Discopersicus n. gen., a New Member of the Family Tylenchidae Örley, 1880 with Detailed SEM Study on Two Known Species of the Genus *Discotylenchus* Siddiqi, 1980 (Nematoda; Tylenchidae) from Iran

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Abstract: Discopersicus iranicus n. gen., n. comb., previously described from Iran as a new species under the genus *Discotylenchus*, is illustrated using light microscope and scanning electron microscope (SEM) observations and further studied using molecular characters. SEM studies revealed the newly proposed genus has oblique amphidial apertures on the lateral sides of the lip region. SEM images are also provided for two species of *Discotylenchus*, namely *D. discretus* and *D. brevicaudatus*, as the first SEM study of the genus. These results confirmed longitudinal amphidial aperture type on lateral sides of the lip region in genus *Discotylenchus*, as noted by Siddiqi while erecting the genus with *D. discretus* as the type species. Molecular phylogenetic analyses using partial small subunit (SSU) and large subunit (LSU) rDNA sequences revealed the affinity of the genus *Discopersicus* n. gen. with members of the subfamily Boleodorinae, as supported by morphological characters (mainly, the oblique amphidial opening).
Key words: 18S rDNA, 28S rDNA, Boleodorinae, new genus, phylogeny, taxonomy.

The family Tylenchidae Örley, 1880 currently includes five subfamilies namely Atylenchinae Skarbilovich, 1959; Boleodorinae Khan, 1964; Ecphyadophorinae Skarbilovich, 1959; Tylenchinae Örley, 1880; and Tylodorinae Paramonov, 1967 (Geraert, 2008). The two subfamilies, Boleodorinae and Tylenchinae, differ from each other mainly in the shape of amphidial aperture of their members: V or oblique, on lateral side of head, usually partly covered by a flap in the former (Boleodorinae) and mostly a longitudinal slit on the lateral side of the head, can also be a transverse slit on the head front, a ventrally curved slit or a pore-like structure in the latter (Tylenchinae) (Geraert, 2008). According to Geraert (2008), there are currently eight valid genera under Boleodorinae and 15 in Tylenchinae. The genus *Discotylenchus* Siddiqi, 1980 belongs to the subfamily Tylenchinae and currently contains six valid species (Siddiqi, 2000). While establishing the genus, Siddiqi (1980) noted presence of minute longitudinal amphidial apertures near the labial disc, in lateral view. In their revision, Raski and Geraert (1987) synonymized *Discotylenchus* with *Filenchus* Andrássy, 1954, and noted that the presence of a perioral disc is an inadequate character to separate two genera. Brzeski (1998) followed the same scheme and transferred six valid species of *Discotylenchus* to *Filenchus*. Again, Siddiqi (2000) emphasized on the validity of the genus *Discotylenchus* and its placement in Tylenchinae. Geraert (2008) accepted it as a valid genus under subfamily Tylenchinae. To date,

there have been no SEM studies on representatives of the genus *Discotylenchus*. During our studies on tylenchids in southwestern Iran, a tylenchid nematode population with a perioral disc was recovered, and after detailed study, it was assigned to the species formerly described as *Discotylenchus iranicus* Ghaemi, Pourjam, Atighi, Pedram and Karssen, 2012. Additional morphological and molecular phylogenetic studies were performed on the recovered population, and based on the newly obtained data, it was revealed that the population belongs to a new genus, belonging to the subfamily Boleodorinae, named in this present study as *Discopersicus* n. gen., currently a monotypic genus with *D. iranicus* (Ghaemi et al., 2012) n. comb. as the type species.

The history of reported tylenchids from Iran is given by Ghaderi et al. (2012) and the recent taxonomic studies are cited by Soleymanzadeh et al. (2016).

Materials and Methods

Nematode sampling, extracting, and mounting

Several soil samples were collected from southwestern Iran. To obtain a cleaner suspension of nematodes, the tray method (Whitehead and Hemming, 1965) was employed for extraction. Nematodes of interest were handpicked under a Nikon SMZ1000 stereomicroscope (Nikon Corp., Tokyo, Japan). The collected specimens were killed in hot 4% formaldehyde solution, transferred to anhydrous glycerin according to De Grisse (1969), and mounted in permanent slides. The specimens were examined with a Nikon Eclipse E600 light microscope (Nikon Corp., Tokyo, Japan). The morphometric symbols and used abbreviations here follow Siddiqi (2000). Specimens of the two species of *Discotylenchus* used for SEM studies were obtained from the material reported by Ghaemi et al. (2012).

