Hemicycliophora ahvasiensis n. sp. (Nematoda: Hemicycliophoridae), and data on a known species, from Iran

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Abstract

Hemicycliophora ahvasiensis n. sp., recovered from the rhizospheric soil of date palm in Khuzestan province, southwest Iran, is described and illustrated based upon morphological, morphometric and molecular data. The new species is characterized by its sheath, closely fitting most of the body, cuticle with or without numerous irregular lines, sometimes appearing as blocks in distal body region. Lateral field without discrete longitudinal lines, but often with continuous broken striae or anastomoses. Continuous lip region with single annulus, slightly elevated labial disc, stylet with posteriorly sloping knobs. Vulva with or without slightly modified lips, spermatheca with sperm and tail conoid, symmetrically narrowing at distal region to form a narrow conical region. Morphologically, the new species looks similar to H. indica, H. labiata, H. siddiqii, H. tenuistriata and H. typica. The latter species appears more similar to the new species under light microscopy, but could be separated using the scanning electron microscopy and molecular data. The new species was also compared with H. epicharoides and H. dulli, two species with close phylogenetic affinities to it. The phylogenetic relationships of the new species were reconstructed and discussed using partial sequences of the D2-D3 expansion segments of large subunit, and internal transcribed spacer regions (LSU D2-D3 and ITS rDNA). Hemicycliophora conida, the second studied species, was recovered from north Iran and characterized by morphological and molecular data.

Keywords

D2-D3-LSU, Hemicycliophora, H. conida, ITS, morphology, morphometrics, phylogeny, sheath nematode, taxonomy.

In their excellent contribution to the systematics of the superfamily Hemicycliophoroidea Skarbilovich, 1959 (Siddiqi, 1980), Chitambar and Subbotin (2014) reviewed the taxonomy of the genus Hemicycliophora (De Man, 1921) and updated data of the currently valid species. In the same year, Subbotin et al. (2014) addressed aspects of the pathogenicity of Hemicycliophora species on associated host plants, the difficulties of morphological identifications due to morphological plasticity, and the lack of scanning electron microscopic (SEM) and molecular data. Currently the genus contains 133 species (132 listed in Chitambar and Subbotin, 2014 and one in Maria et al., 2018).

There are 12 species of Hemicycliophora have been reported from different provinces in Iran. They are H. belemnis Germani & Luc, 1973, H. chilensis Brzeski, 1974, H. conida Thorne, 1955, H. iranica Loof, 1984, H. lutosa Loof & Heyns, 1969, H. megalodiscus Loof, 1984, H. poranga Monteiro & Lordello, 1978, H. ripa Van den Berg, 1981, H. sculpturata Loof, 1984, H. spinituberculata Loof, 1984, H. sturhani Loof,
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1984 and H. vaccinii Reed & Jenkins, 1963. All of these species were characterized using traditional taxonomic methods (Eskandari, 2018). In an effort to document Hemicycliophora species occurring in Iran, two populations were recovered from soil samples obtained from different geographical locations in northern and southern regions. The preliminary morphological studies revealed the population recovered from south Iran resembled H. typica de Man, 1921 under light microscope (LM), but further studies using SEM and molecular data, and comparisons with all known species of the genus, revealed it to be an unknown species, described herein as H. ahvasiensis n. sp. The second species recovered from north Iran belonged to H. conida Thorne, 1955.

Materials and methods

Nematode extraction and morphological observations

Several soil samples were collected from date palm and fruit tree gardens in Khuzestan and Gilan provinces, Iran. The relevant information of the presently studied nematode populations, and those included in phylogenetic analyses, are given in Table 1. Jenkins’ method (Jenkins, 1964) was used to extract the nematodes from soil samples. The collected specimens were killed in hot 4% formaldehyde solution and transferred to anhydrous glycerin according to De Grisse (1969). Observations and measurements were conducted using a Leitz SM-LUX light microscope equipped with a drawing tube. Some of the specimens were photographed using an Olympus DP71 digital camera attached to an Olympus BX51 light microscope equipped with differential interference contrast (DIC).

Scanning electron microscopy (SEM)

Specimens preserved in glycerin were selected for observation according to Abolafia (2015). They were hydrated in distilled water, dehydrated in a graded mixture of ethanol-acetone series, critical point-dried with liquid carbon dioxide, and coated with gold. The mounts were examined with a Zeiss Merlin microscope (5 kV).

