging into the basal expansion that is 14.6–16.7 times longer than wide, 8.3–9.1 times longer than body diameter at neck base, and occupies more than two-thirds (68–71%) of the total neck length; gland nuclei obscure. A small but conspicuous mucro is observed in ventral position inside the pharynx, located at 103–111  $\mu\text{m}$  from the anterior end. Nerve ring situated at 260–279  $\mu\text{m}$  or 20–23% of the total neck length from the anterior end. Pharyngo-intestinal junction consisting of conoid to cylindrical, 26–31  $\times$  17–18  $\mu\text{m}$  cardia and a complex ring-like structure encircling its junction to pharyngeal base. Genital system divoarian, with moderately and equally developed genital branches, the anterior one 561–638  $\mu\text{m}$  or 9–10% of the total body length, the posterior one 564–636  $\mu\text{m}$  or 9–10% of the total body length: ovaries comparatively small, 168–345  $\mu\text{m}$  long, often not reaching the sphincter, with oocytes in two or more rows at its germinative zone, then in a single row; oviduct 252–288  $\mu\text{m}$  or 2.1–2.5 body diameters long, consisting of a long and slender distal section made of prismatic cells and moderately developed *pars dilatata* without visible lumen; a distinct sphincter separates oviduct and uterus; uterus a simple, tube-like structure, 243–296  $\mu\text{m}$  or 2.1–2.6 body diameters long; uterine eggs ovoid, 198–229  $\mu\text{m}$  long or 1.2–1.4 times the body diameter; vagina large, 85–89  $\mu\text{m}$  long, extending inwards to about three-quarters (72–76%) of body diameter, *pars proximalis* 61–66  $\times$  43–67  $\mu\text{m}$ , with straight or somewhat sigmoid walls and encircled by rather weak musculature, *pars refrigens* consisting of two trapezoidal, 12.5–17  $\times$  12.5–14  $\mu\text{m}$  sclerotized pieces with a combined width of 33–37  $\mu\text{m}$ , *pars distalis* 8.5–11  $\mu\text{m}$  long; vulva a transverse slit, with prominent lips whose cuticle often appears visibly irregular and somewhat rough. Prerectum 3.1–3.6, rectum 1.0–1.1 anal body diameters long. Caudal region short and rounded, ventrally nearly straight, dorsally more convex, with two pairs of caudal pores, one suborsal, another sublateral, both at the middle of tail.

#### *Utah population (online supplementary fig. S2, morphometrics in table 1)*

Very similar to the New York population, almost identical in its morphological features, the coincidences in lip region, odontostyle, pharynx, genital system and tail being particularly important. Nonetheless, some morphometric differences are also observed, certainly due to the low number of specimens studied, and herein regarded as geographical variations, including: larger general size (body 6.87–8.81 vs. 6.20–6.63 mm long in New York females); and somewhat longer odontostyle (22.5–25 vs. 21–22.5  $\mu\text{m}$ ). Interestingly, both populations share several minor but relevant features such as the existence of ‘duplex’ amphids (see remarks), odontophore with wide lumen and the presence of mucros at the anterior region of pharynx.

#### **Iberian material of *E. declinatoaculeatus***

##### *Short morphological description for comparative purposes (figs 1–3, morphometrics in table 1)*

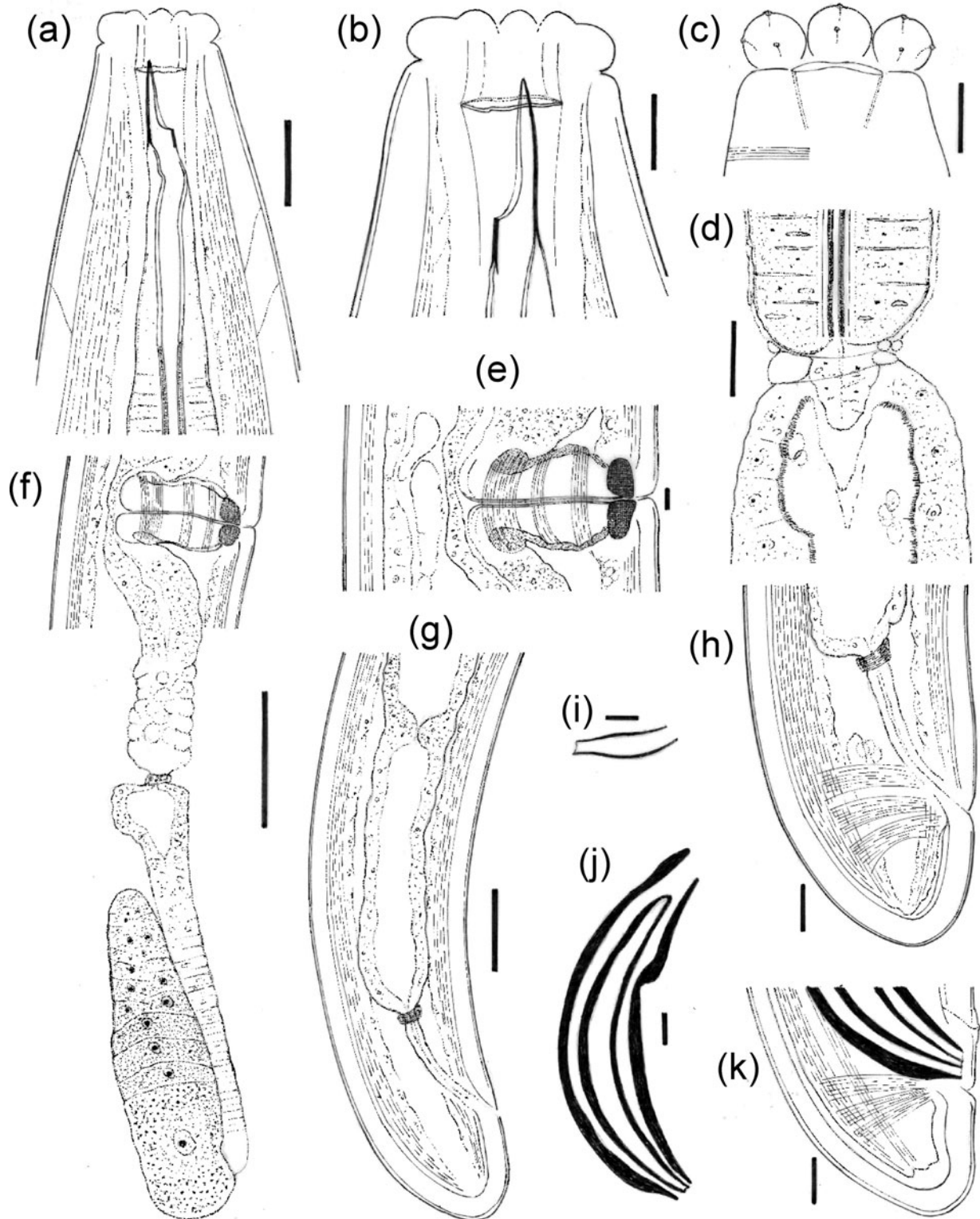
**Female:** Cuticle three-layered, 4.5–8.0  $\mu\text{m}$  thick at anterior region, 7–14  $\mu\text{m}$  at mid-body, and 11–17  $\mu\text{m}$  on tail. Lip region offset by

very deep constriction, 3.0–3.9 times as wide as high, with separate lips and distinct perioral lobes. Amphid fovea funnel-like, its aperture 11.5–14.5  $\mu\text{m}$  or up to one-half (41–54%) of lip region diameter. Cheilostome 9–15  $\mu\text{m}$  long. Odontostyle less than five (3.8–4.9) times as long as wide, hardly shorter (0.8–0.9 times) than lip region diameter, its aperture 18–20  $\mu\text{m}$  or 82–86% of its total length. Pharyngeal basal expansion occupying 65–73% of the total neck length, its gland nuclei obscure in general. Cardia 38–50  $\times$  23–32  $\mu\text{m}$ . Genital branches occupying 7–13% of the total body length: ovaries very variable (101–518  $\mu\text{m}$ ) in length, oviduct 217–305  $\mu\text{m}$  or 1.7–2.4 body diameters long, uterus 155–216  $\mu\text{m}$  or 1.1–1.7 times the body diameter long, uterine egg 187–222  $\times$  85–106  $\mu\text{m}$ . Vagina extending inwards 61–100  $\mu\text{m}$  to up to three-quarters (57–75%) of body diameter, with *pars refringens* having a combined width of 29–40  $\mu\text{m}$ . Vulva a transverse slit. The cuticle surrounding the vulva occasionally bearing some kind of irregularities (weak wrinkles or striation) mainly affecting its inner layer. Prerectum 2.4–5.1, rectum 0.8–1.3 anal body diameters long. Caudal region short and rounded.

