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Dorylaimida, Thornenematidae)

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COUVERTURE / *COVER*:

Anterior body region of *Sylphodorylaimus sylphoides* (Williams, 1959) n. comb.

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# New insights into the evolutionary relationships of *Opisthodorylaimus sylphoides* (Williams, 1959), with proposal of *Sylphodorylaimus* n. gen. (Nematoda, Dorylaimida, Thornenematidae)

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## ABSTRACT

A new nematode genus, *Sylphodorylaimus* n. gen., is proposed to accommodate one species previously classified under *Opisthodorylaimus* Ahmad & Jairajpuri, 1982. The study of two Iranian populations of *O. sylphoides* (Williams, 1959), collected in natural areas, including their morphological, morphometrical and molecular (28S rDNA) characterisation, revealed that females were almost identical to those of other known population, but also that their males, found and described for the first time, displayed long and filiform tail, similar to that observed in females. Since *Opisthodorylaimus* is characterized by showing a conspicuous sexual dimorphism in tail shape, with males having short and rounded tail, *O. sylphoides* must not be retained under this genus. The new taxon is compared with and separated from other genera of Thornenematidae Siddiqi, 1969, and its evolutionary relationships are discussed.

## RÉSUMÉ

*Nouvelles perspectives sur les relations évolutives d'Opisthodorylaimus sylphoides* (Williams, 1959), avec la proposition de *Sylphodorylaimus* n. gen. (Nematoda, Dorylaimida, Thornenematidae).

Un nouveau genre de nématode, *Sylphodorylaimus* n. gen., est proposé pour accueillir une espèce précédemment classée sous *Opisthodorylaimus* Ahmad & Jairajpuri, 1982. L'étude de deux populations iraniennes d'*O. sylphoides* (Williams, 1959), collectées dans des zones naturelles, y compris leur caractérisation morphologique, morphométrique et moléculaire (28S rDNA), a révélé que les femelles étaient presque identiques à celles des autres populations connues, mais aussi que leurs mâles, trouvés et décrits pour la première fois, présentaient une queue longue et filiforme, semblable à celle observée chez les femelles. Étant donné qu'*Opisthodorylaimus* est caractérisé par un dimorphisme sexuel très net sur la forme de la queue, les mâles ayant une queue courte et arrondie, *O. sylphoides* ne doit pas être maintenu dans ce genre. Le nouveau taxon est comparé et séparé d'autres genres de Thornenematidae Siddiqi, 1969, et ses relations évolutives sont discutées.

## KEY WORDS

Dorylaims,  
morphology,  
morphometry,  
nematodes,  
rDNA,  
new combination,  
new genus.

## MOTS CLÉS

Dorylaimides,  
morphologie,  
morphométrie,  
nématodes,  
ADNr,  
combinaison nouvelle,  
genre nouveau.

## INTRODUCTION

*Opisthodorylaimus sylphoides* (Williams, 1959) is a widely distributed dorylaimid (Nematoda, Dorylaimida) monosexual species, repeatedly recorded from many habitats of at least 11 countries in three continents (see Peña-Santiago 2021). Originally described as *Dorylaimus sylphoides* by Williams (1959), it was later transferred to *Thornenema* Andrassy, 1960 and to *Opisthodorylaimus* Ahmad & Jairajpuri, 1982. In the course of their excellent revision of the genus *Opisthodorylaimus*, Carbonell & Coomans (1986) provided a re-examination of Williams' type material and a very detailed morphological description of this and other populations, including beautiful illustrations. Besides, SEM observations of the species were also available (Fadaei-Tehrani & Coomans 2005), as well molecular data (Holterman *et al.* 2008). Thus, it is a perfectly characterised and easily recognisable taxon when compared to members of the family Thornenematidae Siddiqi, 1969 in general and to representatives of its genus in particular.

Ahmad & Jairajpuri (1982) placed their (new) genus *Opisthodorylaimus* in Thornenematidae, but its taxonomical affinities have been matter of some controversy. Thus, Coomans & Carbonell (1988) concluded that it was closely related to *Mesodorylaimus* Andrassy, 1959 and proposed its inclusion in the subfamily Mesodorylaiminae Andrassy, 1969 in Dorylaimidae de Man, 1876. Jairajpuri & Ahmad (1992) classified it in Dorylaimidae, Thornenematinae, meanwhile Andrassy (2007, 2009) argued in favour of its retention in Thornenematidae, Thornenematinae.

Two populations of *O. sylphoides* were collected in the course of a general nematological survey conducted to explore the dorylaimid diversity of some regions of Iran. The study of these populations offered a chance to go further in the elucidation of its identity, with two remarkable novelties. On the one hand, the finding of male specimens for the first time, a key matter in the taxonomy/systematics of long-tailed dorylaims. On the other hand, the opportunity to obtain fresh specimens, suitable for molecular analysis. The results obtained support the proposal of a new genus to accommodate this species. Therefore, the main aim of this contribution is to provide a characterisation of the new taxon and to discuss its evolutionary relationships.

## MATERIAL AND METHODS

## SAMPLING AND PREPARATION OF THE MATERIAL EXAMINED

Soil samples were collected from the rhizosphere of different plants and localities in Iran during 2020. Nematodes were extracted by the modified method of Brown & Boag (1988), killed by heat, fixed in formaldehyde 4%, transferred to dehydrated glycerin according to De Grisse (1969), and mounted on permanent slides using paraffin wax to their handle and study.

## MORPHOLOGICAL AND MORPHOMETRICAL STUDY

Nematodes were observed, measured and photographed using an Eclipse 80i microscope (Nikon, Tokyo, Japan) with dif-

ferential interference contrast optics and a DS digital camera. Morphometrics include Demanian indices and other measurements and ratios, some of them presented in a separate table, and others form part of the literal description of the material examined.