DNA extraction, PCR and sequencing

For molecular analyses, single female specimens were picked out, examined in a drop of distilled water on a temporary slide under the light microscope, transferred to 3μl of TE buffer (10mM Tris-Cl, 0.5mM EDTA; pH 9.0) on a clean slide, and then crushed using a cover slip. The suspension was collected by adding 20μl TE buffer. One DNA sample for the Gilan population and two DNA samples for the Khuzestan population were prepared in this manner. The DNA samples were stored at ~20°C until used as a PCR template. Primers for LSU rDNA D2-D3 amplification were forward primer D2A (5’–ACAAGTACCGTGAGGGAAAGT–3’) and reverse primer D3B (5’–TCGGAAGGAACCAGCTACTA–3’) (Nunn, 1992). Primers for amplification of ITS rDNA were forward primer TW81 (5’–GTTTCCGTA GTGAAAACCTGC–3’) and reverse primer AB28 (5’– ATATGCTTAAGTTCAGCGGGT–3’) as described in Vovlas et al. (2008). The 25μl PCR mixture contained 14.5μl of distilled water, 3μl of 10 x PCR buffer, 0.5μl of 10mM dNTP mixture, 1.5μl of 50mM MgCl2, 1μl of each primer (10 pmol/μl), 0.5μl of Taq DNA polymerase (Cinna Gen, Tehran, Iran, 5 U/μl), and 3μl 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 s, annealing at 52°C for 40 s, and extension at 72°C for 80 s. A final extension was performed at 72°C for 10 min. Amplification success was evaluated by electrophoresis on 1% agarose gel (Aliramaji et al., 2018, 2020). The PCR products were purified using the QIAquick PCR purification kit (Qiagen®) following the manufacturer’s protocol and sequenced directly using the PCR primers with an ABI 3730XL sequencer (Bioneer Corporation, South Korea). The newly obtained sequences of the studied species were deposited into the GenBank database (accession numbers LSU D2-D3 MT901580/MT901581 and ITS rDNA MT901582/MT901583 for the new species and MT901584 for ITS rDNA of H. conida, as indicated in Table 1).

Phylogenetic analyses

The newly obtained sequences of the D2-D3 fragments of LSU rDNA of the both populations, and the selected sequences from GenBank, were aligned by Clustal X2 (http://www.clustal.org/) using the default parameters. The ITS dataset was aligned using MUSCLE as implemented in MEGA6 (Tamura et al., 2013). The editing of both alignments was performed manually. The outgroup taxa were chosen according to previous studies (Subbotin et al., 2014; Van den Berg et al., 2018; Maria et al., 2018). The base substitution model was selected using MrModeltest 2 (Nylander, 2004) based on the Akaikie information criteria. A general time reversible model, including among-site rate heterogeneity and estimates of
Table 1. Information of the species/populations of *Hemicycliophora* studied in present paper and those of ingroup and outgroup taxa used in phylogenetic analyses.