Scanning electron microscopy

After their examination and identification, a few specimens preserved in glycerin were selected for

observation under SEM following the protocol of Álvarez-Ortega and Peña-Santiago (2016). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin scanning electron microscope (Carl Zeiss, Germany).

DNA extracting, PCR, and sequencing

A single live specimen of *Discopersicus iranicus* n. gen., n. comb. 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 crushed using a clean slide cover. The suspension was collected by adding 20 µl AE buffer. DNA samples were stored at −20°C until using as PCR templates. Primers for amplification of the D2/D3 expansion segment of 28S rDNA were the forward primer D2A (5′-ACAAGTACCGT GAGGGAAAGT-3′) and reverse primer D3B (5′-TGCG AAGGAACCAGCTACTA-3′) (Nunn, 1992). Primers for partial amplification of 18S rDNA were forward 18S4 (5′-GCTTGTCTCAAAGATTAAGCC-3′) and reverse 18S1573R (5′ TACAAAGGGCAGGGACGTAAT-3′) pairs (Blaxter et al., 1998 and Mullin et al., 2005, respectively). Details of PCR cycles are according to Yaghoubi et al. (2014). PCR products were cleaned and then sequenced in both directions using the same primers used while PCR with an ABI 3730XL sequencer (Bioneer Corporation, South Korea) and were deposited in the GenBank database (accession number KM502981 for partial 18S rDNA and KM502982 for partial 28S rDNA).

Phylogenetic analyses

The partial 28S (D2/D3 expansion segment) and 18S rDNA sequences of *D. iranicus* n. gen., n. comb. (accession numbers: KM502981 for 18S rDNA and KM502982 for 28S rDNA) were compared with those of other nematode species available in GenBank using the BLAST homology search program. Selected sequences of other tylenchids (representatives of all tylenchid genera were selected for both dataset analyses) and newly obtained sequences of the new genus were aligned using MUSCLE (Edgar, 2004) as implemented in MEGA6 (Tamura et al., 2013). Poorly aligned positions and divergent regions were selected and deleted by Gblocks 0.91b (Castresana, 2000) with all three less stringent options. The model of base substitution was selected using MrModeltest 2 (Nylander, 2004). The Akaike information criterion supported model, a general time reversible model, including among-site rate heterogeneity and assessment of invariant sites (GTR+G+I) and a general time reversible model including among-site rate heterogeneity (GTR+G) were selected for phylogenetic analyses of the SSU and LSU datasets. Bayesian analysis was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), running the chains for 10^7 generations. Burn-in was arbitrarily chosen to be 25% of the results. The

Markov chain Monte Carlo method within a Bayesian framework was used to determine equilibrium distribution and help estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using the 50% majority rule. Burn-in was determined on stabilization of log likelihood using TRACER v1.5 (Drummond and Rambaut, 2007) and of the clades posterior probabilities with AWTY (Nylander et al., 2008). A maximum likelihood (ML) tree was constructed by using RaxmlGUI 1.1 (Silvestro and Michalak, 2011) software under the same evolutionary model as in Bayesian analysis, in 1,000 bootstrap (BS) replicates. Some taxa causing polytomy in 18S phylogenetic tree namely *Abursanema iranicum* Yaghoubi, Pourjam, Pedram, Siddiqi and Atighi, 2014; *Veleshkinema iranicum* Miraeiz Heydari, Álvarez-Ortega, Pedram and Atighi, 2015; *Hexatylus* sp.; *Rubzovinema* sp.; *Deladenus proximus* Bedding, 1974; *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936; *D. drepanocercus* Goodey, 1953 and *Psilenchus hilarulus* de Man, 1921 (accession numbers KF885743, KP300015, KJ636358, KF373732, KF908897, HQ219210, JQ429768, and AY919271, respectively) were deleted from 18S dataset.

RESULTS

Discopersicus n. gen.