## DNA EXTRACTION, PCR AND SEQUENCING

For DNA extraction, a single nematode from Sufiyan, East-Azarbaijan province, was placed in 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 ddH<sub>2</sub>O, 2.85 µl 10X PCR buffer and 1.5 µl proteinase K (600 µg/ml) (Promega, Benelux, the Netherlands). In order to disintegrate the nematode body and facilitate the extraction of DNA, the tubes were stored at -80°C for 1 h, and subsequently incubated at 65°C for 1 h and at 95°C for 15 min. The DNA samples were stored at -20°C until used as the polymerase chain reaction (PCR) template. The D2-D3 expansion fragments of 28S rDNA were amplified using the forward primer D2A (5'-ACAAG-TACCGTGAGGGAAAGT-3') and reverse primer D3B (5'-TCGGAAGGAACCAGCTACTA-3') (Nunn 1992). The PCR cycles and sequencing of amplified fragments were performed according to Archidona-Yuste *et al.* (2016). The PCR products was sequenced in an Applied Biosystems® 3730/3730xl DNA Analyzer in South Korea. The newly generated sequence was deposited in the GenBank database under (OQ550280) accession number.

## PHYLOGENETIC ANALYSIS

For phylogenetic relationships, the newly generated sequence was edited and aligned with another segments of 28S rDNA gene sequences available in GenBank using Muscle software implemented in MEGA6 (Tamura *et al.* 2013). Phylogenetic analysis of the sequences data set was performed based on Bayesian inference (BI) using MrBayes 3.1.2. The best-fitted model of DNA evolution was obtained using MrModel test 2.3 (Nylander 2004) with Akaike-supported model in conjunction with PAUP\* v4.0b10 (Swofford 2003). The BI analysis was run using four Metropolis-coupled Markov chain Monte Carlo (MCMC) for 1 × 10<sup>8</sup> generation. Posterior probabilities (PP) are given on the appropriate clade. Tree was visualized using FigTree v1.4.3

## ABBREVIATIONS

*Material depositories*

UTANC University of Tabriz Nematode Collection, Tabriz;  
UJANC University of Jaén Nematode Collection, Jaén.

*Morphometrics used in text in tables*

L body length in mm;  
a body length/maximum body width;  
b body length/neck length;  
c body length/tail length;  
c' tail length anal body diameter;  
V distance vulva-anterior end × 100 /.

## RESULTS

Order DORYLAIMIDA Pearse, 1936  
 Suborder DORYLAIMINA Pearse, 1936  
 Family THORNENEMATIDAE Siddiqi, 1969  
 Subfamily THORNENEMATINAE Siddiqi, 1969

*Sylphodorylaimus* n. gen.

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DIAGNOSIS. — Thornenematidae, Thornenematinae. Medium-sized nematodes, 1.66–2.48 mm long. Cuticle dorylaimid. Lip region continuous with the adjoining body, with totally amalgamated lips, and lacking both labial and postlabial sclerotization. Amphid fovea cup-like, its aperture *c.* one-half of lip region diameter. Cheilostom with moderately thick, inward curved walls. Odontostyle hardly longer than lip region diameter, with aperture occupying one-third to two-fifths of its length. Guiding ring double. Odontophore rod-like. Pharynx entirely muscular, gradually enlarging into the basal expansion that occupies less than half of the total neck length. Female genital system mono-opistho-ovarian, with drastically reduced anterior branch, very weak *pars refringens vaginae* and short, longitudinal vulva. Tail similar in both sexes, long and filiform. Spicules dorylaimid. Nine to eleven shortly spaced ventromedian supplements with hiatus.

ETYMOLOGY. — The generic substantive is a combination of the type species name *sylphoides*, and *Dorylaimus*, the type genus of the order Dorylaimida.

TYPE AND ONLY SPECIES. — *Dorylaimus sylphoides* Williams, 1959.

## SEPARATION FROM OTHER SIMILAR GENERA

Within Thornenematidae, the new genus shares the lack of sexual dimorphism in tail shape with the genera *Indodorylaimus* Ali & Prabha, 1974 and *Sicaguttur* Siddiqi, 1971, but it differs from these in the absence (vs presence) of a strongly developed cephalic framework, with conspicuous labial and postlabial sclerotization, a significant difference indeed. Besides, *Indodorylaimus* males have much less (1–4) ventromedian supplements, whereas *Sicaguttur* females are di-ovarian.

*Sylphodorylaimus* n. gen. resembles some members of Dorylaimidae, for instance, a few *Prodorylaimus* species, in several aspects (continuous lip region with amalgamated lips, relatively strong odontostyle, both sexes with long tail), but it can be easily distinguished from them in its mono-ovarian (vs always di-ovarian) condition.

The proposal of the new genus raises a doubt about the identity of monosexual species of *Opisthodorylaimus*. Taking into account that females of these species appreciably differ (see below) from those of *S. sylphoides* n. comb., they are provisionally retained under *Opisthodorylaimus* until new evidence is available.

Sen *et al.* (2012) described two new *Indodorylaimus* species from India, namely *I. asaccatus* and *I. bagrii*, with (Sen *et al.* 2012: 12) “moderately sclerotized” lip region and no male, among other traits. The true identity of these two species is however questionable as their poor original descriptions, in-

cluding laconic drawings and pictures, lack enough information. Assuming that labial and postlabial sclerotization may be absent in these species – as suggested by illustrations –, *S. sylphoides* n. comb. resembles these two taxa in its general aspect, but it can easily distinguished from them by its longer body (1.66–2.48 vs 1.38–1.73 mm in *I. asaccatus* and 1.40–1.58 mm in *I. bagrii*), longitudinal (vs transverse) vulva, much longer female tail (338–458 vs 233–257 and 155–257  $\mu$ m), and male present (vs absent).

*Sylphodorylaimus sylphoides*

(Williams, 1959) n. comb.

(Figs 1–3; Tables 1; 2)

*Dorylaimus sylphoides* Williams, 1959: 21.

*Thornenema sylphoides* – Andr ssy 1960: 6. — Eliava 1984: 35 (catalogued).