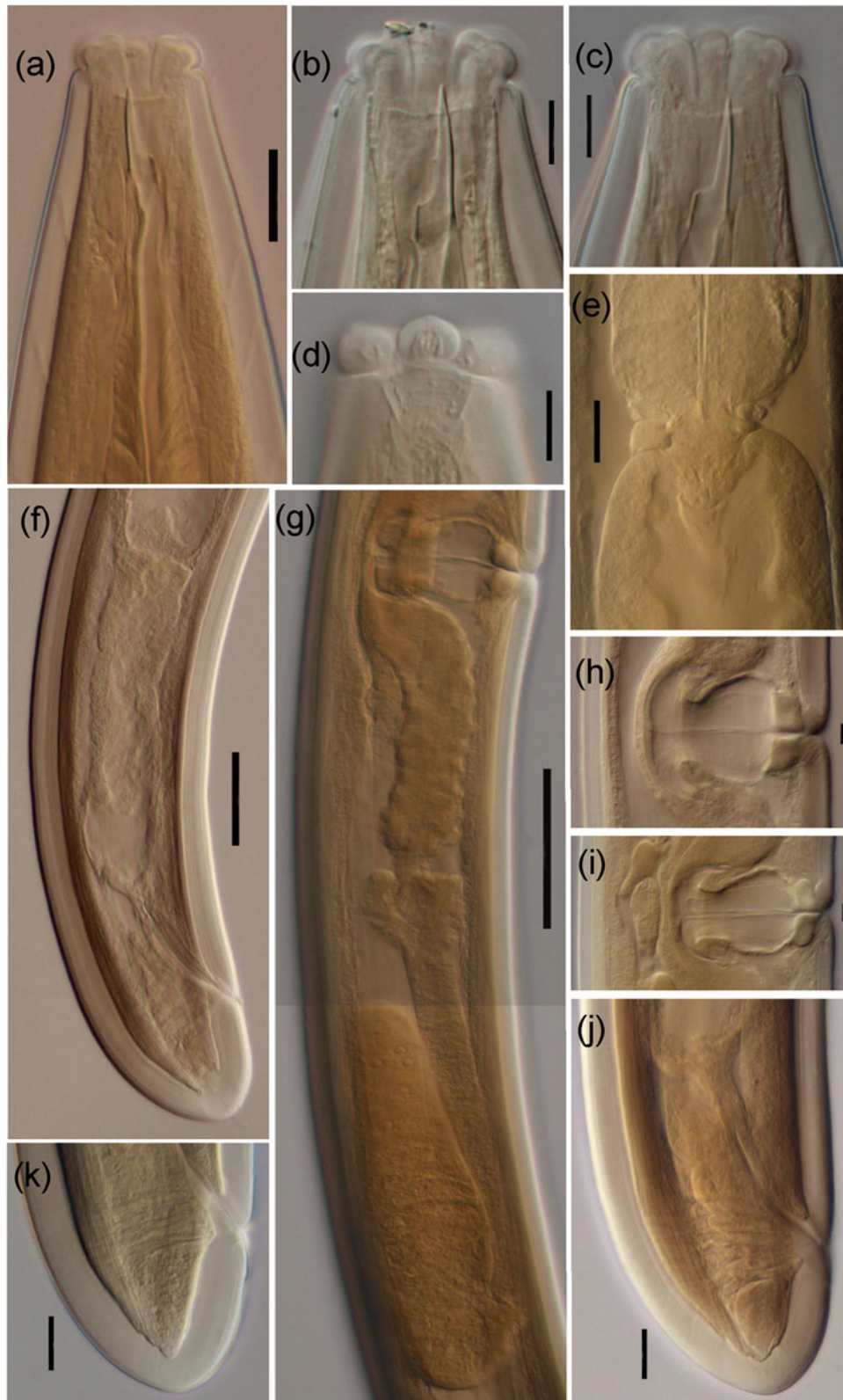
**Male:** General morphology similar to that of female. Prerectum 4.1, cloaca 1.5 body diameters long. Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, located at 19, 29  $\mu\text{m}$  from the cloacal aperture, there is a series of 6, 8, moderately spaced (19–30  $\mu\text{m}$  apart) ventromedian supplements, the most posterior of which is situated at 130, 136  $\mu\text{m}$  from the ad-cloacal pair, therefore with an appreciable hiatus. Spicules dorylaimid, 4.1, 4.7 times as long as wide and 1.9, 2.5 times longer than body diameter at level of the cloacal aperture: head very short, 11, 13% of total length, and hardly longer at its dorsal side, median piece occupying one-third to one-half of the maximum spicule wide, reaching the posterior tip, ventral hump easily perceptible, situated at 43, 51  $\mu\text{m}$  or 29% of the total length from the anterior end, posterior end of spicule 14, 15  $\mu\text{m}$  wide, curvature 128, 130°. Lateral guiding pieces 4.7, 8.0 times as long as wide, slightly tapering at its posterior end. Caudal region short and rounded to conoid.

#### **Molecular characterization**

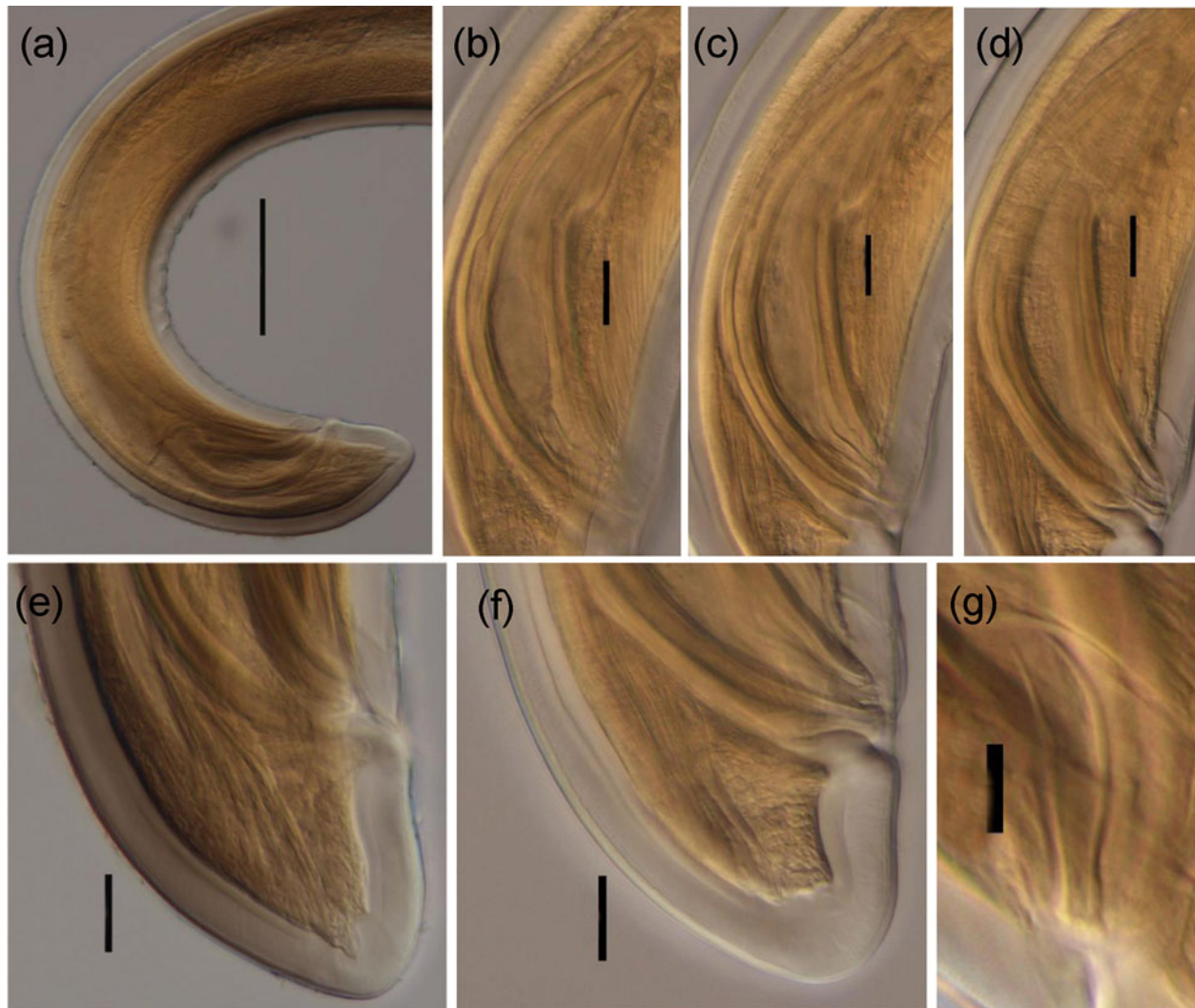
Five D2–D3 of the 28S (ON814779–ON814783), four ITS (ON815469–ON815472), five 18S (ON764419–ON764423) rDNA and four COI gene sequences (ON764415–ON764418) were generated for Iberian specimens of *E. declinatoaculeatus*. Overall intraspecific variation was 1–2 nucleotides and 0–1 indel for D2–D3, 5–7 nucleotides and 0 indel for ITS, and no variation for 18S and COI. D2–D3 domains of the 28S rDNA of the Iberian *E. declinatoaculeatus* populations (ON814779–ON814783) were 99.7–99.9% identical (differing from 1–6 nucleotides and 1–6 indels) with sequences of *E. declinatoaculeatus* (MH727507–MH727508) from Iran, and 95.1% similar to *Sectonema barbatoides* (AY593031). The ITS region sequences (ON815469–ON815472) showed a very low similarity and coverage with other Dorylaimida species, consequently no phylogenetic analysis can be performed. 18S rDNA of the Iberian *E. declinatoaculeatus* populations (ON764419–ON764423) was 99.8–99.9% similar (differing from 1–6 nucleotides and 0 indels) with sequences of *Sectonema* sp. JH-2004 (AY284815) and *S. barbatoides* (AY284814). For COI gene sequences (ON764415–ON764418), the similarity values were 79.7 and 79.1% (differing from 71 to 81 nucleotides and 2 indels) with *Xiphinema humaniense* Wang & Wu, 1992 (ON107534) and *Longidorus pini* Andrés & Arias, 1988 (MH454070), respectively.