| Species            | Host                  | Locality                              | GenBank accession numbers | Reference or identifier                      |
|--------------------|-----------------------|---------------------------------------|---------------------------|---------------------------------------------|
| *H. ahvasiensis* n. sp. | *Phoenix dactylifera* | Khuzestan province, Iran              | MT901580, MT901581, MT901583 | Present study                              |
| *H. californica*   | *Salix sp.*           | Yolo County, CA, USA                  | KF430518, KF430519        | Subbotin et al. (2014)                      |
| *H. conida*        | *Punica granatum*     | Gilan province, Iran                  | –                         | Present study                              |
| *H. conida*        | Unknown plant         | Belgium                               | FN433875–                  | I. Tandingan De Ley et al. (unpub.)          |
| *H. conida*        | Turf grasses          | Football pitch, Madrid, Spain         | KF430447, KF430580        | P. Castillo; Subbotin et al. (2014)         |
| *H. conida*        | Unknown plant         | Clallam County, WA, USA               | KF430448, KF430579        | Subbotin et al. (2014)                      |
| *H. dulli*         | Peat                  | South Africa                          | MT329669, MT329670, MT329671 | M. Rashidifard (unpub.)                    |
| *H. epicharoides*  | *Ammophila arenaria*  | Serranova, Brindisi, Italy            | KF430512                   | Subbotin et al. (2014)                      |
| *H. epicharoides*  | *Ammophila arenaria*  | S. Barrameda, Cádiz, Spain            | –                         | KF430608                                   |
| *H. epicharoides*  | *Pragmites sp.*       | Epiros, Greece                        | –                         | KF430606                                   |
| *H. floridensis*   | *Pinus elliotti*      | Lake City, FL, USA                    | KF430506, KF430536        | Subbotin et al. (2014)                      |
| *H. gracilis*      | *Prunus domestica*    | Hamilton City, Glenn County, CA, USA  | KF430480, KF430562        | Subbotin et al. (2014)                      |
| *H. gracilis*      | *Prunus domestica*    | Butte City, Glenn County, CA, USA     | KF430481                   | Subbotin et al. (2014)                      |
| *H. gracilis*      | Unknown plant         | Brooklyn Park, MN, USA                 | KF430482                   | Subbotin et al. (2014)                      |
| *H. gracilis*      | Unknown plant         | California, USA                       | –                         | FN435301                                   |
| *H. gracilis*      | Unknown plant         | Sacramento County, CA, USA             | –                         | MG019827                                   |
| *H. halophila*     | *Desmoschoenus spiralis* | Taylors Mistake, New Zealand    | KF430444, KF430445, KF430582, KF430583 | Subbotin et al. (2014)                      |
| *H. hellenica*     | *Arundo donax*        | Filippias, Epirus, Greece             | KF430453                   | Subbotin et al. (2014)                      |
| *H. iberica*       | *Populus nigra*       | Arroyo Frío, Jaén, Spain              | KF430461, KF430539, KF430540 | Subbotin et al. (2014)                      |
| *H. iberica*       | *Quercus suber*       | Hinojos, Huelva, Spain                | KF430462                   | Subbotin et al. (2014)                      |
| *H. iberica*       | *Quercus suber*       | Santa Elena, Jaén, Spain              | KF430463, KF430541        | Subbotin et al. (2014)                      |
| Hemicycliophora species | Host Plant | Location | Genbank Accession Numbers | Authors |
|------------------------|------------|----------|---------------------------|---------|
| H. italae              | Ammophila arenaria | Zapponeta, Foggia, Italy | KF430458 | Subbotin et al. (2014) |
| H. labiata             | Poa pratensis | South Korea | MK305971, MK305972 | Mwamula et al. (2020) |
| H. lutososa            | Unknown plant | Gauteng province, South Africa | GQ406240, GQ406241 | Van den Berg et al. (2010) |
| H. lutosoides          | Turf grasses | Madrid, Spain | KF430454 | Subbotin et al. (2014) |
| H. lutosoides          | Juncus sp. | Cádiz, Spain | – | Subbotin et al. (2014) |
| H. obtusa              | Pinus pinea | Moguer, Huelva, Spain | KF430521, KF430578 | Subbotin et al. (2014) |
| H. onubensis¹          | Pinus pinea | Moguer, Huelva, Spain | KF430449, KF430450, KF430587, KF430588 | Subbotin et al. (2014); Van den Berg et al. (2018) |
| H. parvana²            | Turf grasses | New Hanover County, NC, USA | KF430501 | Subbotin et al. (2014); Van den Berg et al. (2018) |
| H. parvana²            | Turf grasses | Carteret County, NC, USA | KF430502 | Subbotin et al. (2014); Van den Berg et al. (2018) |
| H. parvana²            | Bentgrass | Texas, USA | KC329574, KC329575, KC329576 | Ma and Agudelo (2015) |
| H. parvana²            | Prunus persica | Punta Gorda, FL, USA | MG019825 | Van den Berg et al. (2018) |
| H. parvana²            | Andropogon virginicus | Paines Prairie, FL, USA | – | Subbotin et al. (2014); Van den Berg et al. (2018) |
| H. parvana²            | Turf grasses | New Hanover County, NC, USA | – | Subbotin et al. (2014); Van den Berg et al. (2018) |
| H. poranga             | Poa annua | Monterey County, CA, USA | KF430432, KF430434, KF430598 | Subbotin et al. (2014) |
| H. poranga             | Turf grasses | San Francisco, CA, USA | MG019815 | Van den Berg et al. (2018) |
| H. poranga             | Unknown plants | Marin County, CA, USA | MG019816 | Van den Berg et al. (2018) |
| H. poranga             | Salix sp. | Santa Rosa, CA, USA | – | Subbotin et al. (2014) |
| H. poranga             | Apium graveolens | Argentina | – | Subbotin et al. (2014) |
| H. poranga             | Lepidorrhachis mooreana | San Francisco, CA, USA | – | Subbotin et al. (2014) |
| H. raskii              | Grasses | Sacramento County, CA, USA | KF430520, KF430577 | Subbotin et al. (2014) |
| H. robbinsi³           | Turf grasses | Brunswick, NC, USA | KF430488, KF430492 | Subbotin et al. (2014); Van den Berg et al. (2018) |
