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Data on Some Species of the Genus *Coslenchus* Siddiqi, 1978 (Rhabditida, Tylenchidae) from Iran

Yousef Panahandeh, Ebrahim Pourjam, and Majid Pedram

Abstract: Data on five known species of the genus *Coslenchus* are provided. Morphological and morphometric data are given for all studied species. Three of the recovered species were also characterized by molecular phylogenetic data. The species *C. leiocephalus* was studied for the first time since its original description. Males of the species, *C. franklinae* and *C. oligogyrus* were described for the first time and the species *C. oligogyrus* was reported from Iran for the first time. In molecular phylogenetic studies based on partial sequences of 28S rDNA D2/D3 fragments, all species formed a clade with high Bayesian posterior probability in Bayesian inference, indicating the monophyly of the genus. The clade of *Coslenchus* spp. formed a highly supported monophyletic group, a sister clade to two species of the genus *Aglenchus*.

Key words: Ardabil grasslands, Atylenchinae, Bayesian, LSU rRNA gene, phylogeny, Sabalan region.

Siddiqi (1978) erected the genus *Coslenchus* and transferred *Tylenchus costatus* de Man, 1921 to it as the type species. Subsequently, he added six other species namely *C. alacinatus* Siddiqi, 1981, *C. bisexualis* Siddiqi, 1981, *C. franklinae* Siddiqi, 1981, *C. multigyrus* Siddiqi, 1981, *C. pycnocephalus* Siddiqi, 1981, and *C. turkeyensis* Siddiqi, 1981 and provided a key for identification of the species of the genus (Siddiqi, 1981). Andrássy (1982) enriched the genus by adding eight species. Geraert (2008) provided an excellent overview on the genera and species of the family Tylenchidae Örley, 1880. According to him, there are currently 38 well-established species under the genus. A review on the species of the genus occurring in Iran is provided by Karegar and Geraer (1996) and according to Ghaderi et al. (2012), 11 species of the genus occur in Iran. According to molecular phylogenetic studies on species of the genus using D2/D3 domain of 28S rRNA gene, the genus *Coslenchus* has close phylogenetic affinities with genus *Aglenchus* Andrássy, 1954 (Subbotin et al., 2006; Palomares-Rius et al., 2009; Atighi et al., 2012), a morphologically close genus.

During our taxonomic studies on tylenchid fauna of grasslands of Ardabil province, some tylenchid taxa were already recovered and reported (Panahandeh et al., 2014, 2015a, 2015b). The present paper illustrates some *Coslenchus* spp. recovered from the region.

Materials and Methods

Soil samples were collected from several points of grasslands of the Sabalan region, Ardabil province, north western Iran during 2012 to 2015. The nematode specimens were extracted from soil using the tray method (Whitehead and Hemming, 1965) and hand-picked under a Nikon SMZ1000 stereomicroscope. The collected individuals were heat-killed by adding boiling 4% formalin solution, and transferred to anhydrous glycerin according to De Grisse (1969). Measurements and drawings were performed using a drawing tube attached to a Nikon E600 light microscope. For examining the number of the longitudinal ridges, the cross section was provided according to Atighi et al. (2013). Photographs were taken using an Olympus DP72 digital camera attached to an Olympus BX51 light microscopes powered with differential interference contrast (DIC).

For molecular phylogenetic studies, a single nematode specimen of each studied species was picked out and transferred to a small drop of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, QIAGEN Inc., Valencia CA) on a clean slide and squashed using a clean slide cover. The suspension was collected by adding 30 µl AE buffer. DNA samples were stored at −20°C until used as PCR templates. Primers for 28S rDNA D2/D3 amplification were forward primer D2A (5′-ACAAGTACCGTGAGGGAAAGT-3′) and reverse primer D3B (5′-TGCGAAGGAACCAGCTACTA-3′) (Nunn, 1992). The 30 µl PCR mixture contained 16.5 µl distilled water, 3 µl 10× PCR buffer , 0.6 µl dNTP mixture, 1.2 µl 50 mM MgCl2, 1.5 µl of each primer (10 pmoles/µl), 0.75 µl of *Taq* polymerase (CinnaGen, Tehran, Iran, 5 U/µl), and 5 µl of DNA template. The thermal cycling program was as follows: denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. The PCR products were sequenced in both directions using the same primers in both directions with an ABI 3730XL sequencer (Bioneer Corporation, South Korea) and were deposited into the GenBank database (accession numbers KM817175 for *C. franklinae*, KM817176 for *C. leiocephalus*, KM817177 and KM817178 for *C. oligogyrus* female and male). The selected DNA sequences for phylogenetic analyses were aligned using MUSCLE (Edgar, 2004) as implemented in MEGA6 (Tamura et al., 2013). To eliminate the ambiguously aligned parts, the online version of Gblocks 0.91b

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(Castresana, 2000) with all the three less stringent parameters was used (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The model of base substitution was selected using MrModeltest 2 (Nylander, 2004). The Akaike-supported model, a general time reversible model including among-site rate heterogeneity and estimates of invariant sites (GTR + G + I), was selected for the phylogenetic analyses. Bayesian analysis was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), running the chains for one million generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using the 50% majority rule. The stationarity of each run was evaluated using AWTY (Nylander et al., 2008). Tracer v1.5 software (Rambaut and Drummond, 2009) was used to visualize the results of each run, to check the effective sample size of each parameter. A maximum likelihood (ML) tree was reconstructed by using RaxmlGUI 1.1 (Silvestro and Michalak, 2012) software using the same nucleotide substitution model as in the BI in 1,000 bootstrap (BS) replicates for both datasets. For all phylogenetic analyses, *Aphelenchus avenae* Bastian, 1865 (accession number JQ348400) was used as outgroup taxon. The output file of the used phylogenetic program was visualized using Dendroscope V.3.2.8 (Huson and Scornavacca, 2012) and redrawn in CorelDRAW software version16. The Bayesian posterior probability (BPP) and ML BS values exceeding 0.70% and 70%, respectively, are given on appropriate clades in the shape BPP/ML BS.

The used classic taxonomic framework of *Tylenchinae* Örley, 1880 is according to Geraert (2008).

Results

Coslenchus costatus (de Man, 1921) Siddiqi, 1978

Measurements: Listed in Table 1.

The morphometric data and morphological characters of the recovered population of this species from Divan Dashi region are in full agreement with those given for the original population (Siddiqi, 1978), the Iranian population studied by Karegar and Geraert (1996) and the data given by Geraert (2008). It is characterized by having cuticle with 14 longitudinal ridges and four incisures in lateral field, slightly offset head with three or four annuli, large vulval flaps, absence of postvulval uterine sac (PUS) and a filiform tail.

Coslenchus multigyrus Siddiqi, 1981

Measurements: Listed in Table 2.