Diagnosis: Tylenchidae, Boleodorinae. Body almost straight or slightly ventrally arcuate when heat killed. Cuticular annuli fine. Lateral field with four incisures. Lip region continuous with body contour, tapering to an
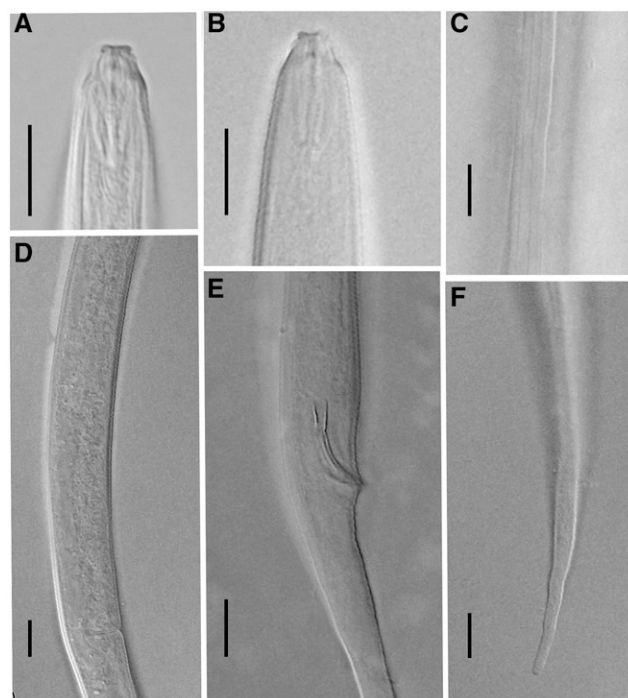

FIG. 1. Light microphotographs of Khouzestan population of *Discopersicus iranicus* (Ghaemi et al., 2012) n. gen., n. comb. A, B. Lip region (lateral view); C. Lateral field at vulva region; D. Part of female reproductive system; E. Male cloacal region; F: Female tail tip. (Scale bars = 10 µm).
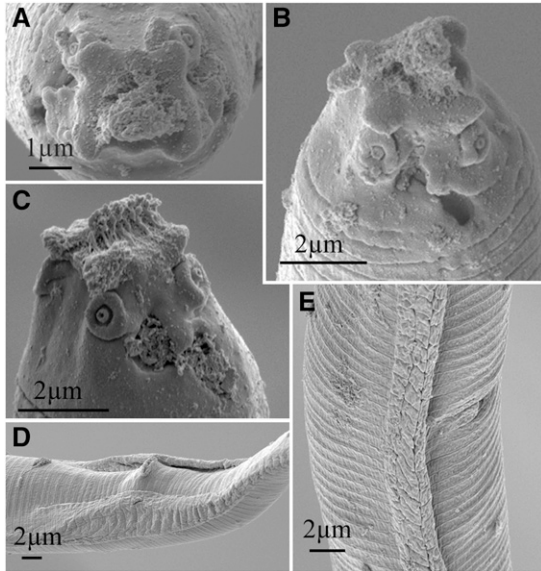
FIG. 2. Scanning electron microscopy of Khouzestan population of *Discopersicus iranicus* (Ghaemi et al., 2012) n. gen., n. comb. A. *En face* view of lip region showing perioral disc; B, C. Lateral view of lip region showing oblique amphidial apertures, labial disc and four cephalic papillae; D. Male cloacal region; E. Cuticle annuli and lateral field at vulva region.

offset and prominent perioral labial disc. Amphidial apertures in the form of two oblique slits on lateral sides of the head under SEM (Fig. 2A,B). Four prominent cephalic papillae behind the disc. Stylet with conus about

32.0% to 38.5% of its total length with minute and rounded knobs. Dorsal pharyngeal gland orifice at 2.5 to 3.0 µm from base of stylet knobs. Pharynx composed of a slender procorpus, oval median bulb with central valve, short isthmus, and saccate basal bulb, offset from intestine. Cardia well developed. Excretory pore located just posterior to hemizonid. Reproductive system monodelphic-prodelphic. Vulva a transverse slit, lacking vulval flaps. Ovary single, prodelphic, outstretched anteriorly, spermatheca almost oval, offset, functional with spheroid sperm cells. Postvulval uterine sac present. Tail elongate conoid with rounded terminus. Spicules arcuate, tylenchoid, gubernaculum simple, and bursa adanal.

Type species: Discopersicus iranicus (Ghaemi, Pourjam, Atighi, Pedram and Karssen, 2012) n. comb. = *Discotylenchus iranicus* Ghaemi, Pourjam, Atighi, Pedram and Karssen, 2012.

urn:lsid:zoobank.org:pub:020B8E18-D371-4F31-A17C-2AC2AFFF038C.

Other species: No other species.

Etymology: The generic name is a combination of the word "disc" which refers to having a perioral disc and the Greek name of Persia (Πρσική), the ancient name of Iran.

Description: Description of Khouzestani population of *D. iranicus* (Ghaemi et al., 2012) n. gen., n. comb (Figs. 1,2). Measurements are given in Table 1.