*Opisthodorylaimus sylphoides* – Carbonell & Coomans 1986: 391 (re-description). — Andr ssy 2009: 247 (catalogued). — Pe a-Santiago 2021: 432 (catalogued).

MATERIAL EXAMINED. — Iran • 7♀, 1♂; Mazandaran province, Zirab County; 36°10'68"N, 052°57'86"E; 500 m a.s.l.; rhizosphere of *Diospyros lotus* L., UTANC • 6♀, 2♂; East-Azarbaijan province, Sufiyan, Roodghat area, Zeinabad village; 38°29'53"N, 46°12'38"E; 1600 m a.s.l.; in the rhizosphere of black cherry trees (*Prunus cerasus* L.); UJANC.

## MORPHOLOGICAL DESCRIPTION

*Adult*

Very slender ( $a = 34\text{--}54$ ) nematodes of medium size, 1.95–2.42 mm long. Body cylindrical, tapering towards both ends but more so posteriorly, the tail being long and filiform in both sexes. Upon fixation, habitus very slightly curved ventrad. Cuticle smooth under light microscope, 1–1.5  $\mu$ m thick in anterior region, 2  $\mu$ m at mid-body and 5–6  $\mu$ m on dorsal side of tail, two-layered, consisting of a thin outer layer with constant thickness throughout the body, and a thicker inner layer, especially distinct at caudal region. Lateral chord 7–12  $\mu$ m or 16–24% of mid-body diameter. Lip region continuous with the adjoining body, somewhat truncate anteriorly, 2.0–2.3 times as wide as high and *c.* one-fourth (23–26%) of body diameter at neck base, with totally amalgamated lips and not protruding (labial and cephalic) papillae. Amphid fovea cup-like, its aperture 6  $\mu$ m, occupying *c.* one-half (55%) of lip region diameter. Cheilostom with moderately thick walls visibly arched inward. Odontostyle typical dorylaimid, 5.7–7.3 times longer than wide, somewhat longer (1.1–1.3 times) than lip region diameter, and 0.59–0.66% of body length; aperture 4.5–6  $\mu$ m or 33–42% of its length. Guiding ring double. Odontophore rod-like, 1.5–1.7 times longer than odontostyle. Conspicuous lacunae are present at both sides of anterior body region. Pharynx entirely muscular, gradually enlarging into the basal expansion that is 6.3–8.9 times longer than wide, 3.2–4.5 times the body diameter at neck base, and occupies less than one-half (42–47%) of the total neck length;



FIG. 1. — *Syphodorylaimus syphoides* (Williams, 1958) n. comb. (Zirab population, male): **A**, caudal region; **B**, entire; **C**, **D**, anterior region, lateral median view; **E**, anterior region, lateral surface view; **F**, lateral guiding piece; **G**, **H**, posterior body region, in part; **I**, spicule. Scale bars: A, H, 50  $\mu$ m; B, 500  $\mu$ m; C, I, 10  $\mu$ m; D-F, 5  $\mu$ m; G, 20  $\mu$ m.