The morphometric data and morphological characters of the recovered population of the species from grasslands of Sardabeh region are in congruence with the given data in its original description by Siddiqi (1981), a population of the species previously reported from Iran by Karegar and Geraert (1996) and the data given by Geraert (2008). It is characterized by having cuticle with 23 to 26 longitudinal ridges and four incisures in lateral field, rudimentary or small vulval flaps, short horn-shaped PUS, and long and filiform tail.

Coslenchus franklinae Siddiqi, 1981
(Figs. 1; 3A–F)

Measurements: Listed in Table 1.

Female: Body slightly arcuate when heat relaxed. Cuticle with 18 longitudinal ridges (except lateral field) and coarse annuli, annulus 2.1 to 2.8 μm wide at mid-body. Lateral field with four incisures, forming two longitudinal ridges, separated from each other by a narrow groove, most conspicuous in cross section. Head anteriorly truncate, continuous with body contour with three annuli. Stylet delicate, with the conus less than half its total length, knobs rounded to slightly posteriorly sloping. Procorpus cylindrical, median bulb oval with refractive valves, isthmus slender and long, basal bulb pyriform and small. Excretory pore at anterior end of basal bulb, immediately after hemizonid. Reproductive system mono-prodelphic, composed of an outstretched ovary with oocytes mostly in two rows (except germinal zone), short oviduct, offset spermatheca appearing as bilobed in lateral view, containing spheroid sperm cells, crustaformeria, uterus, vagina perpendicular to body axis and slightly anteriorly directed, vulva sunken in body with large vulval flaps and short PUS, less than half corresponding body width. Tail regularly tapering toward end with pointed tip.

Male: Similar to female in general morphology, except for reproductive system. Body slightly ventrally bent after fixation. Cuticle with 18 longitudinal ridges (except lateral field) and coarse annuli, annulus 2.1 to 2.6 μm wide at mid-body. Head continuous with body contour, anteriorly truncate, with three annuli. Stylet delicate, similar to that of female. Dorsal gland orifice at 1 μm distance from the knobs. Pharynx and its parts similar to that of female. Testis straight, outstretched, its proximal tip bluntly rounded, spermatocytes in two rows (after germinal zone), *vas deferens* full of spheroid sperm cells. Spicules tylenchoid, small, slightly arcuate ventrally. Gubernaculum crescent shape and fixed. Cloacal lips protruding. Bursa short, adanal, with crenate border. Tail similar to that of female.

Remarks: Morphological characters and range of morphometric data of present population of *C. franklinae* are in full agreement with the data given in its original description (Siddiqi, 1981), the Polish population (Brzeski, 1987), one Iranian population reported by Karegar and Geraert (1996) and the ranges given by Geraert (2008). Male of the species was recovered for the first time and described in present study.

Coslenchus franklinae was originally described by Siddiqi (1981) from Nigeria. Present Iranian population

TABLE 1. Morphometrics of *Coslenchus costatus* and *Coslenchus franklinae* from Iran compared with original description.[a]

Origin	*C. costatus*			*C. franklinae*		
	Iranian population		Geraert (2008)	Iranian population		Siddiqi (1981)
Characters	Female	Male	Female	Female	male	Female
n	11	2	–	20	6	20
L	488 ± 32 (430–525)	432 ± 12 (424–441)	390–600	519 ± 22 (458–550)	500 ± 29 (460–543)	430 (330–490)
a	25.6 ± 1.9 (22.6–29.2)	26.3 ± 1.9 (24.9–24.6)	22–34	28.7 ± 2.1 (25.4–32.9)	31.8 ± 4.1 (24.4–36.2)	28 (25–32)
b	5.3 ± 0.2 (5.0–5.6)	4.9 ± 0.1 (4.9–5.0)	–	5.4 ± 0.3 (4.7–6.2)	5.3 ± 0.3 (4.8–5.8)	5.3 (4.7–5.8)
c	5.6 ± 0.3 (5.2–5.9)	6.6 ± 1.0 (5.9–7.4)	4.2–7.5	4.6 ± 0.2 (4.1–5.0)	4.4 ± 0.2 (4–4.6)	5.2 (4.8–6.1)
c′	8.2 ± 1.1 (6.6–10.0)	6.6 ± 0.8 (6.0–7.2)	6–15	10.7 ± 0.9 (9.3–12.3)	11.8 ± 0.8 (10.9–13.2)	8.5 (6.2–2.8)
V or T	65.6 ± 1.7 (62.9–68.0)	33.0 ± 2.4 (31.4–34.7)	60–71	62 ± 2 (55.2–65.0)	30.5 ± 3.1 (26.1–35.3)	64.5 (62–68)
V′	80.0 ± 1.8 (76.0–82.3)	–	77–83	79.4 ± 2.6 (69.7–81.4)	–	–
Stylet	10.3 ± 0.7 (9.5–12.0)	9.5 ± 0.7 (9–10)	9–13	10.5 ± 0.5 (10.0–11.5)	10.5 ± 0.5 (10–11)	11.5 (11–12)
MB	45.9 ± 1.9 (43.4–49.5)	46.0 ± 1.5 (44.9–47.1)	45–50	47.1 ± 1.5 (45.3–50.0)	47.5 ± 0.8 (46.3–48.5)	45 (43–48)
E. pore	74.6 ± 2.7 (70–79)	74.5 ± 4.9 (71–78)	66–84	77.6 ± 3.5 (72–85)	77.5 ± 3.1 (74–82)	66 (56–71)
Pharynx	92.9 ± 4.5 (85–99)	88.0 ± 1.4 (87–89)	78–101	96.1 ± 3.2 (87–101)	95 ± 2.2 (92–98)	82 (74–92)
Head-vulva	319.9 ± 19.0 (289–351)	–	–	322 ± 18 (285–355)	–	274 (228–310)
Body width	19.1 ± 0.8 (18–20)	10	–	18.1 ± 1.3 (16–20)	16 ± 2.5 (14–21)	–
Rst	–	–	–	89.0 ± 0.9 (8–11)	8.2 ± 0.8 (7–9)	–
Rex	34.3 ± 2.1 (31–37)	32.0 ± 2.8 (30–34)	–	37.8 ± 2.2 (33–43)	36.5 ± 2.3 (32–38)	39 (35–45)
Roes	40.9 ± 2.5 (37–45)	47.0 ± 8.5 (41–53)	–	45.2 ± 2.7 (41–51)	43.5 ± 1.6 (41–46)	47 (42–52)
Rv	118.7 ± 7.3 (106–133)	–	–	131.5 ± 6.5 (119–145)	–	141 (132–152)
Ran	147.2 ± 10.5 (134–170)	148.5 ± 2.1 (147–150)	–	164.5 ± 6.9 (155–183)	161.2 ± 6.4 (150–167)	174 (163–186)
Rvan	28.5 ± 4.8 (23–37)	–	–	33.0 ± 4.2 (29–47)	–	33 (30–37)
Annuli width	2.7 ± 0.2 (2.4–2.9)	–	2.1–3.6	2.5 ± 0.2 (2.1–2.8)	2.4 ± 0.2 (2.1–2.6)	
Vulva-anus	80.4 ± 9.4 (67–97)	–	–	82.4 ± 11.9 (67–125)	–	71 (58–82)
Tail	88.2 ± 8.3 (73–100)	66.0 ± 8.5 (60–72)	71–122	114.0 ± 6.6 (103–127)	114 ± 5.8 (106–120)	81 (59–103)
Tail/vulva-anus	1.1 ± 0.1 (0.9–1.2)	–	1.0–1.8	1.4 ± 0.2 (0.9–1.7)	–	–
Spicule	–	16.5 ± 2.1 (15–18)	–	–	15.8 ± 0.4 (15–16)	–
Gubernaculum	–	7	–	–	6.2 ± 0.4 (6–7)	–

[a] All measurements are in μm and in the form: mean ± SD (range).