**Fig. 1.** *Epacrolaimus declinatoaculeatus* (Kreis, 1924) Andrassy, 2000 (Iberian material, drawings): (a, b) anterior region in lateral, median view; (c) same in surface view; (d) pharyngo-intestinal junction; (e) vagina; (f) female, posterior genital branch; (g) female, posterior body region; (h) female, caudal region; (i) lateral guiding piece; (j) spicule; and (k) male, caudal region. Scale bars: a, d, h, k = 20  $\mu$ m; b, c, i, j = 10  $\mu$ m; e = 5  $\mu$ m; f = 100  $\mu$ m; g = 50  $\mu$ m.



**Fig. 2.** Light micrographs of *Epacrolaimus declinatoaculeatus* (Kreis, 1924) Andrásy, 2000 (Iberian material, female): (a–c) anterior region in lateral, median view; (d) same in surface view; (e) pharyngo-intestinal junction; (f) posterior body region; (g) posterior genital branch; (h, i) vagina; and (j, k) caudal region. Scale bars: a, e, j, k = 20  $\mu$ m; b–d = 10  $\mu$ m; f = 50  $\mu$ m; g = 100  $\mu$ m; h, i = 50  $\mu$ m.



**Fig. 3.** Light micrographs of *Epacrolaimus declinatoaculeatus* (Kreis, 1924; Andr ssy, 2000 (Iberian material, male): (a) posterior body region; (b–d) spicule; (e, f) caudal region; and (g) lateral guiding piece. Scale bars: a = 100  $\mu$ m; b–d, g = 10  $\mu$ m; e, f = 20  $\mu$ m.

The D2–D3 domains of the 28S rDNA alignment (738 base pairs (bp) long) included 47 sequences of Aporcelaimidae and other Dorylaimida species and two outgroup species (*Anatonchus tridentatus* (MG994941) and *Mononchus truncatus* (AY593064)). The Bayesian 50% majority rule consensus tree inferred from the D2–D3 alignment is given in fig. 4. In this tree the Iberian populations of *E. declinatoaculeatus* (ON814779–ON814783) clustered together with sequences from Iran (MH727507–MH727508) in a well-supported clade (PP = 1.00). This clade clustered together with several *Sectonema* species in a robustly supported clade (PP = 1.00). In addition, the unidentified *Sectonema* sp. (JH-2004) was 99.2–99.3% similar (differing from 8–9 nucleotides and 1–2 indels) from *S. barbatooides* (AY593030–AY593032), and clustered together with this species in a well-supported clade (PP = 1.00), and should be considered as conspecific.

The 18S rDNA gene alignment (1640 bp long) included 29 sequences of Aporcelaimidae and other Dorylaimida species and three outgroup species viz. *Aquatides aquaticus* (KJ636342), *Paravulvulus hartingii* (AY284775) and *Anatonchus tridentatus* (AY284768). The Bayesian 50% majority rule consensus tree inferred from the 18S alignment is given in fig. 5. In this tree the

Iberian populations of *E. declinatoaculeatus* (ON764419–ON764423) clustered together with sequences from *S. barbatooides* in a well supported clade (PP = 1.00).

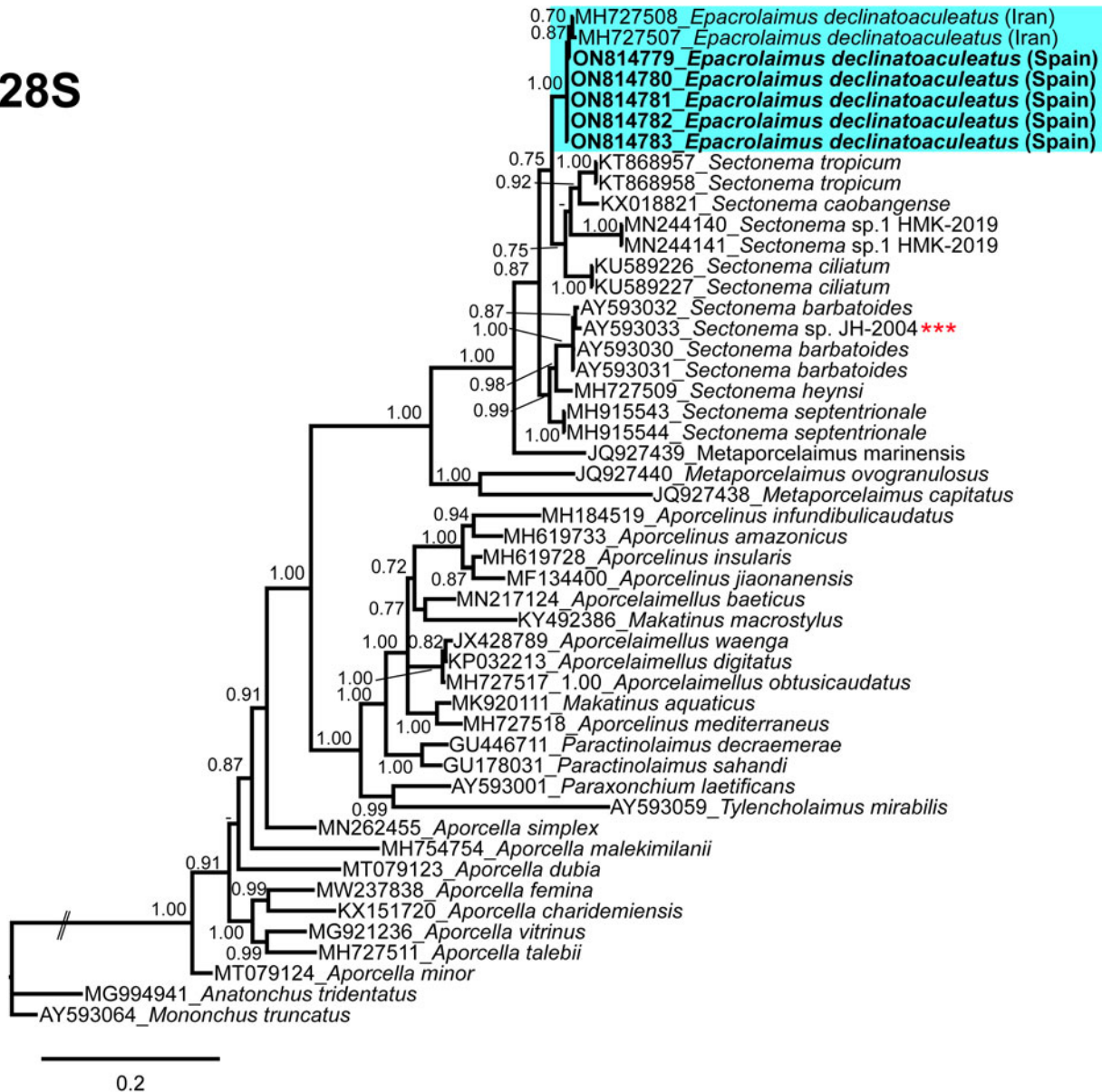
Finally, since no COI sequences for Aporcelaimidae were available in the United States National Center for Biotechnology Information (NCBI), other Dorylaimida species were selected for phylogenetic analysis with this marker, but phylogeny was not well resolved (online supplementary fig. S3), and no conclusion can be obtained until new COI sequences on this group can be provided.