Female: Body almost straight or slightly ventrally arcuate after fixation. Cuticular annuli fine, 0.8 to

TABLE 1. Morphometrics of Khouzestani and type population of *Discopersicus iranicus* (Ghaemi, Pourjam, Atighi, Pedram and Karssen, 2012) n. gen., n. comb. All measurements are in µm and in the form: mean ± SD (range).

| Origin	Khouzestan population		Ghaemi et al. (2012)	
Characters	Female	Male	Female	Male
n	8	10	3	5
L	734.0 ± 34.4 (685–784)	663.0 ± 33.9 (583–707)	707.0 ± 2.8 (681–748)	648 ± 58 (553–700)
a	42.4 ± 3.6 (37.5–48.9)	44.4 ± 1.9 (41.8–46.4)	36.8 ± 1.8 (35–39)	43.5 ± 6.2 (34.6–50.0)
b	5.7 ± 0.5 (5.1–6.5)	5.3 ± 0.3 (4.8–5.7)	4.9 ± 0.2 (4.7–5.2)	4.7 ± 0.2 (4.5–5.0)
c	9.4 ± 0.7 (8.5–10.8)	8.3 ± 0.8 (7.0–9.8)	8.1 ± 0.9 (7.0–9.2)	7.8 ± 0.4 (7.2–8.3)
c'	7.1 ± 0.9 (5.7–8.1)	6.4 ± 0.9 (5.4–8.1)	8.3 ± 1.4 (6.8–10.0)	6.7 ± 0.9 (5.9–8.1)
V or T	76.0 ± 1.2 (74.5–77.9)	–	73.8 ± 2.3 (70.8–76.5)	36.6 ± 5.8 (27.1–42.9)
V'	85.1 ± 1.1 (84.1–87.5)	–	–	–
Head height	4.1 ± 0.7 (3.5–5.0)	3.7 ± 0.3 (3.5–4.0)	4.1 ± 0.6 (3.5–5.0)	3.7 ± 0.3 (3.5–4.0)
Head width	6.4 ± 0.4 (6–7)	6.5 ± 0.5 (6–7)	7.1 ± 0.5 (6.5–7.5)	6.5 ± 0.8 (5.5–7.5)
Stylet	12.4 ± 0.6 (12.0–13.5)	12.1 ± 0.7 (11.0–13.5)	14.6 ± 0.5 (14–15)	14.0 ± 0.8 (14–15)
Conus	4.4 ± 0.7 (4–6)	4.3 ± 0.4 (3.5–5.0)	5.0 ± 0.6 (4.5–6.0)	5.0 ± 0.7 (4–6)
m	35.5 ± 4.1 (32.0–38.5)	35.2 ± 2.4 (31.8–39.1)	–	–
DGO	2.7 ± 0.3 (2.5–3.0)	2.5 ± 0.6 (2–3)	2.4 ± 0.5 (2–3)	26.0 ± 0.2 (2.5–3.0)
Median bulb	54.3 ± 1.3 (52–56)	53.2 ± 3.7 (48–59)	56.0 ± 3.2 (52–59)	55 ± 5 (46–58)
MB	42.9 ± 2.5 (39.7–46.0)	42.5 ± 1.5 (39.1–44.4)	–	–
Excretory pore	98.0 ± 6.5 (87–109)	96.0 ± 4.8 (90–103)	92 ± 5 (89–100)	90.0 ± 9.5 (75–100)
Pharynx	129.0 ± 9.1 (115–141)	125.0 ± 7.8 (114–135)	143 ± 6 (135–148)	137 ± 12 (116–148)
Head-vulva	558.0 ± 31.8 (510–600)	–	–	–
Body width (BW)	17.4 ± 1.8 (14–20)	15.0 ± 0.7 (14.5–16.0)	19 ± 1 (18–20)	15 ± 1 (14–16)
PUS	14.6 ± 2.5 (11–18)	–	13.5 ± 3.0 (10–16)	–
PUS/BW	0.8 ± 0.1 (0.7–1.0)	–	0.7 ± 0.2 (0.5–0.9)	–
Anal body width	11.2 ± 1.0 (10–12)	12.6 ± 1.1 (11–14)	–	–
Vulva–anus	98.0 ± 6.1 (86–105)	–	97 ± 6 (92–105)	–
Tail	78.6 ± 5.7 (68–85)	80.0 ± 5.6 (70–89)	88 ± 8 (81–100)	83 ± 4 (77–89)
Spicules	–	19.8 ± 1.4 (17.5–22.0)	–	19 ± 1 (18–21)
Gubernaculum	–	5.2 ± 0.9 (4–7)	–	5.7 ± 0.7 (5.0–6.5)
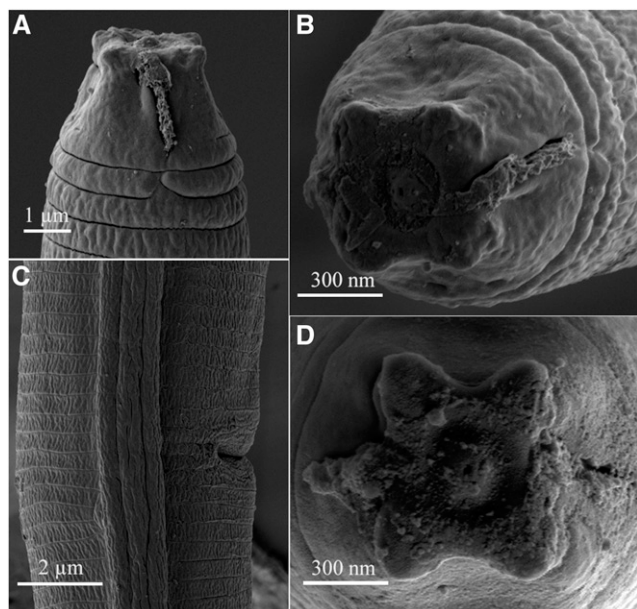
FIG. 3. Scanning electron microscopy of Iranian population of *Discotylenchus discretus* Siddiqi, 1980 reported by Ghaemi et al. (2012). A, B. Lip region showing longitudinal amphidial aperture; C. Body annuli and lateral field at vulva region; D. *En face* view of lip region showing perioral disc.