TABLE 2. Morphometrics of *Coslenchus leiocephalus* and *Coslenchus multigyrus* from Iran compared with original description.[a]

Origin	*C. leiocephalus*		*C. multigyrus*	
	Iranian population	Brzeski (1998)	Iranian population	Geraert (2008)
Character	Female	Female	Female	Female
n	21	46	10	–
L	424 ± 20 (390–479)	460 (380–510)	530 ± 29 (494–579)	410–650
a	24.6 ± 1.7 (22.2–28.2)	27 (22–33)	29.6 ± 2.3 (26.3–32.0)	25–40
b	5.2 ± 0.2 (4.8–5.5)	5.3 (5.0–5.7)	5.8 ± 0.4 (5.4–6.6)	–
c	5.2 ± 0.2 (4.6–5.4)	5.0 (4.5–6.0)	4.4 ± 0.2 (4.1–4.9)	3.4–5.4
c′	7.8 ± 1.0 (6.7–10.1)	9.2 (7.0–1.1)	11.3 ± 1.2 (8.9–12.8)	8–17
V	66.3 ± 1.4 (63.9–71.1)	64 (62–69)	62 ± 1 (60.5–63.7)	55–67
V′	82.3 ± 1.7 (79.4–88.7)	81 (78–85)	80.1 ± 1.0 (79–81.8)	79–83
Stylet	10.9 ± 0.7 (10.0–12.5)	10.8 (10–12)	10.5 ± 0.6 (10–11.5)	10–12
MB	48.8 ± 3.9 (36.6–60.5)	47 (44–54)	47.2 ± 1.2 (45.4–49)	44–50
E. pore	71.0 ± 4.2 (65–80)	63–76	78.3 ± 4.2 (71–85)	71–78
Pharynx	82.1 ± 2.6 (78–91)	84 (73–92)	92.0 ± 4.1 (85–98)	74–101
Head-vulva	281 ± 13 (260–321)	–	328 ± 20 (304–369)	–
Body width	17.3 ± 1.1 (15–19)	–	18.0 ± 1.7 (16–22)	–
Rst	5.5 ± 0.6 (4–6)	–	5.9 ± 0.7 (5–7)	–
Rex	27.8 ± 1.6 (25–30)	34 (26–41)	37.7 ± 2.3 (34–41)	–
Roes	32.5 ± 2.0 (29–36)	–	44.9 ± 2.6 (39–49)	–
Rv	101 ± 5 (94–112)	123 (97–154)	156.0 ± 9.5 (142–175)	–
Ran	123.2 ± 4.9 (116–132)	152 (115–185)	192.7 ± 12.4 (177–216)	–
Rvan	22.2 ± 1.7 (19–26)	28 (18–34)	37.7 ± 3.8 (33–44)	–
Annuli width	2.8 ± 0.1 (2.5–3.0)	1.9–2.8	2.1 ± 0.1 (1.9–2.4)	1.9–2.8
Vulva-anus	62.2 ± 4.7 (55–75)	–	81.5 ± 4.6 (75–89)	–
Tail	82.4 ± 5.2 (75–93)	93 (73–110)	120.0 ± 9.6 (107–141)	99–143
Tail/vulva-anus	1.3 ± 0.1 (1.2–1.6)	1.3 (1–1.6)	1.5 ± 0.1 (1.3–1.8)	1.2–1.7

[a] All measurements are in μm and in the form: mean ± SD (range).

was recovered from rhizosphere of unknown grasses in Shafi Gonei and Arkhalti region in Sabalan grasslands, Ardabil province, northwestern Iran.

Coslenchus leiocephalus Brzeski, 1998

(Figs. 2; 3G–K)

Measurements: Listed in Table 2.

Female: Body straight or slightly arcuate ventrally when heat relaxed. Cuticle annuli prominent, 2.5 to 3.0 μm wide at mid-body, having 22 longitudinal ridges except lateral field, the latter with two bands. Head unstriated, separated from body contour by a shallow constriction. Cephalic framework delicate. Stylet developed, its conus 30% to 50% of total length, knobs rounded and large. Dorsal gland orifice at 1.0 to 1.5 μm distance from knobs. Pharynx composed of a slender procorpus, ovoid median bulb with distinct valve, narrow slender isthmus and pyriform and small terminal bulb. Nerve ring encircling middle of isthmus. Excretory pore at the level with anterior end of terminal bulb. Hemizonid just posterior to excretory pore. Intestine simple. Reproductive system mono-prodelphic, composed of an outstretched ovary with oocytes mostly in one row (except germinal zone), oviduct, offset spermatheca without sperm, crustaformeria, uterus, anteriorly directed vagina with swollen walls, no PUS, vulva sunken in body with large vulval flaps and hardly visible epiptygmata. Tail regularly tapering with hair like terminus.

Males: Not found.

Remarks: The recovered population of *C. leiocephalus* from Divan Dashi and Agh Masjed from the rhizosphere of milkvetch (*Astragalus* sp.) and white clover (*Trifolium* sp.) in grasslands of Sabalan region, Ardabil province, northwestern Iran, studied herein, is in full morphological and morphometric agreement with the data given in its original description by Brzeski (1998). The species is only known from its type locality and present study represents the second report of the species after its original description.

A number of 18 to 20 longitudinal ridges (except lateral fields) is reported for the species in the original description, while presently studied population had 22 longitudinal ridges on its cross sections with no variation.

Coslenchus oligogyrus Brzeski, 1987

(Figs. 4,5)

Measurements: Listed in Table 3.