#### Brief discussion

Iberian material of *E. declinatoaculeatus* comprised specimens from several locations of the southern Iberian Peninsula. They were collected in natural soils of a few mountain systems throughout the last three decades, and their state of preservation is variable, but often good or acceptable. The finding of two males is especially interesting for comparative purposes.

The general morphology and the morphometrics of Iberian specimens fit those of type population, displaying total

28S



**Fig. 4.** Phylogenetic relationships of *Epacrolaimus declinatoaculeatus* (Kreis, 1924) Andrassy, 2000 with species of Aporcelaimidae and other Dorylaimida. Bayesian 50% majority rule consensus tree as inferred from D2 and D3 expansion domains of 28S rRNA sequence alignment under the GTR + G model ( $-\ln L = 8257.7420$ ; AIC = 16725.483920;  $\text{freqA} = 0.248$ ;  $\text{freqC} = 0.216$ ;  $\text{freqG} = 0.290$ ;  $\text{freqT} = 0.2451$ ;  $R(a) = 0.7607$ ;  $R(b) = 2.9321$ ;  $R(c) = 1.7427$ ;  $R(d) = 0.3983$ ;  $R(e) = 6.3661$ ;  $R(f) = 1.0000$ ;  $\text{Pinva} = 0.000$ ; and  $\text{Shape} = 0.5850$ ). Posterior probabilities more than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in boldface type, and coloured box indicates clade association of the studied species. Scale bar = expected changes per site. \*\*\* = originally identified as *Sectonema* sp. JH-2004, according to these results needs to be identified as *Sectonema barbatoides* Heyns, 1965.

coincidence or a wide overlapping in most features. Especially relevant is the agreement between both series of females in key traits such as lip region shape and width, cheilostom, incurved aperture of odontostyle, lengths of odontostyle and odontophore, morphology of female genital tract, uterine egg, etc. Interestingly, however, Iberian nematodes have somewhat shorter uterus than American ones (155–216  $\mu\text{m}$  or 1.1–1.7 times the body diameter vs. 243–296  $\mu\text{m}$  or 2.1–2.6 body diameters long, respectively), vulva position with a much wider range ( $V = 44\text{--}57$  vs.  $V = 50\text{--}56$ ), appreciably longer spicules (148, 175 vs. 122  $\mu\text{m}$ , calculated from Thorne's original illustration), and less ventromedian supplements (6, 10 vs. 11). These differences are provisionally interpreted as intraspecific, geographical variations due to the low

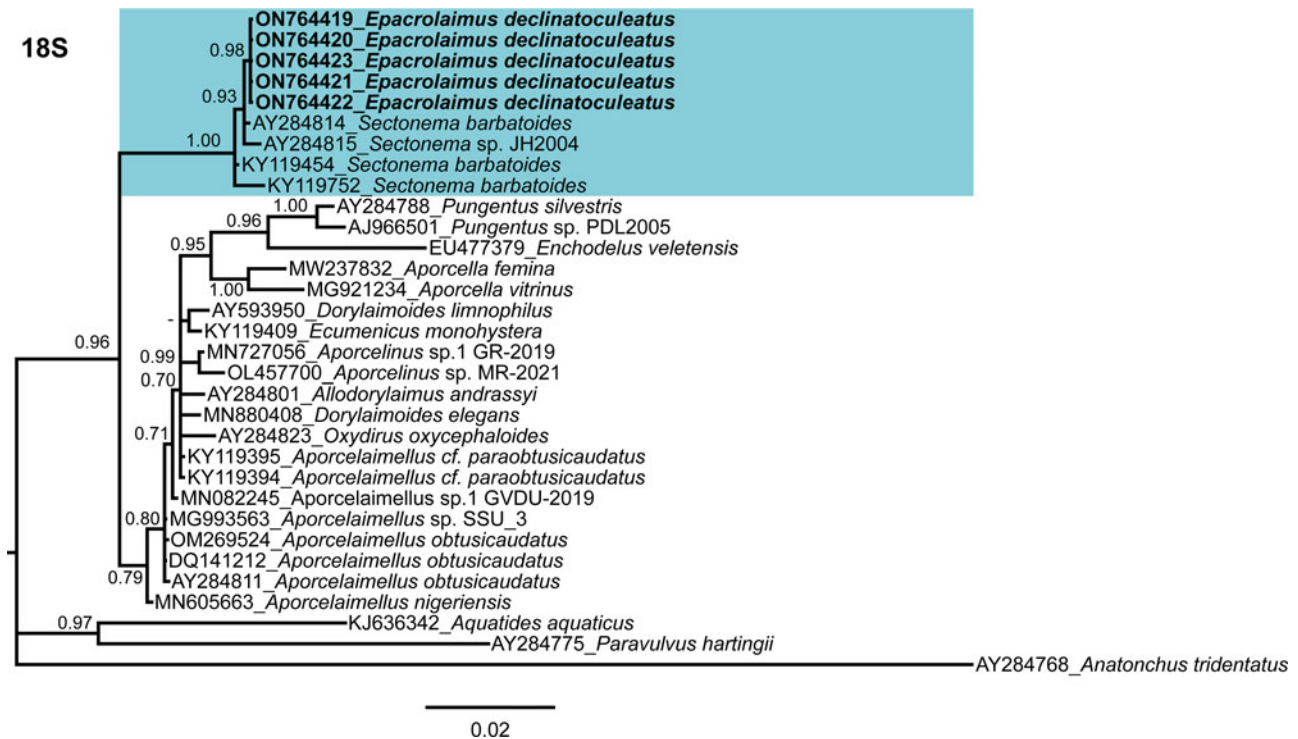
number of American females studied and the poor available information about the only American male described by Thorne (1937, 1939). Nevertheless, if these differences were confirmed in the future by means of molecular analyses, the existence of two separate forms, one American and another European, might be realistic and solidly supported.