0.9 μm wide. Lateral field with four incisures. Lip region continuous with body contour, smooth, tapering to an offset, and prominent perioral labial disc, 3 to 5 μm width. SEM images show amphidial apertures in the form of two oblique slits on lateral sides of the head (Fig. 2A,B). Four prominent cephalic papillae anterior to amphidial openings, behind the disc. Stylet with conus about 32.0% to 38.5% of its total length with minute and rounded knobs. Dorsal pharyngeal gland orifice at 2.5 to 3.0 μm from base of stylet knobs. Procorpus slender, median bulb oval with central valve, isthmus short, basal bulb saccate, offset from intestine, and cardia well developed. Excretory pore 87 to 109 μm from anterior end, located just posterior to hemizonid. Deirids present, located posterior to excretory pore level. Nerve ring at midpoint of isthmus. Reproductive system monodelphic-prodelphic, composed of outstretched ovary, oviduct (not clearly seen), spermatheca almost oval, offset, functional with rounded sperm cells, crustaformeria, uterus, vagina perpendicular to body axis, extending for less than half body width into the body, vulva a transverse slit and no vulval flap, postvulval uterine sac present, *ca* 0.7 to 1.0 times corresponding body width. Vulva-anus distance 86 to 105 μm. Phasmid not seen. Tail elongate conoid with rounded terminus.

Male: General morphology similar to that of female, except for characters associated with sexual differences. Testis single, outstretched. Spicules arcuate, tylenchoid. Gubernaculum simple, bursa adanal. Tail elongate conoid with rounded terminus.
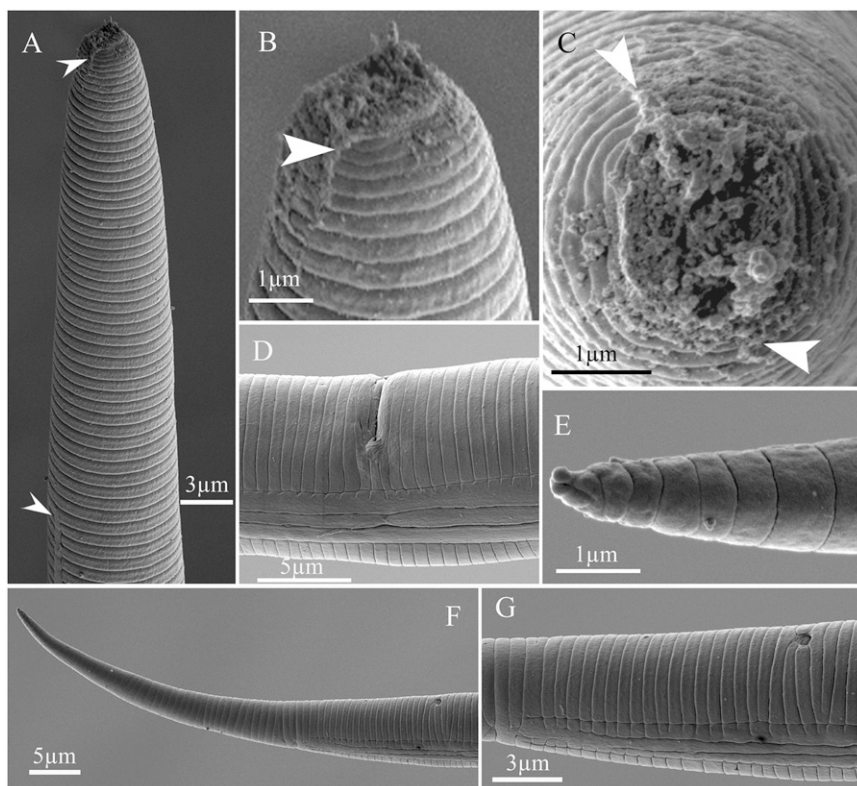