Females: Body slightly ventrally bent after heat relaxation. Cuticle annuli prominent, annulus 2.4 to 2.9 μm wide at mid-body, with 10 longitudinal ridges except lateral field, the latter with two bands separated by a narrow groove, appearing as four incisures in cross section, the two middles ones very close to each other. Cephalic region bearing three annuli. Stylet thin, its conical part 35% to 45% of total length with small rounded to slightly posteriorly directed knobs. Dorsal gland orifice at 1 to 2 μm distance from the knobs. Procorpus cylindrical, posteriorly joining to an oval median bulb with moderately developed valve, isthmus slender, and narrow basal bulb saccate. Excretory at the level with anterior end of basal bulb. Hemizoind just

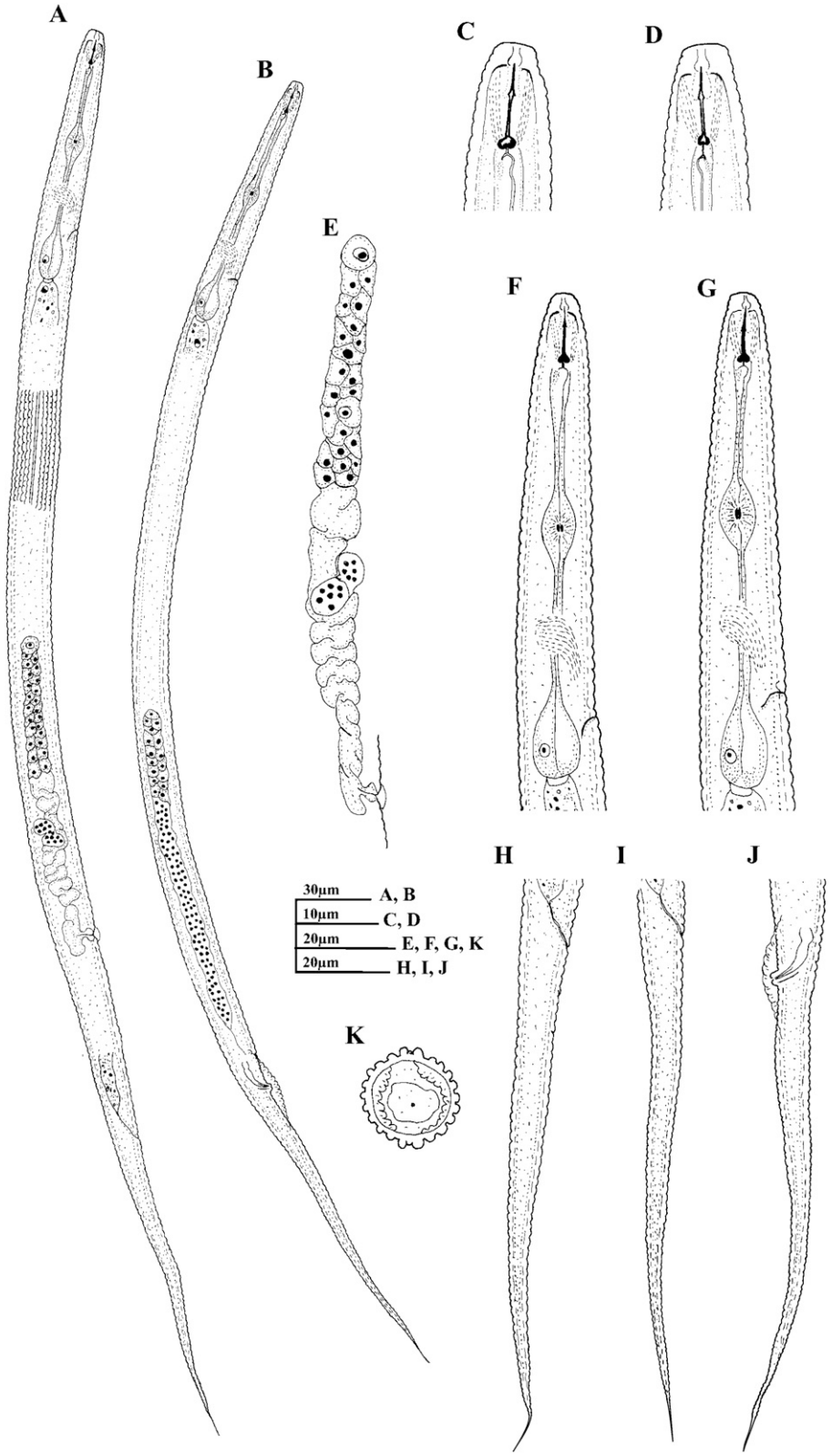

Fig. 1. Iranian population of *Coslenchus franklinae*. A. Female entire body. B. Male entire body. C, D. Female anterior end. E. Female reproductive system. F, G. Female pharyngeal region. H, I. Female tail. J. Male tail and cloacal region. K. Female mid-body cross section.

anterior to excretory pore. Reproductive system monoprodelphic, composed of an outstretched ovary with oocytes mostly in one row in proximal half and two rows in distal part, oviduct, oval spermatheca sometimes appearing bilobed containing spheroid sperm, crustaformeria, uterus, anteriorly directed vagina, no PUS, and vulva sunken in body with large vulval flaps. Tail conical, regularly tapering with filiform terminus.