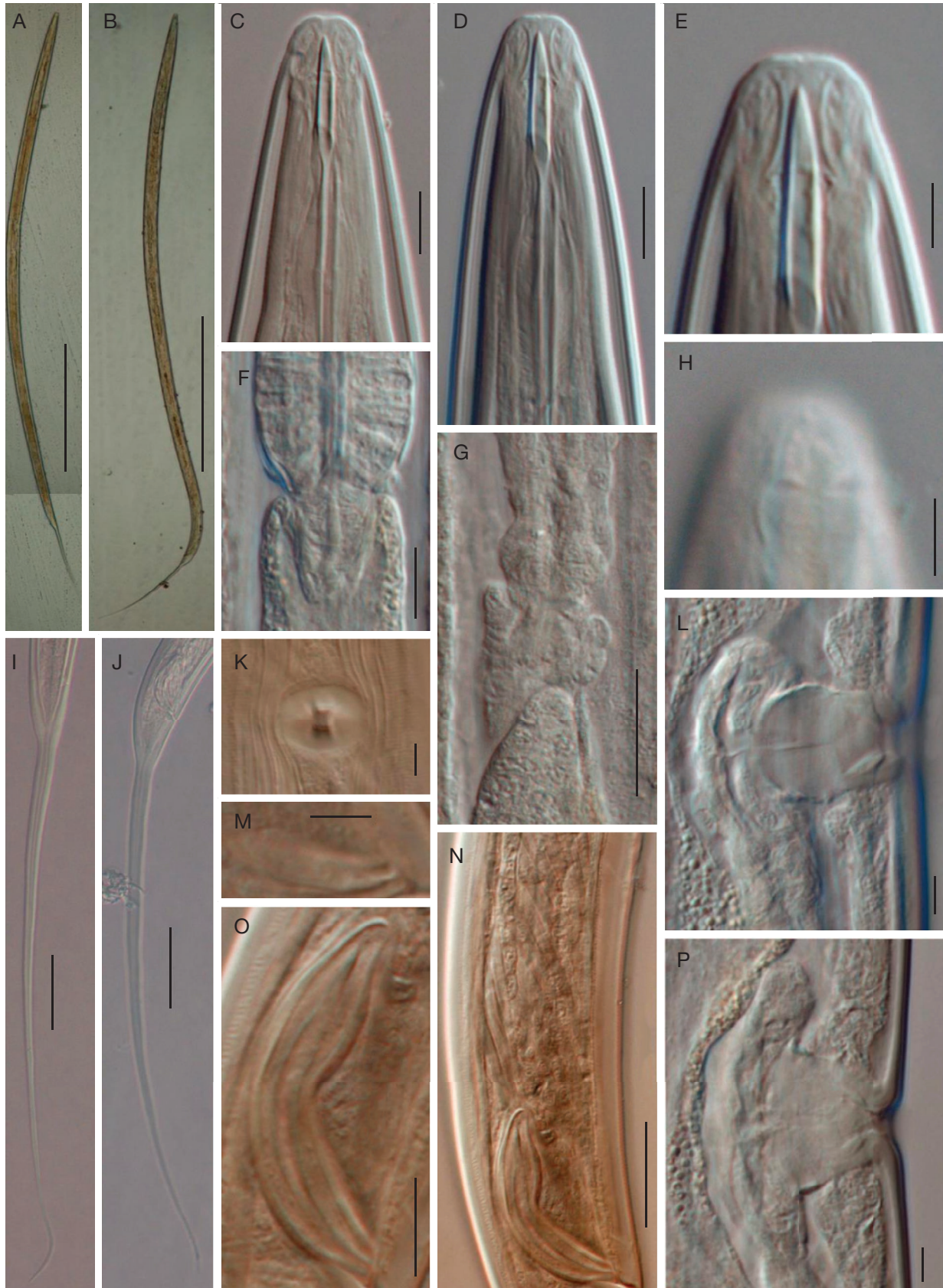


FIG. 2. — *Sylphodorylaimus syphoides* (Williams, 1958) n. comb. (Zirab population): **A**, female, entire; **B**, male, entire; **C–E**, anterior region, lateral median view; **F**, pharyngo-intestinal junction; **G**, oviduct-uterus junction; **H**, anterior region, lateral surface view; **I**, female, caudal region; **J**, male, caudal region; **K**, vulva, ventral view; **L**, **P**, vagina region; **M**, lateral guiding piece; **N**, male, posterior body region in part; **O**, spicule. Scale bars: A, B, 500 µm; C, D, N, O, 10 µm; E, H, K, L, M, P, 5 µm; F, G, 20 µm; I, J, 50 µm.

TABLE 1. — Morphometrics of *Sylphodorylaimus sylphoides* (Williams, 1959) n. comb. from Iran. Measurements in  $\mu\text{m}$ , except L in mm, and in the form: average  $\pm$  standard deviation (range).

Province/ Population	Mazandaran/ Zirab County		East Azarbaijan/ Sufiyan	
	7♀	1♂	6♀	2♂
n				
Character				
L	2.29 $\pm$ 0.08 (2.16-2.42)	2.12	2.09 $\pm$ 0.02 (1.95-2.22)	2.14, 2.21
a	48.7 $\pm$ 3.6 (43-54)	47	38.6 $\pm$ 3.6 (34-44)	40, 51
b	5.7 $\pm$ 0.1 (5.7-5.8)	5.6	5.5 $\pm$ 0.2 (5.1-5.9)	4.4, 5.6
c	5.5 $\pm$ 0.5 (4.7-6.0)	5.9	5.1 $\pm$ 0.2 (4.9-5.7)	5.8, 6.8
c'	16.5 $\pm$ 1.4 (15.5-19.1)	14.3	15 $\pm$ 0.1 (14-16)	12.3, 13.5
V	37.2 $\pm$ 0.2 (35-38)	—	35.5 $\pm$ 1.2 (34-37)	—
Lip region diameter	11.5 $\pm$ 0.4 (11-12)	11	10.8 $\pm$ 0.9 (10-12)	11, 12.5
Odontostyle length	14.5 $\pm$ 0.4 (14-15)	15	14.8 $\pm$ 0.9 (14-16)	14, 15
Odontophore length	23.5 $\pm$ 0.2 (22-24)	23	22.6 $\pm$ 1.3 (21-24)	22, 23
Neck length	400 $\pm$ 11 (381-416)	380	373 $\pm$ 19 (350-397)	359, 394
Pharyngeal expansion length	178 $\pm$ 10 (166-188)	168	168 $\pm$ 8 (153-178)	156, 163
Body diameter at – neck base	47.8 $\pm$ 4.1 (42-53)	47	53.6 $\pm$ 2.1 (51-59)	42, 54
– midbody	47.5 $\pm$ 4.3 (42-53)	45	53.4 $\pm$ 3.8 (51-59)	43, 54
– anus/cloaca	25.7 $\pm$ 1.2 (24-27)	25	26.4 $\pm$ 1.9 (25-30)	25, 27
Distance vulva – anterior end	845 $\pm$ 50 (762-900)	—	752 $\pm$ 52 (685-831)	—
Prerectum length	82.8 $\pm$ 35.5 (48-140)	143	61.0 $\pm$ 3.8 (57-68)	150, 162
Rectum/cloaca length	30.8 $\pm$ 3.1 (27-34)	40	29.5 $\pm$ 4.6 (23-36)	43, 45
Tail length	421 $\pm$ 27 (388-458)	357	400 $\pm$ 10 (387-416)	322, 366
Spicule length	—	40	—	34, 41
Ventromedian supplements	—	10	—	9, 11

gland nuclei located as follows: DO = 56-59, DN = 59-62, S<sub>1</sub>N<sub>1</sub> = 68-71, S<sub>1</sub>N<sub>2</sub> = 76-79, S<sub>2</sub>N = 86-87. Nerve ring situated at 128-139  $\mu\text{m}$  or 31-36% of the total neck length from the anterior end. Pharyngo-intestinal junction consisting of a short and rounded cardia surrounded by intestinal tissue, all together forming a 10.5-16.5  $\times$  9-12.5  $\mu\text{m}$  structure bulging into intestinal lumen.