#### Iranian material of *E. declinatoaculeatus*

##### Short morphological description for comparative purposes (fig. 6, table 1)

Cuticle three-layered, 7.0–9.5  $\mu\text{m}$  thick at anterior region, 7–15  $\mu\text{m}$  at mid-body, and 14–20  $\mu\text{m}$  on tail. Lip region offset by





**Fig. 5.** Phylogenetic relationships of *Epacrolaimus declinatoculeatus* (Kreis, 1924) Andrassy, 2000 with species of Dorylaimida. Bayesian 50% majority rule consensus tree as inferred from 18S rRNA gene sequence alignment GTR + I+ G model ( $-\ln L = 5296.16057$ ; AIC = 10744.321140; freqA = 0.2764; freqC = 0.2048; freqG = 0.2576; freqT = 0.2612; R(a) = 1.2859; R(b) = 2.8763; R(c) = 1.4423; R(d) = 0.2586; R(e) = 5.2463; R(f) = 1.0000; Pinva = 0.4940; and Shape = 0.6560). Posterior probabilities more than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in boldface type, and coloured box indicates clade association of the studied species. Scale bar = expected changes per site.

very deep constriction, 2.7–3.6 times as wide as high, with separate lips and distinct perioral lobes. Amphid fovea funnel-like, its aperture 7.0–9.5  $\mu\text{m}$  or 43–52% of lip region diameter. Cheilostom 10.0–13.5  $\mu\text{m}$  long. Odontostyle 4.1–4.5 times as long as wide, shorter (0.7–0.8 times) than lip region diameter, its aperture 18.0–18.5  $\mu\text{m}$  or 81–84% of its total length. Pharyngeal basal expansion occupying 67–69% of the total neck length, its gland nuclei located as follows: DN = 44–48; S<sub>1</sub>N<sub>1</sub> = 65–69; S<sub>1</sub>N<sub>2</sub> = 76–77; and S<sub>2</sub>N = 86–87. Cardia 28–40  $\times$  20–32  $\mu\text{m}$ . Genital branches occupying 6–9% of the total body length: ovaries 156–310  $\mu\text{m}$  long, oviduct 187–299  $\mu\text{m}$  or 1.3–2.1 body diameters long, uterus 151–266  $\mu\text{m}$  or 1.1–1.8 times the body diameter long. Vagina extending inwards 76–93  $\mu\text{m}$  or 55–73% of body diameter, with *pars refringens* having a combined width of 36–40  $\mu\text{m}$ . Vulva a transverse slit. Prerectum 3.0–3.8, rectum 0.9–1.1 anal body diameters long. Caudal region short, rounded to somewhat conoid.

### Brief discussion

Very well-preserved specimens, in which some morphological traits, especially the location of pharyngeal gland nuclei is easily observed. Their description and morphometrics are totally comparable in general with those of American and Iberian females. Even the presence of a conspicuous mucro inside the pharynx is a shared feature with American nematodes (see above). Uterus length (151–266  $\mu\text{m}$  or 1.1–1.8 times the body diameter), however, compares to that of Iberian females, being visibly shorter than that of American ones. Female tail shows an unexpected or unusual variation, ranging from 46 to 77  $\mu\text{m}$  long ( $c = 84$ –156,  $c' = 0.6$ –1.0; fig. 6g, h), but widely overlapping with ranges of

other populations too. Regarding the arrangement of pharyngeal gland nuclei, it is in good concordance with that provided by Loof & Coomans (1970; see also Andrassy, 2000) for *A. vorax*.

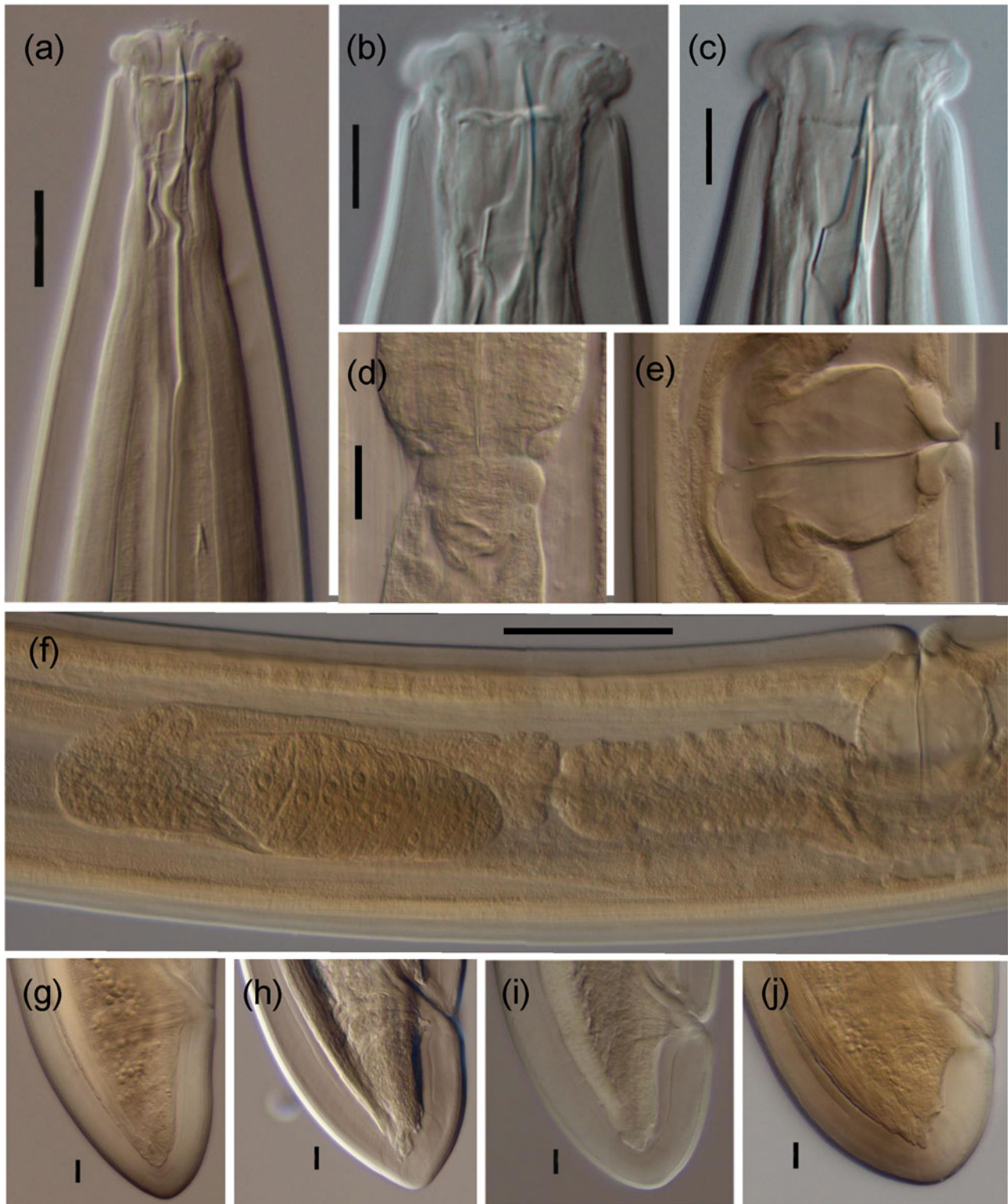
### Analysis of other populations previously identified as *A. vorax* (morphometrics in online supplementary table S1)

After its original description, this species was later recorded in several European enclaves. The main morphometrics of the corresponding populations/specimens are compiled in supplementary table S1.

Thorne (1937, 1939) corroborated the original data provided by Thorne & Swanger (1936), and recorded the very rare male of the species for the first time, literally ‘A single male... observed among over 100 specimens’. This male is herein characterized as follows: in addition to the ad-cloacal pair, situated at 16  $\mu\text{m}$  from the cloacal aperture, there is a series of 11 irregularly spaced ventromedian supplements, the most posterior of which is located at 154  $\mu\text{m}$  from the adcloacal pair, with appreciable hiatus. Spicule dorylaimid, robust, about 3.7 times longer than wide and 1.7 times the body diameter. Tail short and rounded, slightly more conoid than that of female.

Heyns (1965) mentioned (p. 20) that he studied specimens from South Africa, The Netherlands and California, USA, but the author only provided a series of good illustrations that fit those of type specimens.

Altherr (1968, 1974) examined one female and several juveniles from freshwater habitats in Germany, providing only basic morphometrics of them. The female was slightly smaller (body 5.80 mm long) than those of type specimens, but other key morphometrics (odontostyle 22  $\mu\text{m}$  long,  $V = 53$ ) fit those of



**Fig. 6.** Light micrographs of *Epacrolaimus declinatoaculeatus* (Kreis, 1924; Andrásy, 2000 (Iranian material, female): (a–c) anterior region in lateral, median view, with an easily perceptible mucro behind the odontophore base; (d) pharyngo-intestinal junction; (e) vagina; (f) posterior genital branch; and (g–j) caudal region. Scale bars: a, d = 20  $\mu$ m; b, c, g–j = 10  $\mu$ m; e = 5  $\mu$ m; f = 100  $\mu$ m.

American females. Thus, and with the due caution, these German nematodes might be rightly identified.

In their monographic contribution on the position of pharyngeal gland nuclei in dorylaims, Loof & Coomans (1970) gave data

of specimens from The Netherlands and Germany: DO = 38–40; DN = 44–46; S<sub>1</sub>N<sub>1</sub> = 64–67; S<sub>1</sub>N<sub>2</sub> = 75–77; and S<sub>2</sub>N = 87–88.