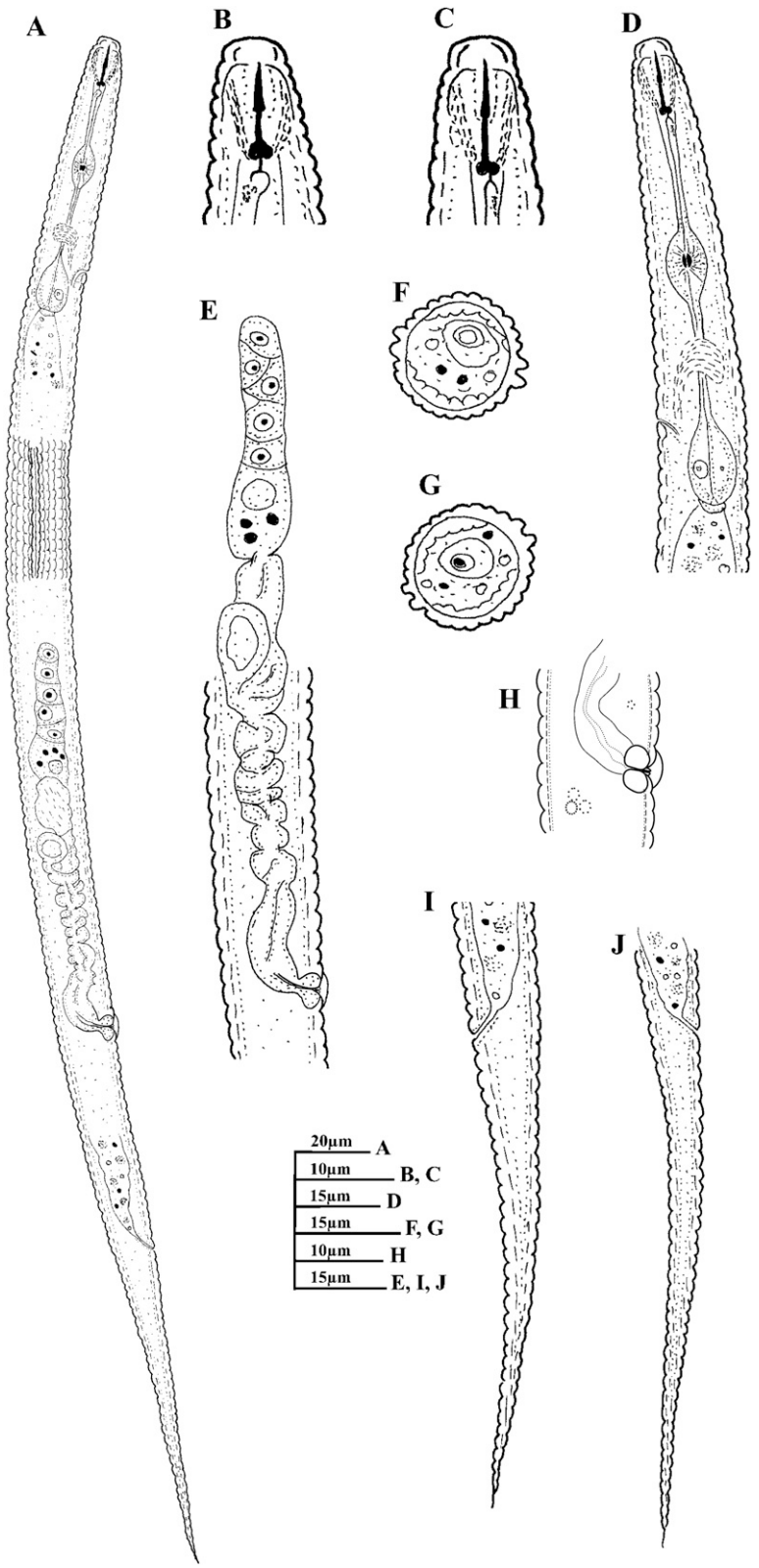

FIG. 2. Iranian population of *Coslenchus leiocephalus*. Female: A. Entire body. B, C. Anterior end. D. Pharyngeal region. E. Reproductive system. F, G. Mid-body cross section. H. Vulval region. I, J. Tail.

Males: General morphology similar to that of female, except for sexual dimorphism. Body more ventrally bent in distal part after heat relaxation. Cuticle with 10 longitudinal ridges (except lateral field) and coarse annuli, annulus 2.2 to 2.5 µm wide at mid-body. Head similar to that of female. Stylet thin, with rounded to slightly posteriorly sloping knobs. Procorpus cylindrical, median bulb oval with moderately developed valve, isthmus

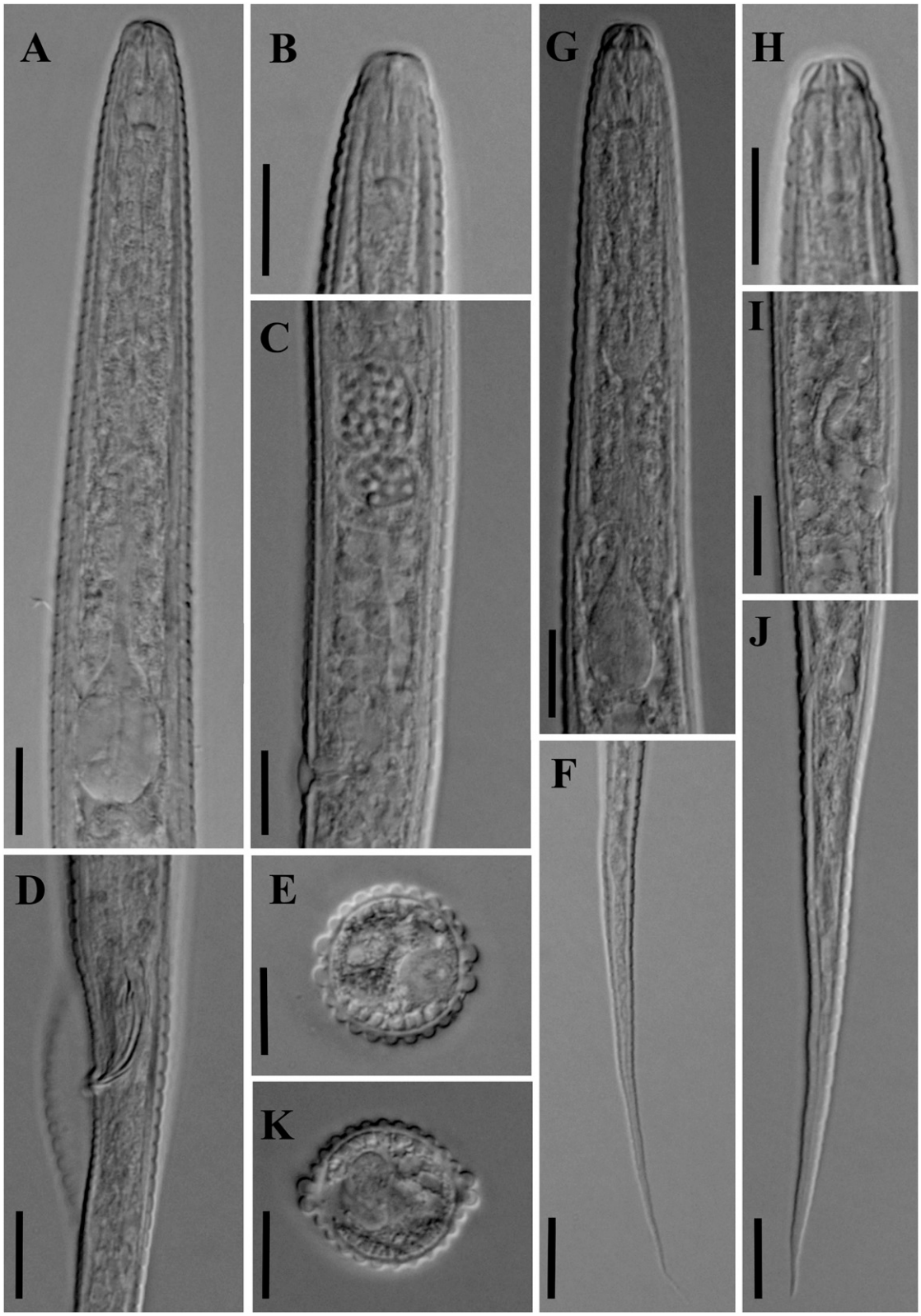

Fig. 3. Iranian population of *Coslenchus franklinae* (A–F). A. Female pharynegeal region. B. Female anterior end. C. Female part of reproductive system. D. Male cloacal region. E. Female mid-body cross section. F. Female tail. Iranian population of *Coslenchus leiocephalus* (G–K). G. Female pharynegeal region. H. Female anterior end. I. Female vulval region. J. Female tail. K. Female mid-body cross section. All scale bars = 10 μm.