*Female*

Genital system mono-opistho-ovarian, with anterior branch reduced to a vestigial uterine sac 6.5-17.5  $\mu\text{m}$  long, less than one-half of body diameter. Posterior branch well developed, 180-243  $\mu\text{m}$  long, occupying 8-10% of body length. Ovary variably long, 77-154  $\mu\text{m}$ , with oocytes first in two or more rows, then in a single row. Oviduct 89-121  $\mu\text{m}$  or 1.9-2.4 body diameters long, consisting of slender portion made of prismatic cells and a moderately developed *pars dilatata* with visible lumen. Uterus a simple tube-like structure, 71-84  $\mu\text{m}$  or 1.5-1.8 body diameters long. Vagina extending inwards 22-26  $\mu\text{m}$  to *c.* one-half (46-57%) of body diameter: *pars proximalis* 12-15.5  $\times$  14-18  $\mu\text{m}$ , with slightly arched walls encircled by very weak musculature; *pars refringens* hardly perceptible, with (in lateral view) two small 5  $\times$  2  $\mu\text{m}$ , weakly sclerotized pieces with a combined width of 10-11  $\mu\text{m}$ ; *pars distalis* 4-5  $\mu\text{m}$  long. Vulva a very anterior, *c.* 4  $\mu\text{m}$  long, somewhat rectangular, longitudinal opening. Prerectum 1.8-5.8, rectum 1.1-1.4 anal body diameters long. Tail long and filiform with acute tip, first tapering more abruptly until about one body diameter behind the anus, then very gradually, inner core extending to 69-75% of total length, so with a visible terminal hyaline portion, caudal pores two pairs, one sublateral, another subdorsal, at the middle of the wider portion of tail.

*Male*

Prerectum 5.7, cloaca 1.6 times the body diameter at level of cloacal aperture long. Genital system diorchic, with opposite testes. In addition to the ad-cloacal pair, located at 8.5  $\mu\text{m}$  from the cloacal aperture, presence of a series of 9-11, shortly spaced, 7-11  $\mu\text{m}$  apart, ventromedian supplements, the most posterior of them located at 68  $\mu\text{m}$  from the ad-cloacal pair, in front of the level of spicule anterior end, thus with a long hiatus. Spicule dorylaimid, curved ventrad, 4.7 times longer than wide, 1.6 times the body diameter at cloacal aperture; head 4  $\mu\text{m}$  or 10% of spicule length, 1.1 times as long as wide, with its dorsal side visibly longer than the ventral one and curved; median piece occupying 59% of spicule width; posterior end 3.5  $\mu\text{m}$ ; ventral hump located at 13  $\mu\text{m}$  or 32% from the spicule anterior end; curvature 129°. Lateral guiding piece 10.5  $\mu\text{m}$  long, relatively coarse, 3.5 times longer than wide, and visibly bifurcate at the end. Tail similar to that of female.

REMARKS

Morphologically, the material examined is very homogeneous, with no relevant difference observed. Morphometrically, it is very similar too, but Sufiyan females display slightly smaller general size than Zirab ones (body 1.95-2.22 vs 2.16-2.42 mm long, respectively) and are less slender (a = 34-44 vs a = 43-54), both always within the known ranges of the species (see below).

Taking the Carbonell & Coomans' (1986) description as reference for comparative purposes, the Iranian females herein examined are identical to type and other European and Iraqi populations studied by these authors, with similar lip region, pharynx, female genital system and caudal region. Nevertheless, the vulva was described (Carbonell & Coomans 1986: 391) as circular, but the ventral view (Fig. 2K) suggests that