Coomans & van der Heiden (1971) studied in detail the structure and formation of the feeding apparatus in *A. vorax*, providing

very interesting information about the matter, but without either additional morphological data or morphometrics.

Mateo & Campoy (1983) described one Iberian female, but its morphometrics (body 1.9 mm long,  $c = 47$ ,  $V = 66$ ) in no way fit those of type material. Obviously, this female belonged to another species.

Bongers (1988) and Loof (1999) simply compiled morphometrics of previous authors.

Finally, the species was also recorded in The Netherlands (Loof & Oostenbrink, 1962; Andrásy, 1978), South Africa (Heyns, 1971) and Hungary (Andrásy, 1973, 1978), but no additional information about these populations was provided.

#### *Analysis of other populations previously identified as E. declinatoaculeatus or their synonyms (morphometrics in online supplementary table S1)*

The main morphometrics of the corresponding populations/specimens are compiled in online supplementary table S1, whereas their identity is discussed in the following:

Kreis (1924) originally described this species on the basis of only one female found in the Swiss Alps. The autor provided Demanian indices and a very short literal description: lip region offset by deep constriction and about one-fifth of body diameter at neck base, rounded lips, odontostyle apparently with incurved aperture, pharynx gradually enlarging, genital system diovarian, vulva located at about 45% of the total body length, and tail short and rounded.

Thorne & Swanger (1936), Schneider (1939), Meyl (1961) and Loof (1999) presented data provided by other authors, but they did not provide any additional information.

Altherr (1950) studied one young female (body 2.60 mm long) that in general (lip region, odontostyle and tail) fits well the specimens described by Kreis (1924), but the vulva is much more posterior ( $V = 58$ ). Thus, a doubt persists about identity of this specimen.

Brzeski (1964) described one Polish female much smaller (body 3.5 mm long,  $a$ -ratio = 33) than Kreis' (1924) specimen. Vulva position is similar ( $V = 46$ ), but other key features (lip region and odontostyle) seem not to be totally comparable. Actually, both Loof (1999) and Andrásy (2000) raised a doubt about the true identity of this female.

Other authors (Stegarescu, 1966; Andrásy, 1978, 2002; Ergashboev & Costin, 1981) simply mentioned the occurrence of the species in Europe, Hungary and Russia, respectively.

Andrásy (2000, see also 2009) studied and described five females and one male from Hungary, gave many new morphological traits, discussed in detail the taxonomy of this species, and concluded that it was identical with *A. vorax*, which became its junior synonym. Interestingly, this author described one male bearing particularly large spicule (215  $\mu\text{m}$  long).

Martínez-Olías *et al.* (2005) described ten females and two males from Spain, provided the first scanning electron microscopy study of the species showing (confirming) the existence of perial lobes, and gave new information about the morphology of female genital system.

#### **General discussion**

##### *Confirmation of the synonymy of A. vorax and Dorylaimus declinatoaculeatus*

Kreis (1924) originally described *D. declinatoaculeatus* from freshwater habitats of Switzerland. His literal description of the

species, based on only one female and one juvenile, was very poor in details, but his illustrations provided more relevant information about some key diagnostic features such as the morphology of lip region, odontostyle, caudal region, etc. Particularly interesting is the drawing of anterior body region, with the odontostyle displaying an apparently incurved aperture. In their description of *A. vorax*, Thorne & Swanger (1936) were aware of the similarity of the new species and *D. declinatoaculeatus* – in fact, they transferred this species to *Aporcelaimus* –, but they separated them by the vulva position ( $V = 54$  vs.  $V = 45$ , respectively) and uterine egg size (longer vs. shorter than body diameter). Both species were later recorded by several authors (see ST1 and text above), but no comparative analysis was made until Andrásy (2000), who did not consider the differences observed by Thorne & Swanger (1936) to be significant enough to maintain a separate status for them, and proposed their synonymy. Present observations support Andrásy's proposal of synonymization. Thus, vulva position is rather variable in Iberian females, its total range ( $V = 44$ – $57$ ) covering the values originally provided for both species. Nevertheless, an appreciable difference exists in uterine egg length, which is almost equal to body diameter in *D. declinatoaculeatus* according to Kreis' (1924) fig. 10c, that is, about 148  $\mu\text{m}$  long, but longer in American females of *A. vorax* (198–229  $\mu\text{m}$ ,  $n = 3$ ) and Iberian specimens (196–222  $\mu\text{m}$ ,  $n = 2$ ).

##### *Morphological characterization of E. declinatoaculeatus*

This species is a typical member of the Holarctic fauna, having been recorded in North America, many enclaves in Europe and now in Iran, its presence in South Africa having not yet been confirmed. It displays an easily recognizable morphological pattern characterized by the incurved nature of odontostyle aperture, presence of perial lobes, hardly variable morphometrics of lip region (24–31  $\mu\text{m}$  wide), odontostyle (21–25  $\mu\text{m}$  long) and location of pharyngeal gland nuclei. Nevertheless, this taxon also shows appreciable variations in some morphological traits (vagina shape and tail shape) and in several morphometrics (body length, uterus length, vulva position, tail length and spicule length). Leaving aside the uterus length (visibly longer in American females) and spicule size, morphometric ranges often overlap when populations are compared in spite of the low number of specimens available in general, an indication that they probably represent intraspecific (geographical) variation. Differences in uterus and spicule length should be a matter of further analysis when additional specimens become available as they indicate the existence of more relevant, perhaps interespecific, variation. Andrásy (2000, p. 16) noted that some specimens presented two nucleoli in DN and  $S_1N_1$ , but this rare feature has not been observed in the material herein examined. Vagina may appear more or less cylindrical (fig. 2i and online supplementary fig. S1g) to almost spherical (figs 2h, 4e and online supplementary fig. S1h), but it seems to be a physiological rather than anatomical difference as it occurs in females of the same population. Tail shape may be another relevant differential feature (see the case of Iranian specimens, fig. 6g–j), whose confirmation would require the study of a higher number of specimens.

##### *Evolutionary relationships of Epacrolaimus*

Molecular studies herein provided, especially those obtained with the analysis of D2–D3 sequences, have resulted in new relevant

data about the phylogeny of the type species of the genus, *E. declinatoaculeatus* (fig. 4). First, the new sequenced Iberian populations (ON814779–ON814783) clustered together in a maximally supported clade (PP = 1.00) with Iranian ones (MH727507–MH727508), so confirming their morphological characterization. Second, *Epacrolaimus* sequences also form a maximally supported (PP = 1.00) clade ((*Epacrolaimus* + *Sectonema*) + *Metaporcelaimus*), which is a remarkable novelty, although the inner evolutionary relationships of this clade are not totally resolved, with one sequence of *Metaporcelaimus* appearing closer to *Sectonema* than to other sequences of the same genus. Third, the topology of the tree shows that *Sectonema* sequences appeared to be split into two subclades, one of them consisting of Vietnamese (Indomalayan) species and with low support (PP = 0.75), another with Palaearctic taxa and highly supported (PP = 0.99), a pattern similar to that observed by Álvarez-Ortega & Peña-Santiago (2019). Fourth, and somewhat surprisingly, *Epacrolaimus* (Palaearctic) sequences appear closer to the Indomalayan subclade of *Sectonema* representatives than to the Palaearctic subclade of the same genus, although further studies should be conducted to confirm these results as the clade (*Epacrolaimus* + Indomalayan *Sectonema*) presents low support (PP = 0.75). Fifth, the sequences of other aporcelaimid genera (*Aporcelaimellus*, *Aporcelinus*, *Aporcella* and *Makatinus*), form part of separate clades.

The molecular tree presented in fig. 5, derived from the analysis of 18S sequences, confirmed in general the results obtained with D2–D3 sequences, but its resolution is lesser. Thus, and on the one hand, the new sequenced Iberian populations of *E. declinatoaculeatus* (ON764419–ON764423) clustered together with several species of the genus *Sectonema* in a maximally supported (PP = 1.00) clade (*Epacrolaimus* + *Sectonema*). On the other hand, other aporcelaims, the members of the genera *Aporcelaimellus*, *Aporcella* and *Aporcelinus*, do not share their most recent ancestor with the subclade (*Epacrolaimus* + *Sectonema*).