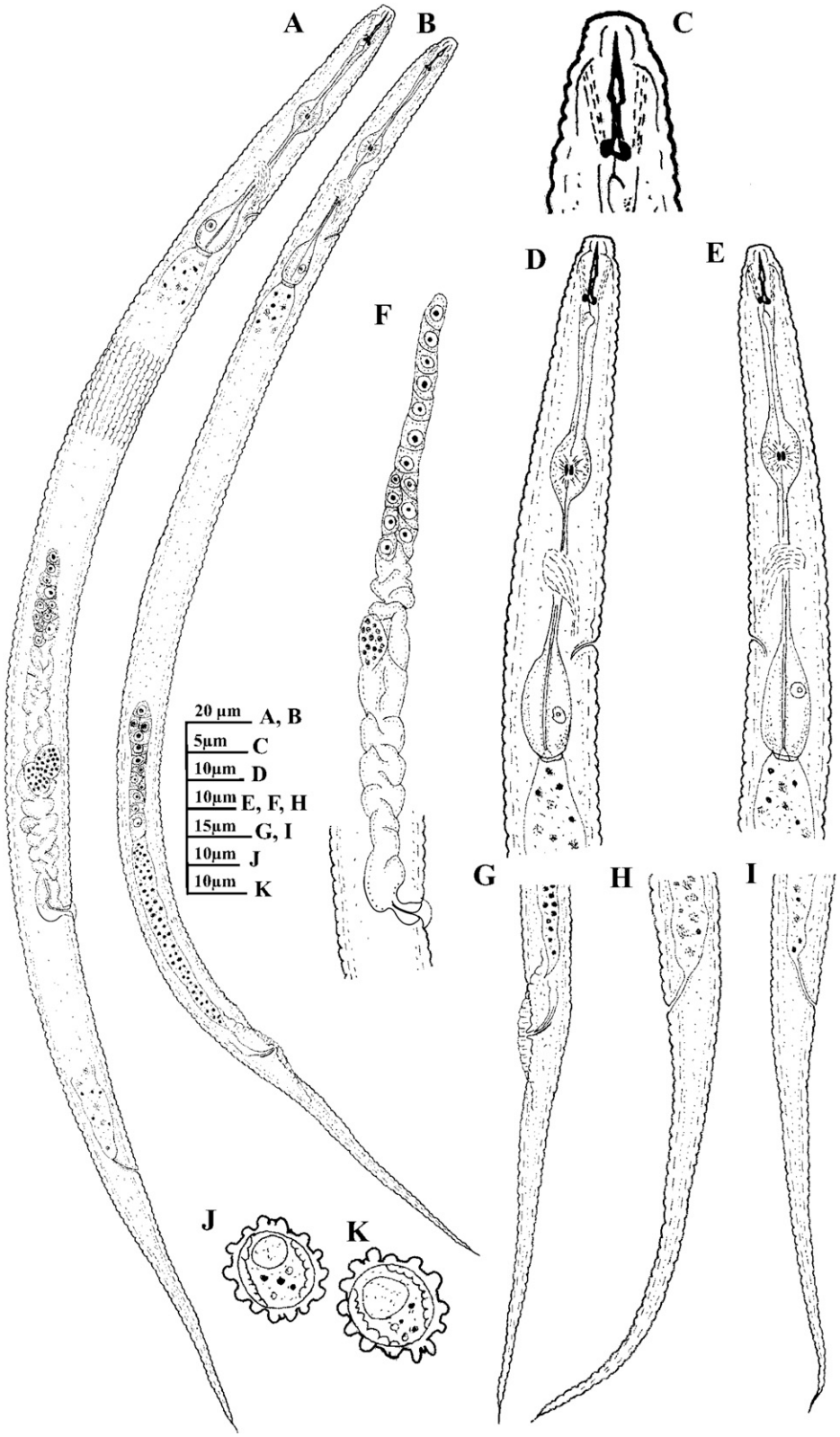

FIG. 4. Iranian population of *Coslenchus oligogyrus*. A. Female entire body. B. Male entire body. C. Female anterior end. D, E. Female pharyngeal region. F. Female reproductive system. G. Male tail and cloacal region. H, I. Female tail. J, K. Female mid-body cross section.

slender, and terminal bulb saccate. Testis straight, spermatocytes in one or two rows after germinal zone, *vas deferens* full of spheroid sperm cells. Spicules tylenchoid, small, slightly arcuate ventrally. Gubernaculum crescent shaped and fixed. Cloacal lips protruding. Bursa short, adanal, with crenate border. Tail similar to that of female.

Remarks: The Iranian population of *C. oligogyrus* is in full morphological and morphometeric agreement