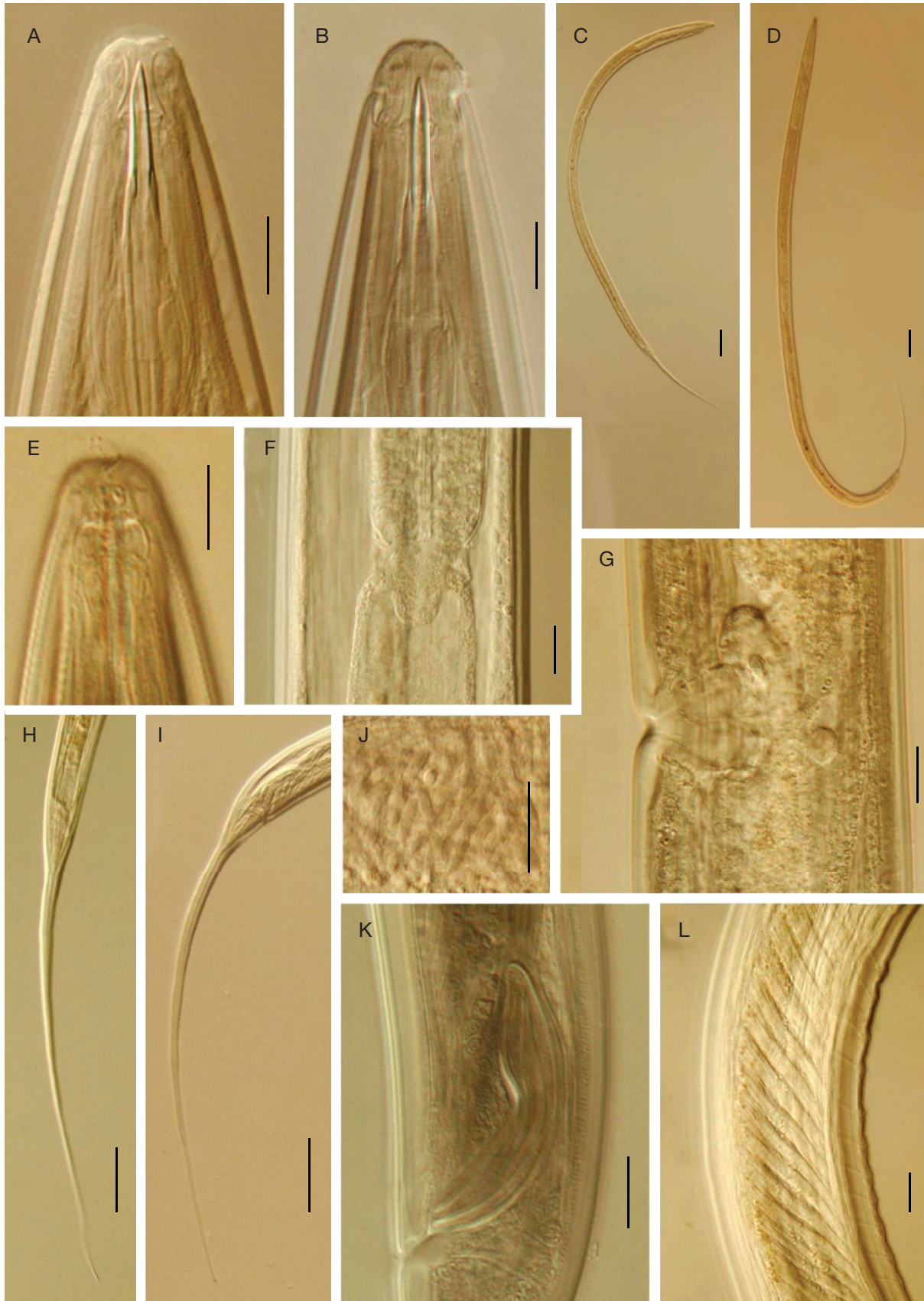


FIG. 3. — *Sylphodorylaimus sylphoides* (Williams, 1959) n. comb. (Sufiyan population): **A**, female, anterior region in lateral median view; **B**, male, anterior region in median ventral view; **C**, female, entire; **D**, male, entire; **E**, anterior region, lateral surface view; **F**, pharyngo-intestinal junction; **G**, vagina region; **H**, female, caudal region; **I**, male, caudal region; **J**, sperm cells; **K**, spicule; **L**, ventromedian supplements. Scale bars: A, B, E-G, J-L, 10  $\mu$ m; C, D, 100  $\mu$ m; H, I, 50  $\mu$ m.

TABLE 2. — Morphometrics of previously known populations of *Sylphodorylaimus sylphoides* (Williams, 1959) n. comb. Total ranges, measurements in µm except L in mm. Reference: 1, Williams (1959); 2, Carbonell & Coomans (1986); 3, Vinciguerra & Gianneto (1987); 4, Popovici (1990); 5, Andrassy (1991); 6, Andrassy (2009); 7, Abolafia & Peña-Santiago (1996); 8, Fadaei-Tehrani & Coomans (2005); \*, specimens of two or more locations.

Population	Mauritius	France	Italy	Iraq	Italy	Romania	Hungary	Spain	Iraq
Reference	1, 2	2	2	2	3	4	5, 6	7	8
n	2♀ (paratypes)	5♀*	2♀	5♀	?♀	3♀	?♀	12♀*	8♀
Character									
L	1.66-1.89	2.01-2.18	2.15-2.34	2.00-2.25	2.15-2.34	2.23-2.48	1.88-2.06	1.8-2.2	2.2-2.5
a	42-56	45-52	51-54	57-64	47-67	44-50	50-58	39-49	50-58
b	5.4-5.9	5.7-6.0	5.9-6.3	5.6-6.0	5.9-6.3	4.6-5.4	5.6-6.1	4.1-6.7	5.5-6.5
c	3.5-3.9	3.6-5.9	5.7	5.0-5.4	5-6	4.9-5.3	4.4-5.4	4.5-5.9	4.5-5.5
c'	20-23	14-24	16	15-18	16	14-18	17-21	12-18	15-19
V	36-38	35-37	36-37	35-36	36-37	35-38	34-35	34-37	34-38
Lip region diameter	11-12	11-14	11	10-12	?	11	9-10	10.5-11.5	?
Odontostyle length-dorsal side	17-19	16-20	16	16-18	?	14-17	14-16	13-15	12-16
Odontophore length	19-20	19-24	22-23	19-24	?	23-24		17-22	?
Neck length	319-324	333-369	357-370	355-370	?	392-413	330-340	300-481	?
Pharyngeal expansion length	141-143	144-160	146-152	148-159	?	181-188	-	112-229	?
Body diameter at neck base	35-39	39-47	41-45	32-38	?	42-45	-	38-60	?
- midbody	35-39	39-48	41-45	32-37	?	44-45	-	44-63	?
- anus/cloaca	21-24	23-25	23-26	22-27	?	24-27	-	23-30	?
Prerectum length	38-43	73-172	?	87-111	?	37-122	-	70-221	?
Rectum/cloaca length	30-36	29-34	?	26-30	?	26-30	-	23-31	?
Tail length	473-500	355-550	379-408	381-440	?	362-431	350-460	318-446	?

it is a short, somewhat rectangular longitudinal opening, a difference that is difficult to appreciate when the specimens are observed, as usual, in lateral view.

Morphometrically, the species displays remarkable intraspecific variability affecting most relevant measurements and ratios (compiled in Table 2), the body (1.66-2.48 mm) and tail lengths (350-550 µm,  $c = 3.5-5.9$ ,  $c' = 14-24$ ) showing especially wide ranges whereas other relevant traits as lip region width (9-14 µm), odontostyle length (12-20 µm) or vulva position ( $V = 34-38$ ) present less variation. When different populations are compared, the ranges of their morphometrics are congruent or largely overlap in general, therefore these populations have been regarded as conspecific and their small differences as geographical variations (Carbonell & Coomans 1986).

#### MOLECULAR CHARACTERISATION

After sequencing and editing, one 28S rDNA gene partial sequence (accession OQ550280) *c.* 800 bp in length was obtained. This sequence was compared with those available from GenBank. Upon BLAST search, it shows high similarity (98-99% identity, three indels/no gap) with sequences (AY593008-AY593010) of European specimens of the same species. Molecular analyses (Fig. 4), also confirmed the identity of the Iranian specimens. Thus, the available sequence forms a maximally supported clade ( $P = 93\%$ ) with other sequences obtained from European nematodes (Holterman *et al.* 2008).