This contribution provides the first COI sequences of representatives of the genus *Epacrolaimus* and the family Aporcelaimidae. Unfortunately, only a few accessions of dorylaims (Dorylaimida) are available from NCBI to date for comparative purposes. Therefore, their analysis (molecular tree presented in online supplementary fig. S3) did not produce any relevant result and no conclusions can be obtained.

As mentioned in the introductory section, when originally proposed by Andrassy (2009), *Epacrolaimus* was regarded as very close morphologically to *Aporcelaimus*, from which its type species, *E. declinatoaculeatus* was transferred. Unfortunately, no D2–D3 *Aporcelaimus* sequence is available for comparative purposes, therefore the relationship between these two genera cannot be confirmed by means of molecular analyses to date. Conversely, the results herein provided show that *Epacrolaimus* is very close to *Sectonema*, two quite different genera under a morphological perspective as the nature of their protruding stomatal structure seems not to be comparable: a typical axial odontostyle in *Epacrolaimus* vs. a mural tooth in *Sectonema*. Álvarez-Ortega & Peña-Santiago (2019) noted however a remarkable variation of the protruding structure in *Sectonema* species, with two recognizable patterns. One of these patterns, observed in the type species of the genus, *Sectonema ventrale*, and other species too, consists of a reduced odontostyle rather than a mural tooth *sensu stricto*. Interestingly, this reduced odontostyle shows an incurved aperture, comparable to that observed in *Epacrolaimus*, a very unusual feature in dorylaims. Nevertheless, the reduced odontostyle

observed in *Sectonema* lacks a perceptible dorsal arm, which is present and conspicuous in *Epacrolaimus*. Thus, further studies should be conducted to confirm the evolutionary relationships between the two genera, with special emphasis on the variation observed in the stomatal protruding structure of *Sectonema* representatives.

In a more general perspective, regarding the inner phylogeny of Dorylaimida, present results confirm that the family Aporcelaimidae is not a monophyletic taxon (*cf.* Holterman *et al.*, 2008; Álvarez-Ortega *et al.*, 2013; Álvarez-Ortega & Peña-Santiago, 2019, among others), with several genera (*Aporcelaimellus*, *Aporcelinus*, *Aporcella* and *Makatinus*) forming part of other clades than that including *Epacrolaimus*, *Metaporcelaimus* and *Sectonema*. It is only an example of the intricate internal evolutionary relationships of dorylaims (Dorylaimida) in general and Dorylaimina in particular. Moreover, the results herein obtained, far from elucidating the tree branching of aporcelaims (Aporcelaimidae), produce a doubt about the identity of *Epacrolaimus* and *Sectonema*, two easily recognizable (separable) genera on the basis of the nature of their protruding stomatal structure (see above), which however forms a very robust clade when their molecular data are analysed.

## Conclusion

The genus *Epacrolaimus* is a typical representative of the Holarctic fauna, with its type species, *E. declinatoaculeatus*, displaying a wide distribution in separate enclaves of the Northern Hemisphere, where it was repeatedly recorded as either *Aporcelaimus declinatoaculeatus* or its (confirmed) junior synonym *A. vorax*. Morphologically, *E. declinatoaculeatus* is recognizable and characterized by a peculiar combination of relevant traits: incurved odontostyle aperture; perioral liplets or lobes; odontostyle 21–25 µm long; and comparatively anterior location of pharyngeal gland nuclei. Nonetheless, it also shows some interesting geographical variations affecting a few features and morphometrics that should be a matter of additional analysis. Its molecular characterization has revealed a closer evolutionary relationship with members of the genera *Sectonema* and *Metaporcelaimus* than with other representatives of the family Aporcelaimidae. Especially intriguing is the relationship between *Epacrolaimus* and *Sectonema*, two genera that significantly differ in the nature of their protruding stomatal structure, a relevant trait of dorylaimid anatomy, but display a close evolutionary relationship, a dilemma that raises a doubt about the identity of these two genera and would be a matter of further studies for its elucidation.

**Supplementary material.** To view supplementary material for this article, please visit <https://doi.org/10.1017/S0022149X2200058X>.

**Acknowledgements.** Our gratitude to Drs Z. Handoo (United States Department of Agriculture, Beltsville, Maryland, USA) for the loan of type material of *Aporcelaimus vorax*, and G. Niknam (University of Tabriz, Tabriz, Iran) for the permission to include the study of several Iranian specimens of *Epacrolaimus declinatoaculeatus* in this study.

**Financial support.** This work was supported by the University of Jaén, Spain, through the research program ‘PAIUIJA 20121/2022: EI\_RNM02\_2021’.