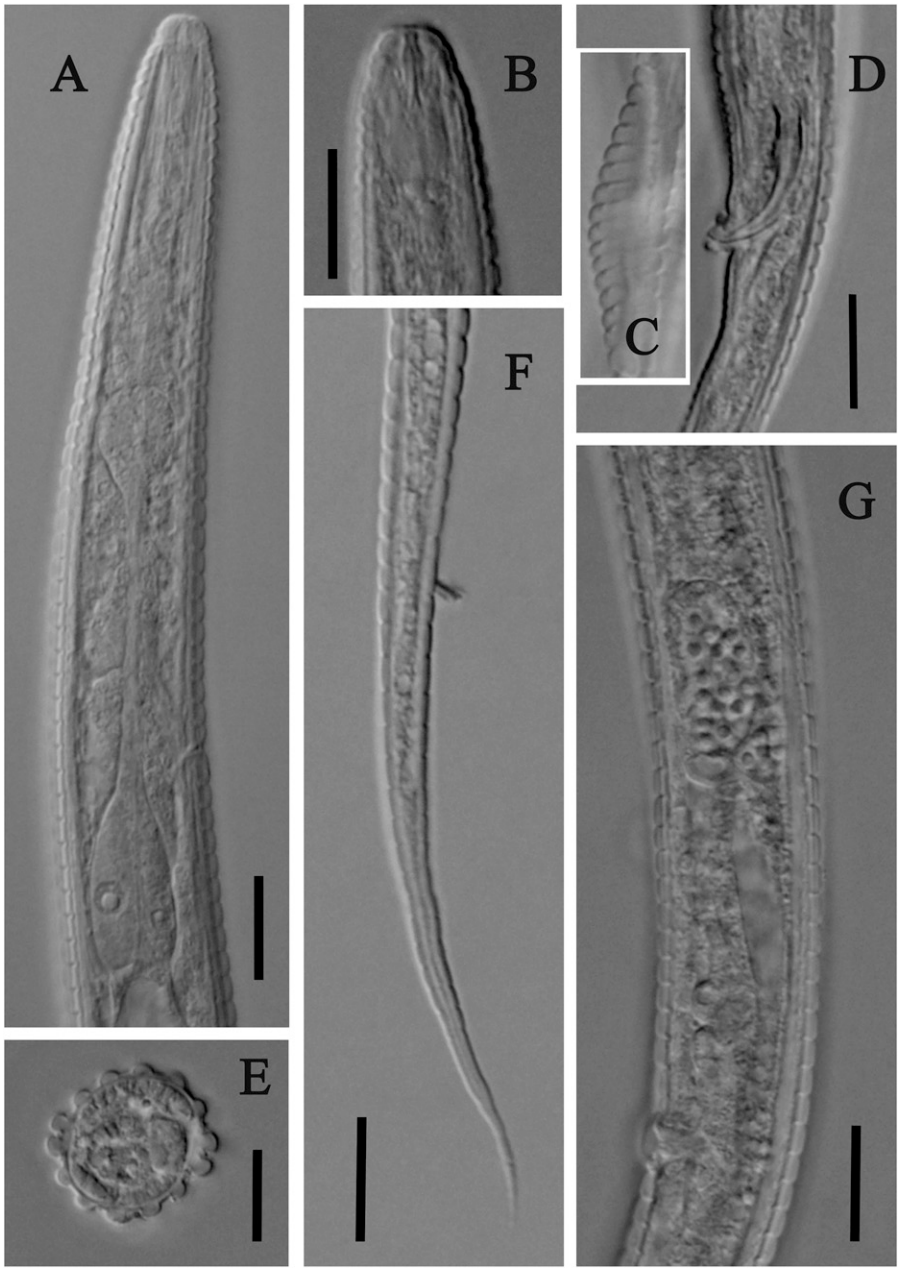

Fig. 5. Iranian population of *Coslenchus oligogyrus*. A. Female pharynegeal region. B. Female anterior end. C. Male bursa. D. Male cloacal region. E. Female mid-body cross section. F. Female tail. G. Female part of reproductive system. All scale bars = 10 μm.

with its original description (Brzeski, 1987) and the data given by Geraert (2008).

Our population was recovered from Palangloo grasslands in Sabalan region, Ardabil province, northwestern Iran.

Male of the species was recovered for the first time and described in present study.

Molecular phylogenetic status: The 28S rDNA D2/D3 sequences of almost all species of the genus *Coslencus* deposited in GenBank database and several other species/genera of Tylenchina Chotwood, 1950 were selected and used in phylogenetic analyses. In total, 53 species/isolates (including one aphelenchid outgroup species) were analyzed. The 28S dataset was composed of 666 total characters of which 435 characters were variable. The average nucleotide composition was as follows: 22.0% A, 18.9% C, 31.8% G, and 24.4% T.

Figure 6 represents the phylogenetic tree reconstructed using the abovementioned dataset. Using *Aphelenchus avenae* as the outgroup taxon, two moderately and weakly supported main clades A and B are inferred in Bayesian tree (0.69 and 0.51 BPP, respectively). The phylogenetic relationships between the genera of major clade A are appropriately resolved, and the clade is divided to two minor clades a and b. The clade Aa contains currently sequenced species of two genera *Tylenchus* Bastian, 1865 and *Filenchus* Andrássy, 1954, two members of Tylenchinae Örley, 1880 (sensu

TABLE 3. Morphometrics of *Coslenchus oligogyrus* from Iran compared with original description.[a]

Origin	Iranian population		Brzeski (1987)
Characters	Female	Male	Female
n	16	10	20
L	504 ± 23 (468–556)	463.7 ± 30.5 (428–527)	520 (460–560)
a	28.6 ± 4.0 (23.4–35.9)	33.2 ± 2.0 (30.7–36.5)	25 (23–30)
b	5.2 ± 0.2 (4.9–5.6)	5.0 ± 0.2 (4.7–5.4)	5.0 (4.6–5.7)
c	5.1 ± 0.3 (4.7–5.9)	4.7 ± 0.1 (4.5–4.9)	5.7 (5.4–6.0)
c′	9.5 ± 1.5 (7.7–12.2)	11.1 ± 0.9 (9.5–12.8)	8 (7–9)
V or T	63.4 ± 1.6 (60.6–65.5)	28.8 ± 1.6 (27.0–31.4)	65 (61–66)
V′	78.9 ± 1.7 (76.6–82.1)	–	79 (74–80)
Stylet	10.1 ± 0.8 (8–11)	9.6 ± 0.4 (9–10)	11 (10–12)
MB	46.3 ± 1.8 (43.1–51.2)	46.3 ± 1.0 (44.4–47.3)	47 (42–48)
E. pore	77.4 ± 3.0 (72–83)	71.6 ± 3.2 (68–77)	83 (77–88)
Pharynx	97.6 ± 4.6 (86–103)	93.5 ± 4.1 (89–102)	102 (91–109)
Head-vulva	319 ± 16 (292–363)	–	–
Body width	17.9 ± 1.9 (15–20)	14.0 ± 0.9 (13–15)	–
Rst	8.4 ± 1.1 (6–10)	8.8 ± 0.8 (8–10)	–
Rex	35.6 ± 1.8 (32–39)	37.2 ± 2.0 (34–40)	32 (30–34)
Roes	43.6 ± 2.6 (39–48)	47.0 ± 2.4 (43–50)	–
Rv	124.7 ± 4.2 (115–132)	–	109 (101–117)
Ran	159.3 ± 5.8 (145–166)	159.8 ± 5.0 (154–169)	137 (125–145)
Rvan	34.5 ± 3.8 (29–42)	–	27 (24–37)
Annuli width	2.6 ± 0.1 (2.4–2.9)	2.3 ± 0.1 (2.2–2.5)	3.5 (3.0–3.8)
Vulva-anus	84.9 ± 7.2 (72–96)	–	–
Tail	99.6 ± 8 (85–114)	99.1 ± 7.5 (87–112)	90 (76–100)
Tail/vulva-anus	1.2 ± 0.1 (1.0–1.4)	–	1.0 (0.8–1.1)
Spicule	–	14.9 ± 1.2 (13–17)	–
Gubernaculum	–	6.4 ± 0.5 (6–7)	–

[a] All measurements are in μm and in the form: mean ± SD (range).

Geraert, 2008). The clade does not contain the genus *Malenchus* Andrássy, 1968, a conflicting observation with the classic taxonomic placement for the genus inside Tylenchinae. The clade Bb contains two genera *Aglenchus* Andrássy, 1954 and *Coslenchus*, the members of Atylenchinae Skarbilovich, 1959 (sensu Geraert, 2008). No other genus/genera of Atylenchinae is sequenced for its/their 28S rDNA D2/D3 fragment. The clade also received the high BPP (0.99) and 65% ML BS values, indicating the monophyly of the two genera. The monophyly of currently sequenced species of the genus *Coslenchus* for their 28S rDNA D2/D3 genomic fragment is also confirmed. The three recently sequenced species/isolates of the genus all placed inside the clade of *Coslenchus*.

The phylogenetic relationships of the members of the major clade B are not fully resolved due to polytomy, a common phenomenon in molecular phylogenetic analyses of Tylenchina, and especially Tylenchidae, and an observation out of the aims of present study to discuss on.