#### DISCUSSION

The study of the two Iranian populations herein examined provides relevant novelties affecting the controversial identity of the family Thornenematidae and brings up again the tax-

onomy of long-tailed dorylaims (Ahmad & Jairajpuri 1982; Coomans & Carbonell 1988; Jairajpuri & Ahmad 1992; Andrassy 2007, 2009). The finding of a bisexual population of *S. sylphoides* n. comb. represents a remarkable novelty as the species had been repeatedly recorded until now, but only females were hitherto known (Carbonell & Coomans 1986). Interestingly, male tail is long and filiform, totally similar to that observed in females, therefore with no sexual dimorphism affecting tail shape in this species. It represents a key relevant trait to elucidating its taxonomy and evolutionary relationships. The filiform-tailed nature of male tail is regarded as a plesiomorphic trait meanwhile the short and rounded male tail reported in type species of *Opisthodorylaimus*, namely *O. maqsoodi* Ahmad & Jairajpuri, 1982, is its corresponding apomorphic state. The shortening of male tail derives from an important change occurred along the postnatal development of the species (Shafqat *et al.* 1991), a process that certainly took place several times and in different ages throughout the evolutionary history of a few dorylaimid taxa (Coomans *et al.* 2001; Peña-Santiago & Álvarez-Ortega 2014). Thus, *S. sylphoides* n. comb. displays a recognisable morphological pattern, easily distinguishable from those observed in other described thornenematid genera, therefore supporting of *Sylphodorylaimus* n. gen. on the basis of relevant morphological evidence; by having long-tailed male, this species does not fit the diagnosis of *Opisthodorylaimus*, whose type species presents rounded-tailed male and sexual dimorphism of tail shape. Other bisexual species of *Opisthodorylaimus*, namely *T. cavalcantii* (Lordello, 1951) and *T. paracavalcantii* Carbonell & Coomans, 1986, obviously have rounded-tailed too, and the remaining species are monosexual, with only females known.

The transference of *O. sylphoides* to the new genus entails homogenisation of the genus *Opisthodorylaimus* as this species

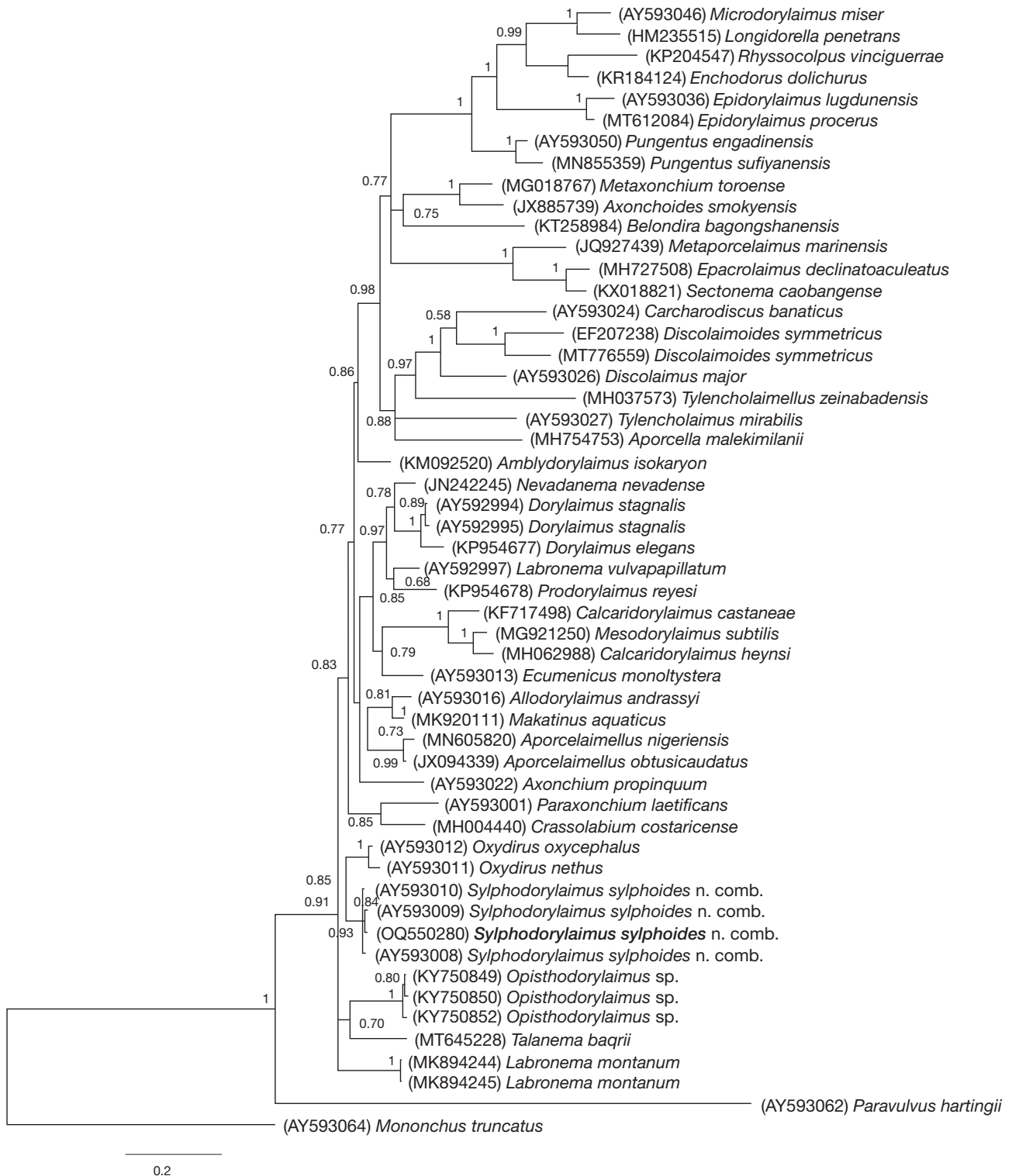


Fig. 4. — Phylogenetic relationships of *Sylphodorylaimus sylphoides* (Williams, 1959) n. comb. Bayesian 50% majority rule consensus tree as inferred from D2-D3 expansion segments of 28S rDNA sequence alignments under the GTR + G + I model. Posterior probabilities are given for appropriate clades. Newly obtained sequence is indicated by bold letter.