FIG. 4. Scanning electron microscopy of Iranian population of *Discotylenchus brevicaudatus* Brzeski, 1985 reported by Ghaemi et al. (2012). A. Lateral view of anterior region showing longitudinal amphidial apertures and beginning of lateral fields (indicated by upper and lower arrows, respectively); B, C. Lip region showing longitudinal amphidial aperture in more detail (indicated by arrows); D. Body annuli and lateral field at vulva region showing crenation; E, F. Tail tip and posterior body region; G. Lateral field at anus region.

Relationships: Discopersicus n. gen. is unique among members of the family Tylenchidae in having both a perioral labial disc and oblique amphidial apertures. The new genus is placed in the subfamily Boleodorinae based on the structure of its amphidial apertures; this placement being supported by phylogenetic analyses. The new genus is distinguished from all other valid genera in the subfamily Boleodorinae by having a perioral disc. In addition, it differs from *Boleodorus* Thorne, 1941 in having a stylet base with minute and rounded knobs (*vs* flange-like), and pharynx with well-developed median bulb (*vs* weakly developed) and bearing refractive valve plates (*vs* lacking). From *Neopsilenchus* Thorne and Malek, 1968, in its stylet conus (*vs* not conical, with wide lumen) and stylet base with minute rounded knobs (*vs* absent), and vulva more posterior (V greater than 70 *vs* 60%–65 %). *Discopersicus* n. gen. can also be distinguished from *Discotylenchus* by the nature of its amphidial apertures (oblique slit *vs* longitudinal apertures), stylet length (longer than 10 μm *vs* shorter), and V ratio (greater than 70% *vs* usually less than 70%, except in *D. brevicaudatus* Brzeski, 1985).