**Conflicts of interest.** None.

**Ethical standards.** 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

- Altherr E** (1950) Les nématodes du Parc National Suisse (Nématodes libres du sol) [The nematodes of the Swiss National Park (Free-living soil nematodes)]. *Ergebnisse der Wissenschaftlichen Untersuchung des Schweizerischen Nationalparks* **22**(3), 3–46. [In French.]
- Altherr E** (1968) Nématodes de la nappe phréatique du réseau fluvial de la Saale (Thuringe) et psammiques du Lac Stechlin (Brandebourg du nord) [Groundwater nematodes from the Saale river system (Thuringia) and psammics from Lake Stechlin (northern Brandenburg)]. *Limnologica* **6**(1), 247–320. [In French.]
- Altherr E** (1974) Nématodes de la nappe phréatique du réseau fluvial de la Saale (Thuringe), II [Groundwater nematodes of the Saale river system (Thuringia), II]. *Limnologica* **9**(1), 81–132. [In French.]
- Álvarez-Ortega S and Peña-Santiago R** (2019) Morphology, phylogeny and taxonomy of the genus *Setonema* (Nematoda, Aporcelaimidae). *Zoologica Scripta* **48**(4), 535–544.
- Álvarez-Ortega S, Subbotin SA and Peña-Santiago R** (2013) Morphological and molecular characterization of *Aporcelaimellus simplex* (Thorne & Swanger, 1936) Loof & Coomans, 1970 and a new concept for the genus *Aporcella* Andrassy, 2002 (Dorylaimida, Aporcelaimidae). *Nematology* **15**(2), 165–178.
- Álvarez-Ortega S, Subbotin SA and Peña-Santiago R** (2018) Morphological and molecular characterization of two new species of the genus *Aporcelinus* Andrassy, 2009 (Nematoda, Dorylaimida, Aporcelaimidae) from the USA, with new insights on the phylogeny of the genus. *Journal of Helminthology* **94**, e22.
- Álvarez-Ortega S, Subbotin SA and Inserra RN** (2021) Morphological and molecular characterization of *Xiphinemella esseri* Chitwood, 1957 (Dorylaimida: Leptonchidae) from Florida, with the first molecular study of the genus. *Journal of Nematology* **53**, e2021-32.
- Andrássy I** (1973) 100 neue Nematodenarten in der ungarischen Fauna [100 new nematode species in the Hungarian fauna]. *Opuscula Zoologica Budapestinensis* **11**(1), 7–48. [In German.]
- Andrássy I** (1978) Nematodes. pp. 98–118. In Illies J (Ed.) *Limnofauna Europaea – A checklist of the animals inhabiting European inland waters, with accounts of their distribution and ecology (except Protozoa)*. Stuttgart, Gustav Fischer Verlag.
- Andrássy I** (2000) Four large-sized species of the family Aporcelaimidae (Nematoda, Dorylaimida) with proposal of a new genus, *Epacrolaimus* gen. n. *Opuscula Zoologica Budapestinensis* **32**(1), 3–26.
- Andrássy I** (2002) Free-living nematodes from the Fertő-Hanság National Park, Hungary. pp. 21–97. In Makunda S (Ed.) *The fauna of the Fertő-Hanság National Park*. Budapest, Hungarian National History Museum.
- Andrássy I** (2009) *Free-living nematodes of Hungary. III. Pedozoologica Hungarica n° 5*. Budapest, Hungarian Natural History Museum.
- Archidona-Yuste A, Navas-Cortés JA, Cantalapiedra-Navarrete C, Palomares-Rius JE and Castillo P** (2016) Unravelling the biodiversity and molecular phylogeny of needle nematodes of the genus *Longidorus* (Nematoda: Longidoridae) in olive and a description of six new species. *PLoS One* **11**, e0147689.
- Bongers T** (1988) *De Nematoden van Nederland* [The Nematodes of the Netherlands]. Utrecht, KNNV. [In Dutch.]
- Brzeski MW** (1964) Einige neue und seltene Nematoden aus der Überfamilie Dorylaimoidea I. Unterfamilie Dorylaiminae (Nematoda: Dorylaimidae) [Some new and rare nematodes from the superfamily Dorylaimoidea I. Subfamily Dorylaiminae (Nematoda: Dorylaimidae)]. *Annales Zoologici* **22**(1), 1–22. [In German.]
- Cai R, Archidona-Yuste A, Cantalapiedra-Navarrete C, Palomares-Rius JE and Castillo P** (2020) New evidence of cryptic speciation in needle and dagger nematodes of the genera *Longidorus* and *Xiphinema* (Nematoda: Longidoridae). *Journal of Zoological Systematics and Evolutionary Research* **58**(4), 869–899.
- Castresana J** (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**(4), 540–552.
- Coomans A and van der Heiden A** (1971) Structure and formation of the feeding apparatus in *Aporcelaimus* and *Aporcelaimellus* (Nematoda: Dorylaimida). *Zeitschrift für Morphologie der Tiere* **70**(2), 103–118.
- Darriba D, Taboada GL, Doallo R and Posada D** (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**(8), 772.
- De Ley P, Félix MA, Frisse LM, Nadler SA, Sternberg PW and Thomas WK** (1999) Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* **1**(6), 591–612.
- Ergashboev I and Costin LH** (1981) [Nematodes of the Nurec reservoir during the filling period.]. pp. 105–107. In Platonova TA and Tsalolikhin SY (Eds) *Evolution, taxonomy, morphology and ecology of freelifving nematodes*. St Petersburg, Zoological Institute, Academy of Sciences of the USSR. [In Russian.]
- Hall TA** (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* **41**(1), 95–98.
- Heydari F, Gharibzadeh F, Pourjam E and Pedram M** (2020) New and known species of the genus *Pungentus* Thorne & Swanger, 1936 (Dorylaimida, Nordiidae) from Iran. *Journal of Helminthology* **94**, e32. 1–9.
- Heys J** (1965) On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimid nematodes. *Entomology Memoirs Department of Agriculture Technical Services Republic of South Africa* **10**(1), 1–51.
- Heys J** (1971) *A guide to the plant and soil nematodes of South Africa*. Cape Town, A.A. Balkema.
- Holterman M, Van Der Wurff A, Van Den Elsen S, Van Megen H, Bongers T, Holovachov O, Bakker J and Helder J** (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* **23**, 1792–1800.
- Holterman M, Rybarczyk K, van den Elsen S, van Megen H, Mooyman P, Peña-Santiago R, Bongers T, Bakker J and 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.
- Holterman M, Van Der Wurff A, Van Den Elsen S, Van Megen H, Bongers T, Holovachov O, Bakker J and Helder J** (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* **23**, 1792–1800.
- Katoh K, Rozewicki J and Yamada KD** (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinformatics* **20**(4), 1160–1166.
- Kreis HA** (1924) Die Seen im Aela- und Tinzenhorngebiet II [The lakes in the Aela and Tinzenhorn area II]. *Nematodes. Jahresbericht der Naturforschenden Gesellschaft Graubündens n. F.* **63**(1), 23–68. [In German.]
- Lazarova SS, Malloch G, Oliveira CMG, Hübschen J and Neilson R** (2006) Ribosomal and mitochondrial DNA analyses of *Xiphinema americanum*-group populations. *Journal of Nematology* **38**(4), 404–410.
- Loof PAA** (1999) *Süswasserfauna von Mitteleuropa 4/2-2. Nematoda, Adenophorea (Dorylaimida)* [Freshwater fauna of Central Europe 4/2-2. Nematoda, Adenophorea (Dorylaimida)]. Heidelberg, Spektrum Akademischer Verlag. [In German.]
- Loof PAA and Coomans A** (1970) On the development and location of the oesophageal gland nuclei in Dorylaimina. pp. 79–161. In *Proceedings of the IX International Nematology Symposium* (Warsaw, Poland, 1967).
- Loof PAA and Oostenbrink M** (1962) Bijdrage tot de kennis van de aaltjesfauna van de Nederlandse bodem [Contribution to the knowledge of the nematode fauna of the Dutch messengers]. *Verslag van den Plantenziektenkundigen Dienst Wageningen* **136**(1), 176–184. [In Dutch.]
- Martínez-Olías J, Liébanas GM, Guerrero P, Abolafia J and Peña-Santiago R** (2005) Sobre la presencia del género *Epacrolaimus* Andrassy, 2000 (Nematoda: Dorylaimida) en la Península Ibérica [On the presence of the genus *Epacrolaimus* Andrassy, 2000 (Nematoda: Dorylaimida) in the Iberian Peninsula]. *Fundamental* **6**(1), 121–124. [In Spanish.]
- Mateo MD and Campoy A** (1983) Estudio de los nematodos libres de las Peñas de Echaury (Navarra) [Study of the free nematodes of the Peñas de Echaury (Navarra)]. *Publicaciones de Biología de la Universidad de Navarra, Serie Zoológica* **9**(1), 1–64. [In Spanish.]
- Meyl AH** (1961) Die freilebenden Erd- und Süswassernematoden (Fadenwürmer) [The free-living terrestrial and freshwater nematodes

- (roundworms)]. *Tierwelt Mitteleuropas, I. Band, Lief. 5a. Freilebende Nematoden* [The animal world of Central Europe. Nematodes]. Leipzig, Quelle and Meyer. [In German.]
- Mullin PG, Harris TS and Powers TO** (2005) Phylogenetic relationships of Nysolaimina and Dorylaimina (Nematoda: Dorylaimida) inferred from small subunit ribosomal DNA sequences. *Nematology* **7**(1), 59–79.
- Pedram M** (2017) Description of *Enchodorus yeatsi* n. sp. (Dorylaimida, Nordiidae) from Southern Iran and its molecular phylogenetic study. *Journal of Nematology* **49**(1), 21–26.
- Pedram M, Pourjam R and Vinciguerra MT** (2012) Description of a new species of the rare genus *Epacrolaimus* Andrassy, 2000 (Dorylaimida, Aporcelaimidae) and new data on male of *Paraxonchium laetificans* (Andrassy, 1956) Altherr & Loof, 1969 (Dorylaimida, Paraxonchiidae) from Iran. *Zootaxa* **3327**(1), 53–61.
- Rambaut A** (2014) *FigTree v1.4.2, a graphical viewer of phylogenetic trees*. Available at <http://tree.bio.ed.ac.uk/software/figtree/> (accessed on 24th august 2022).
- Ronquist F and Huelsenbeck JP** (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**(12), 1572–1574.
- Schneider W** (1939) Würmer oder Vermes II. Fadenwürmer oder Nematoden. I. Freilebende und pflanzenparasitische Nematoden [Worms or Vermes II. Roundworms or Nematodes. I. Free-living and plant-parasitic nematodes]. *Tierwelt Deutschlands (F. Dahl) Jena* **36**(1), 1–260. [In German.]
- Stegarescu OP** (1966). Recherche nématologique dans les vignobles de la Moldavie [Nematological research in the vineyards of Moldova] In: Rapoport EH (Ed.) *Monografias I: Progresos en Biología del Suelo*: pp. 191–193. Lasco, Montevideo.
- Thorne G** (1937) Notes on free-living and plant parasitic nematodes. III. *Proceedings of the Helminthological Society of Washington* **4**(1), 16–18.
- Thorne G** (1939) A monograph of the nematodes of the superfamily Dorylaimoidea. *Capita Zoologica* **8**(5), 1–261.
- Thorne G and Swanger HH** (1936) A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n. gen., *Dorylaimoides* n. gen., and *Pungentus* n. gen. *Capita Zoologica* **6**(4), 1–223.
- Varela-Benavides I and Peña-Santiago R** (2019) *Metaxonchium toroense* n. sp. (Nematoda, Dorylaimida, Belonidiridae) from Costa Rica, with the first molecular study of a representative of the genus. *Journal of Helminthology* **93**(1), 100–108.
- Vrain TC, Wakarchuk DA, Levesque AC and Hamilton RI** (1992) Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* **15**(6), 563–573.