| H. robbinsi³           | Turf grasses | Indian Hills, CA, USA | KF430491 | Subbotin et al. (2014); Van den Berg et al. (2018) |
| H. robbinsi³           | Turf grasses | San Antonio, TX, USA | – | Subbotin et al. (2014); Van den Berg et al. (2018) |
| Species      | Genus                     | Location                        | GenBank Accession Numbers | Authors                                      |
|--------------|---------------------------|---------------------------------|---------------------------|----------------------------------------------|
| H. robbinsi  | *Borrichia* sp.           | St Augustine, FL, USA           | –                         | KF430550                                    |
| H. robbinsi  | *Phoenix roebelenii*      | Fort Lauderdale, FL, USA        | –                         | KF430552                                    |
| H. signata   | Grasses                   | Chembas district, Mozambique    | MG019824                  | Van den Berg et al. (2018)                   |
| H. similis   | *Fragaria x ananassa*     | Cartaya, Huelva, Spain          | KF430465                  | Subbotin et al. (2014)                       |
| H. subbotini | *Cinnamomum camphora*     | Zhejiang Province, China        | MG01275–MG01277           | Maria et al. (2018)                          |
| H. thienemanni | *Salix sp.*             | Moscow, Russia                  | KF430469–KF430471         | Subbotin et al. (2014)                       |
| H. thienemanni | *Populus nigra*          | Castell de Locubin, Jaén, Spain | –                         | Subbotin et al. (2014)                       |
| H. thornei   | *Vitis vinifera*          | La Rambla, Córdoba, Spain       | KF430452                  | Subbotin et al. (2014)                       |
| H. typica    | Grasses                   | Gauteng province, South Africa  | KF430515                  | Subbotin et al. (2014)                       |
| H. vaccinii  | *Pinus pinaster*          | Carnota, Coruña, Spain          | –                         | Subbotin et al. (2014)                       |
| H. vaccinii  | *Pinus pinaster*          | Montegudo Isl., Pontevedra, Spain | KF430459, KF430460  | Subbotin et al. (2014)                       |
| H. vidua     | *Camellia* sp.            | South Carolina, USA             | –                         | Cordero López et al. (2013)                  |
| Hemicycliophora sp. | Unknown plant            | Iran                            | KY284835                  | E. Miraeiz, R. Heydari (unpub.)               |
| Hemicycliophora sp. 1 | Grasses                   | Terovo, Epirus, Greece          | AY780974                  | Subbotin et al. (2005); Subbotin et al. (2014) |
| Hemicycliophora sp. 2 | Unknown plant            | Birdlings Flat, New Zealand     | KF430516, KF430517        | Subbotin et al. (2014)                       |
| Hemicycliophora sp. 3 | *Zea mays*               | Tingle Farms, Wilco, AZ, USA    | –                         | Subbotin et al. (2014)                       |
| Hemicycliophora sp. 5 | Turf grasses              | Carteret County, NC, USA        | –                         | Subbotin et al. (2014)                       |
| Hemicycliophora sp. 6 | *Nothofagus* forest      | Kaitoke Waterworks, New Zealand | KF430446                  | Subbotin et al. (2014)                       |
| Hemicycliophora sp. 7 | *Pinus pinea*            | Almonte, Huelva, Spain          | KF430451                  | Subbotin et al. (2014)                       |
| Hemicycliophora sp. 8 | Unknown plant            | Henrieville, UT, USA            | KF444173                  | Subbotin et al. (2014)                       |
| Hemicycliophora sp. 8 | Turf grasses              | Monterey, CA, USA               | KF430494                  | Subbotin et al. (2014)                       |
| Hemicycliophora sp. 9 | *Tritolium repens*       | Preveza, Greece                 | KF430509, KF4305511, KF430514 | Subbotin et al. (2014)                       |
| Hemicycliophora sp. 9 | *Agrostis* sp.           | Jaroslav region, Russia         | –                         | Subbotin et al. (2014)                       |
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| Species/Genus | Host Plant | Location | Accession Numbers | Authors |
|---------------|------------|----------|-------------------|---------|
| Hemicycliophora sp. 9 | Unknown plant | Brake, Germany | AY780973 | Subbotin et al. (2005); Subbotin et al. (2014) |
| Hemicycliophora sp. 10 | *Salix* sp. | Yolo County, CA, USA | KF430483, KF430485–KF430486, MG019828 | Subbotin et al. (2014); Van den Berg et al. (2018) |
| Hemicycliophora sp. 11 | *Andropogon virginicus* | Paines Prairie, FL, USA | KF430493, KF430557, KF430558 | Subbotin et al. (2014) |
| Hemicycliophora sp. 12 | Grasses | Saint Paul, MN, USA | KF430474 | Subbotin et al. (2014) |
| Hemicycliophora sp. 12 | Unknown plant | Brooklyn Park, MN, USA | KF430475 | Subbotin et al. (2014) |
| Hemicycliophora sp. 12 | Unknown plant | Sedona, AZ, USA | KF430476 | Subbotin et al. (2014) |
| Hemicycliophora sp. 13 | *Neoregelia* sp. | Los Angeles County, CA, USA | KF430507, KF430508 | Subbotin et al. (2014) |
| Hemicycliophora sp. 15 | Unknown plant | Vicinity of Trois-Rivières, Quebec, Canada | MG019819 | Van den Berg et al. (2018) |
| Hemicycliophora sp. 16 | Unknown tree | east of Temecula, CA, USA | MG019818, MG019829 | Van den Berg et al. (2018) |
| Hemicycliophora sp. 17 | Unknown tree | Pismo Beach, San Luis Obispo County, CA, USA | –, MG019830 | Van den Berg et al. (2018) |
| Hemicycliophora sp. 18 | Unknown plant | Vicinity of Quebec City, Quebec, Canada | MG019820 | Van den Berg et al. (2018) |
| *Gracilacus bilineata* | *Bambusa* sp. | Taiwan | –, EU247525 | Chen et al. (2008) |
| *Paratylenchus bukowinensis* | Unknown plant | Monopoli, Italy | AY780943 | Subbotin et al. (2005) |
| *Paratylenchus minutus* | *Annona squamosa* | Taiwan | –, EF126180 | Chen et al. (2009) |
| *Paratylenchus nanus* | Unknown plant | Niebüll, Germany | AY780946 | Subbotin et al. (2005) |
| *Trophotylenchulus floridensis* | *Pinus elliottii* | Crystal river, Florida, USA | –, JN112261 | Tanha Maafi et al. (2012) |