Remarks: The presently studied population of *Discopersicus iranicus* n. gen., n. comb. was recovered from soil samples collected about the rhizosphere of sugarcane (*Saccharum officinarum* L.) in Khouzestan province, southwestern Iran (GPS coordinates: N:32°09.592, E:48°33.977) during May 2012. Except slight difference in stylet length (12.0–13.5 *vs* 14–15 μm), no remarkable morphological or morphometric differences were observed between the recovered population and the type population described by Ghaemi et al. (2012) (see Table 1). Detailed SEM studies of the new genus revealed it has oblique amphidial opening slits (Fig. 2B,C), similar to the nature of amphidial openings described by Brzeski and Sauer (1982) for three genera *Basiria* Siddiqi, 1959, *Boleodorus* and *Neopsilenchus* with SEM observations. The nature of the four sublateral cephalic papillae at the anterior end of the new genus is also similar to that observed in the three aforementioned genera of Boleodorinae (*op. cit.*). Details of amphidial opening characters of two species of *Discotylenchus,* namely *D. discretus* Siddiqi, 1980 and *D. brevicaudatus*, reported and illustrated by Ghaemi et al. (2012) were also studied with SEM in present study. The presence of a perioral disc usually interferes with observation of the amphidial apertures using common light microscopic studies, but, newly obtained photographs reveal longitudinal amphidial apertures for both species of *Discotylenchus* (Figs. 3,4), a fundamentally different shape from the amphidial
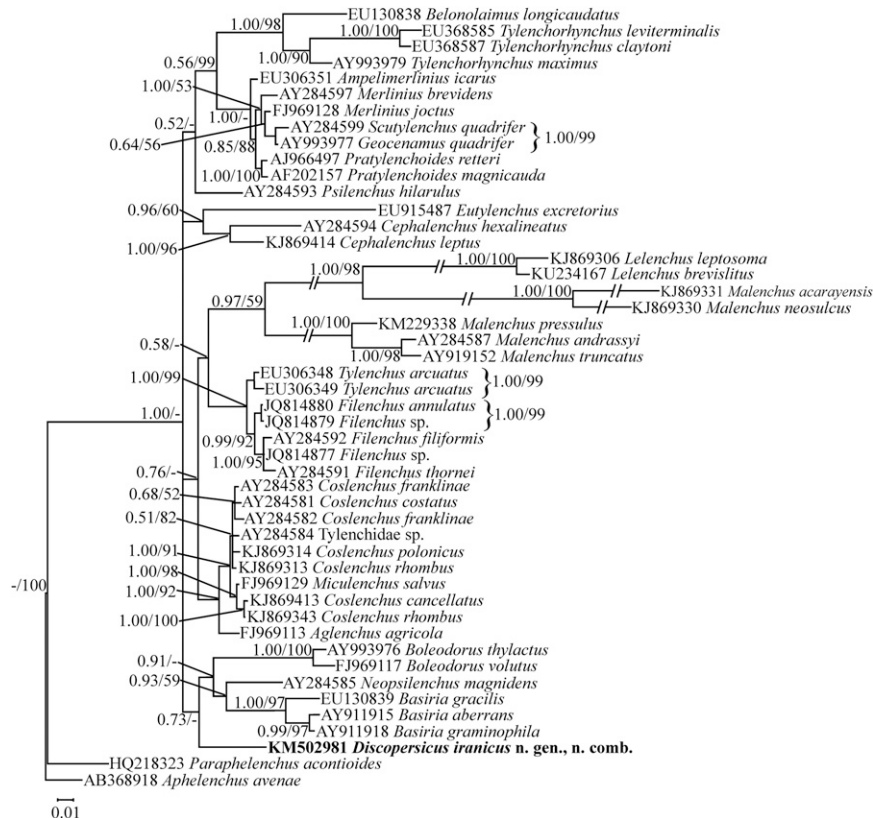

FIG. 5.   Consensus 50% majority rule Bayesian phylogenetic tree of *Discopersicus iranicus* (Ghaemi, Pourjam, Atighi, Pedram and Karssen, 2012) n. gen., n. comb. based on small subunit (SSU) rDNA sequences under GTR + I + G model. Bayesian posterior probabilities (BPP) and maximum likelihood (ML) bootstrap (BS) values more than 50% are given on appropriate clades in the form BPP/ML BS. The new sequence is in bold font.

opening form, typical for the members of Boleodorinae, including the newly proposed genus.

Molecular phylogeny of Discopersicus n. gen.

Partial 18S rDNA phylogeny: To determine the phylogenetic relationships of *Discopersicus* n. gen. with other tylenchid nematode species, a 1,522 nt long partial sequence of 18S rDNA was used. Figure 5 represents a Bayesian phylogenetic tree inferred from the multiple alignment of 18S partial sequences of 48 taxa (including one isolate of the new genus