DISCUSSION

Currently there are different classic taxonomic frameworks for Tylenchidae (e.g., the frameworks given by Siddiqi [2000], Geraert [2008]). The family has also attracted less attention from the aspects of molecular phylogenetic studies, and currently just few representatives of the family are sequenced for their genomic or non-genomic regions. Some genera are also rare and there are not access to their live material. During the past few years, some species/genera of the family are included in molecular phylogenetic studies, and usually the sequences of 18S rDNA have been used (Palomares-Rius et al., 2009; Ashrafi et al., 2012; Atighi et al., 2012). In some recent studies, the sequences of 28S rDNA and especially the sequences of the D2/D3 fragments are used (Palomares-Rius et al., 2009; Panahandeh et al., 2014, 2015a, 2015b; Soleymanzadeh et al., 2016). In present study, some discrepancies observe between the classic taxonomic frameworks on placement of some genera or species, as already documented in aforementioned studies using 28S data. For example, the *Malenchus*/*Lelenchus* Andrássy, 1954 formed a sister clade to the clade of *Cephalenchus* Goodey, 1962/*Eutylenchus* Cobb, 1913, and the relation of these four genera with the rest genera is not fully resolved. The phylogenetic affinities of two genera *Malenchus*/*Lelenchus* is also under open question needing further studies, broader sampling, and exploitation of different phylogenetic approaches. On the other hand, *Malenchus* is classified under two different subfamilies by Siddiqi (2000) and Geraert (2008); however, phylogenetic studies showed unresolved phylogenetic relation of the genus with Tylenchinae genera (regarding the taxonomic framework of Geraert [2008]). Unfortunately, Duosulciinae Siddiqi, 1979 sensu Siddiqi

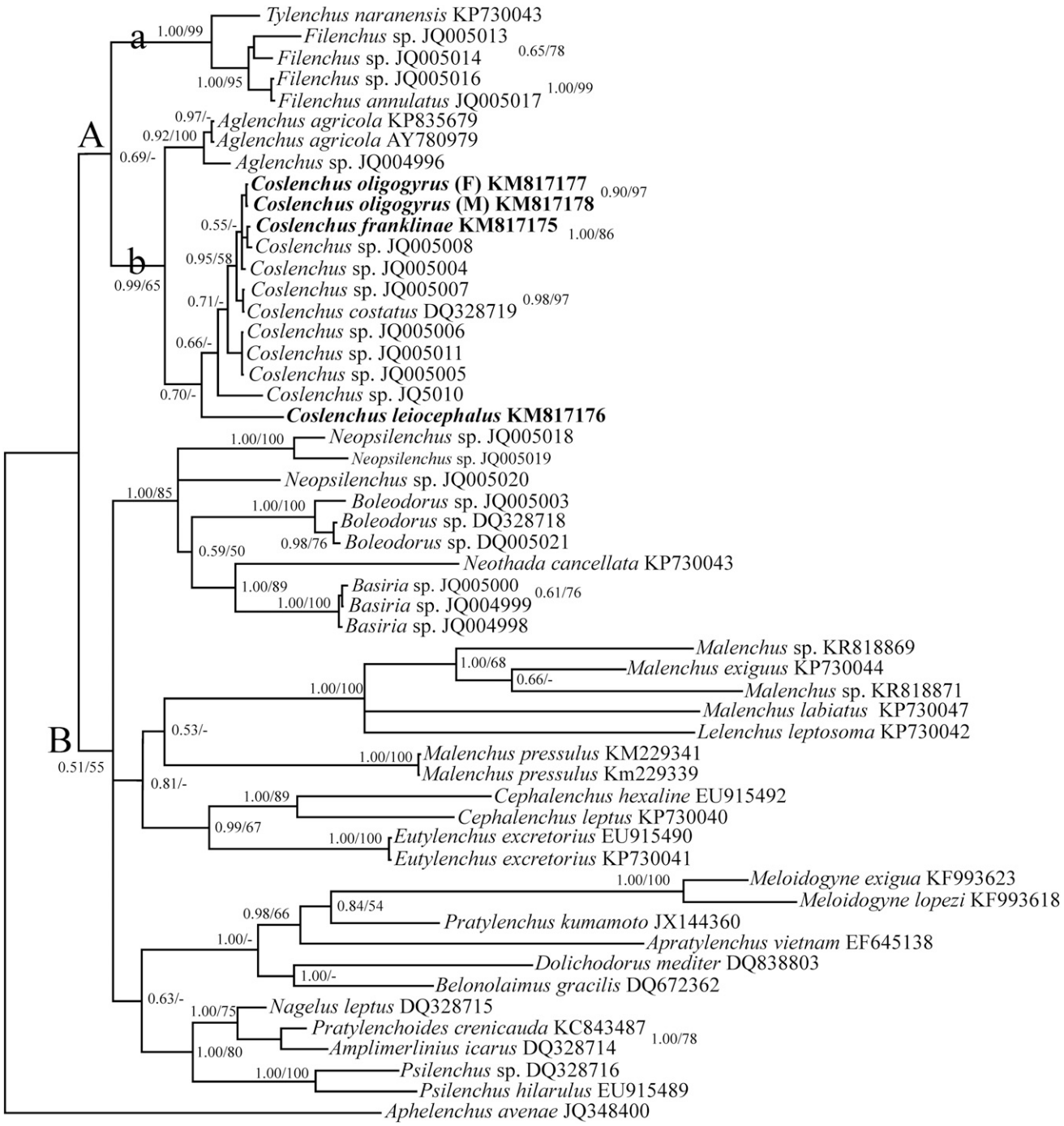

FIG. 6. Bayesian 50% majority rule consensus tree inferred from 53 sequences of the D2–D3 domains of the 28S rDNA under the GTR + I + G model. Bayesian posterior probability (BPP) and maximum likelihood bootstrap (ML BS) values are given for each appropriate clade in the shape BPP/ML BS. The newly sequenced taxa/isolates are in bold font.

(2000) members, still do not have sequences for 28S rDNA deposited in GenBank (except *Malenchus*), so that the relations of the genera under the subfamily being studied.

In the study of Atighi et al. (2013), *Filenchus* revealed to be a monophyletic genus using 28S data, whereas 18S data proved it as a polyphyletic genus, a phylogenetic controversy. Briefly, the discrepancies between 18S/28S phylogenies and the rudimentary disagreements of classis *versus* modern phylogenetic frameworks as discussed historically by Atighi et al. (2013) still persist. A discussion on deep phylogeny of Tylenchidae is not the aim of present study, and we simply showed that the recently recovered and sequenced species of *Coslenchus* form a monophyletic group with the rest currently sequenced species of the genus, and finally, we emphasize that phylogeny of Tylenchidae needs a deep sampling of representatives of the genera and also needs to

exploit data from several genomic/nongenomic regions and different phylogenetic approaches, especially multilocus phylogenetic analyses.

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