Note: ¹Originally identified as *H. ripa* ²Originally identified as *H. wyei* ³Originally identified as *Hemicycliophora* sp. 4.

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Invariant sites (GTR + G + I), was selected for the both phylogenies.

The Bayesian analysis was performed to infer the phylogenetic trees using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003), running the chains for two 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 the Bayesian framework were used to determine equilibrium distribution and help estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using the 50% majority rule. Bayesian posterior probability (BPP) values higher than 0.50 are given on appropriate clades. The output files of the phylogenetic program was visualized using Dendroscope v3.2.8 (Huson and Scornavacca, 2012) and re-drawn in CorelDRAW software version 17.

### Results

#### Systematics

*Hemicycliophora ahvasiensis* n. sp. (Figures 1–4; Table 2).
Figure 1: Line drawings of *Hemicycliophora ahvasiensis* n. sp. Female. A: Anterior body region; B: Spermatheca; C: Vulval region; D–F: Variation of posterior body end morphology. (Scale bar = 20 μm).

**Description**

**Female**

Body straight to slightly ventrally arcuate following heat fixation. Cuticular sheath closely appressed over entire or most of body. Under LM, annuli rounded, with or without longitudinal lines, appearing as blocks mostly in the distal body region. Block-like differentiations are more prominent in distal body region under SEM. Lateral field with no longitudinal lines, but having broken or continuous striae or anastomoses. Amphidial openings large, partly plugged. Lip region continuous with body contour,
-figure2.jpg

**Figure 2:** Light photomicrographs of *Hemicycliophora ahvasiensis* n. sp. Female. A: Entire body; B, C: Anterior body region; D: Reproductive system (the arrow indicates the spermatheca); E: Spermatheca; F: Vagina; G: Posterior body region.