TABLE 3. — Morphometrics of *Opisthodorylaimus* Ahmad & Jairajpuri, 1982 species (females). Total ranges, measurements in µm except L in mm. Reference: 1, Carbonell & Coomans (1986); 2, Ahmad & Jairajpuri (1982); 3, Andrásy (2007).

Species	<i>baqrii</i>	<i>caudatus</i>	<i>chamoliensis</i>	<i>cavalcantii</i>	<i>filicaudatus</i>	<i>maqsoodi</i>	<i>mitis</i>	<i>papuanus</i>	<i>paracavalcantii</i>
Reference	1	2	2	1, 2, 3	1	2, 3	3	3	1
n	7♀	14♀	2♀	48♀	5♀	15♀	11♀	6♀	6♀
Character									
L	1.20-1.45	1.28-1.57	1.21, 1.24	0.95-1.30	0.66-0.77	1.30-1.47	1.23-1.37	1.32-1.49	1.33-1.45
a	38-47	31-44	34, 30	25-36	30-37	28-33	28-36	28-36	34-40
b	4.0-5.3	4.0-4.6	4.6, 4.3	3.5-4.8	4.1-5.1	4.1-4.5	4.3-4.7	4.1-4.6	4.1-4.6
c	8-11	11-15	5, 5	9-19	2.3-2.6	4.0-5.7	3.7-4.6	5.6-8.1	16-23
c'	6.5-7.9	4-5	12, 11	2.0-5.2	21-30	10-12	10-16	7.5-8.5	2.5-3.0
V	42-44	44-48	36, 40	40-50	33-38	40-45	38-42	40-46	48-52
Lip region diameter	9.5-11.5	12-13	13, 13	10-14	7-8	13-15	10-15	12-13	12-14
Odontostyle length-dorsal side	10.5-11	18-19	18, 18	11-14	10-11	19-21	15-20	17-18	14-16
Odontophore length	16-17	21-23	21, 21	15-18	14	23-25	?	?	16-18
Neck length	236-290	315-345	262, 285	226-289	138-161	299-360	285-306	286-354	304-324
Pharyngeal expansion length	110-119	?	?	103-155	48-77	?	?	?	152-167
Body diameter at neck base	28-34	?	?	29-37	19-24	?	?	?	34-37
mid-body	29-35	?	?	32-40	19-24	48-52	35-40	40-48	36-40
anus/cloaca	18-22	27-30	21, 23	17-26	12-14	26	?	?	24-27
Prerectum length	42-70	39-48	39, 36	30-75	22-47	41-45	?	?	37-73
Rectum/cloaca length	27-34	38-44	31, 32	24-39	12-20	29-39	?	?	28-36
Tail length	120-149	90-115	246, 254	55-115	289-373	260-311	280-360	180-220	62-80

was its least atypical member. Actually, females of *S. sylphoides* n. comb. are easily distinguishable from those of other representatives of *Opisthodorylaimus* (compared in Table 3), for instance, showing an appreciably larger general size (body length 1.66-2.48 vs 0.66-1.57 mm). Besides, *S. sylphoides* n. comb. presented a more anterior vulva position ( $V = 34-38$ ), only comparable to that of other two species, namely *O. chamoliensis* Ahmad & Jairajpuri, 1982 ( $V = 36-40$ , but body length 1.21-1.24 mm) and *O. filicaudatus* Carbonell & Coomans, 1986 ( $V = 33-38$ , body length 0.66-0.77 mm long), whereas it separates from the remaining species ( $V = 38-52$ ). Finally, but not less interesting, *S. sylphoides* n. comb. is a genuine member of Palearctic (France, Georgia, Germany, Hungary, Iraq, Iran, Italy, Netherlands, Romania, Spain) nematode fauna in spite of its original description in Mauritius Archipelago, which is its only record out of Palearctic range so far. Conversely, and leaving aside the type species of the genus, *O. cavalcantii* (Lordello, 1955), the remaining *Opisthodorylaimus* species display a pantropical distribution (Cameroon, Ecuador, Guadeloupe Island, India, Ivory Coast, Papua-New Guinea, Seychelles Archipelago and Uganda), with only one of them, *O. maqsoodi* Ahmad & Jairajpuri, 1982, sporadically recorded in temperate habitats of Italy and Korea. Regarding the type species, it is widely spread in pantropical areas too (Brazil, Cameroon, India, Indonesia, Ivory Coast, Kenya, Malaysia, Nigeria, Venezuela), and more occasionally found in temperate enclaves (Australia, Azerbaijan, Georgia, South Africa, United States).

Morphologically, *Sylphodorylaimus* n. gen. displays a unique combination of key traits within the subfamily Thornenematinae: absence of both labial and postlabial sclerotiza-

tion, mono-opistho-ovarian females, and long and filiform tail in both sexes. From a cladistic approach, only the drastic reduction of anterior genital branch should be interpreted as an apomorphic state. Nevertheless, its lip region with amalgamated lips, the arched walls of its cheilostome, and the arrangement of its pharyngeal gland nuclei might be also regarded as apomorphic conditions. Andrásy (2007) put emphasis on two of these apomorphic features, namely the arrangement of pharyngeal gland nuclei and the nature of female genital system, to support a higher affinity of *Opisthodorylaimus* with other thornenematid taxa than with Dorylaimidae. This same idea is applicable to *Sylphodorylaimus* n. gen.

Unfortunately, results of molecular analysis (Fig. 4), provide no robust evidence to the elucidation of evolutionary relationships of the new genus. On the one hand, the resolution of Dorylaimina superclade branching is deficient. On the other hand, *S. sylphoides* n. comb. sequences form part of a small, and not highly supported (PP = 84%) clade also including two sequences of the genus *Oxydirus* Thorne, 1939 (Belondiridae Thorne, 1939), a scenario that, far from clarifying or confirming previous ideas, introduces new uncertainties.

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