and two aphelenchid taxa as outgroup species). The dataset was composed of 1,679 total characters, of which 617 characters were variable and 463 characters were parsimony-informative with an average nucleotide composition of 25.1 % A, 21.9 % C, 27.3 % G, and 25.7 % T. As expected, *Discopersicus* n. gen. has formed a clade with species of *Basiria*, *Boleodorus*, and *Neopsilenchus*, representatives of Boleodorinae with moderate (0.73) Bayesian posterior probability (BPP); but with no significant support in ML tree. Further future sequences of the subfamily might help
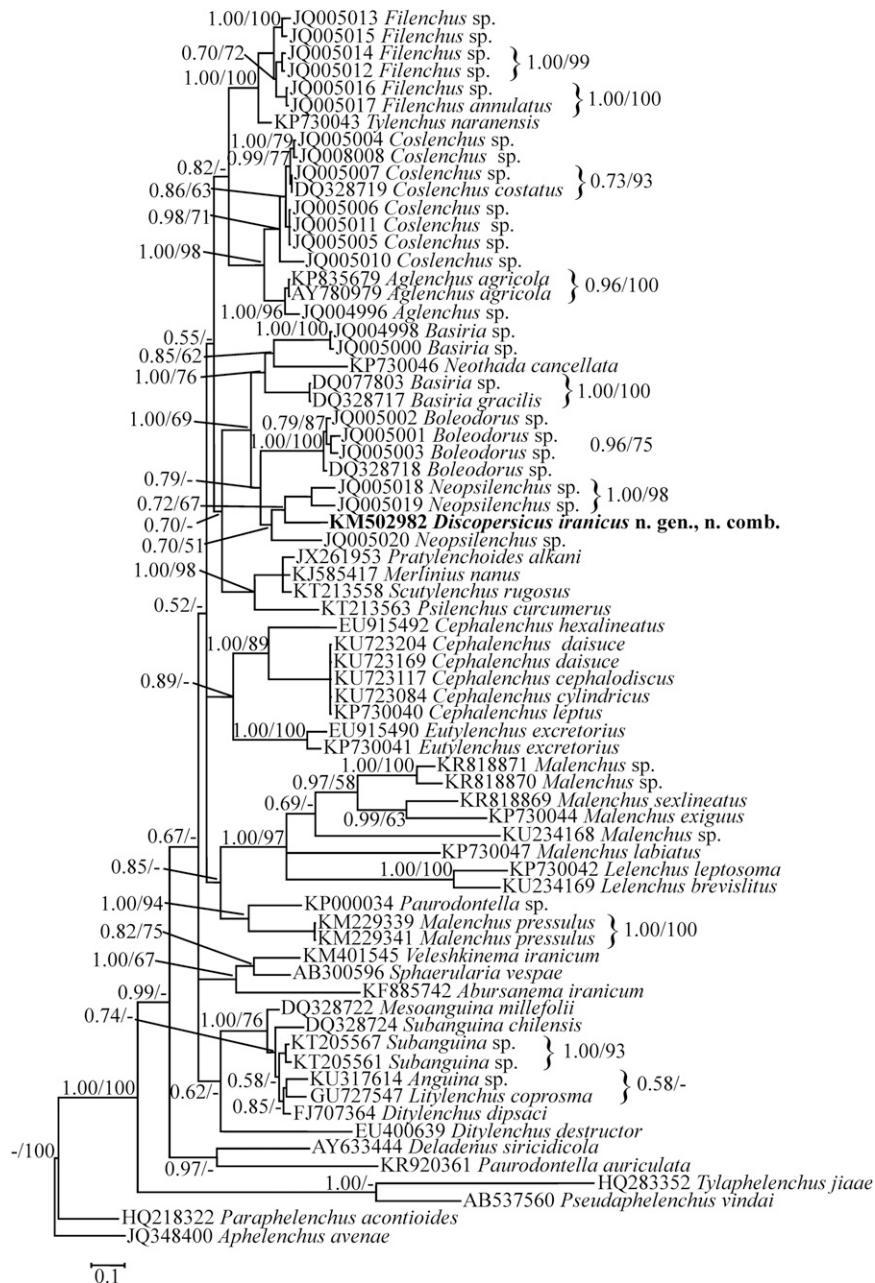

FIG. 6.   Consensus 50% majority rule Bayesian phylogenetic tree of *Discopersicus iranicus* (Ghaemi, Pourjam, Atighi, Pedram and Karssen, 2012) n. gen., n. comb. based on large subunit (LSU) (D2/D3) rDNA sequences under GTR + G model. Bayesian posterior probabilities (BPP) and maximum likelihood (ML) bootstrap (BS) values more than 50% are given on appropriate clades in the form BPP/ML BS. The new sequence is in bold font.

to depict a more reliable phylogenetic relation between the members.

Partial 28S rDNA (D2/D3 expansion segment) phylogeny: To reconstruct the 28S rDNA tree, a 471 nt partial sequence of D2/D3 region was used. Figure 6 represents the phylogenetic tree of 67 tylenchid taxa/isolates (including one isolate of *Discopersicus* n. gen.) and four aphelenchids as outgroup taxa. The dataset was composed of 781 total characters of which 612 characters were variable and 512 characters were parsimony-informative. The average nucleotide composition was 20.4% A, 22.3% C, 32.1% G and 25.3% T. In present tree, the new genus has occupied a placement inside *Neopsilenchus* spp. The main clade of Boleodorinae in this tree (containing new genus and representatives of the genera *Neopsilenchus*, *Boleodorus*, *Basiria*, and *Neothada*) received full (1.00) and moderate (69%) BPP and ML BS, respectively.

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