bearing one wide annulus. Labial disc slightly elevated. Stylet with posteriorly sloping knobs, having moderate to large cavity at base. Pharynx criconematoid, with pharyngeal corpus absent, metacorpus (median bulb) ovoid bearing central valves, short isthmus surrounded by the nerve ring and reduced pyriform basal bulb. Cardia short, surrounded by intestinal tissue. Excretory pore five to 10 annuli posterior to the pharynx base. Hemizonid indistinct. Reproductive system monodelphic-prodelphic, outstretched, composed by long ovary with oocytes arranged in one or two rows, spermatheca round to oval, filled
with spheroid sperm cells, vulva with not or slightly modified lips, vulval sleeve slightly elongate, one to two annuli long. Body portion behind vulva slightly narrowing towards distal region. Distance between vulva to anus about five anal body diam. Tail conoid, symmetrically narrowing at about 35% of its length at distal region to form a narrower conical section ending to a finely rounded to sharp terminus.

**Male**

Not found.
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Figure 4: Scanning electron micrographs of Hemicycliophora ahvasiensis n. sp. Female. A–F: Mid-body annuli ornamentation.

Juvenile

One juvenile specimen was found in the population that is similar to female except by a smaller body size and undeveloped sexual organs.

Type host and locality

This population was recovered from the rhizospheric soil of date palm (Phoenix dactylifera L.) collected from Ahvaz city in Khuzestan province, southwest Iran. The GPS information of the sampling site is 31°18′11.1″N, 48°39′10.1″E.

Etymology

The specific epithet of the new species refers to the original city name in Latin where it was discovered.

Type material

The holotype and 12 paratype females were deposited into the nematology laboratory of the Department of Plant Protection, Shahid Chamran University of Ahvaz, Ahvaz, Iran. Three paratype females deposited at the Wageningen Nematode Collection (WaNeCo), Wageningen, The Netherlands. Two paratype females deposited at the Nematode Collection of the Department of Animal Biology, Plant Biology and Ecology of the University of Jaén, Jaén, Spain. The ZooBank Life Science Identifier (LSID) for this publication is as follows: http://zoobank.org/urn:lsid:zoobank.org:pub:EEF9C9E9-90B8-4EC1-8BD9-A403FD8D58E4.

Diagnosis and relationships

Hemicycliophora ahvasiensis n. sp. is mainly characterized by a cuticle with or without longitudinal lines on annuli. Instead of lateral lines there may be broken or continuous striae or anastomoses on lateral sides of the body. The lip region is continuous with body contour and has a single annulus, slightly elevated labial disc, and plugged amphidial openings. Other characters include posteriorly sloping stylet knobs, vulva with or without slightly modified lips and short vulval sleeve, spermatheca full of sperm and conoid tail, symmetrically narrowing at about 35% of its length at the distal region to form a narrower conical region. The polytomous identification codes of the new species from Chitambar and Subbotin (2014) are: A4, B2, C3, D1, E1, F1, G23, H1, I12, J1, K23, L3, M2, N1, O1, P1, Q2, R2, S3, T1, U2, V1, W1, X1, Y-.

In general morphology, the new species is close to H. indica Siddiqi, 1961, H. labiata Colbran, 1960, H. siddiqii Deswal & Bajaj, 1987, H. tenuistriata Doucet, 1982 and H. typica. A comparison of the new species with the aforementioned species is as follows:

From H. indica, by a shorter body (767–893 vs 800–1500 μm), lower R, Rph, Rex and RV (212–247 vs 270–320, 35–48 vs 47–69, 42–50 vs 51–67 and 38–59 vs 64–81), respectively, lateral field without line(s) (vs with three lines), lip region with one annulus (vs two or three annuli), short vulval sleeve (vs elongate) and tail symmetrically narrowing at about 35% of its length at distal region to form a narrower conical section (vs uniformly narrowing).

From H. labiata, by annuli with or without longitudinal lines (vs not), lateral field lacking line(s) (vs having one line), lip region with one annulus (vs two or three annuli), short vulval sleeve (vs moderately long) and body not constricted immediately posterior to vulva (vs constricted).

From H. siddiqii, by lateral field lacking line(s) (vs having one line), a longer body (767–893 vs 650–780 μm).
Table 2. Morphometrics of *Hemicycliophora ahviensis* n. sp. from Khuzestan province, Iran.

| Character                                      | Female holotype | Female paratypes   | Juvenile |
|-----------------------------------------------|-----------------|--------------------|----------|
| n                                             | 1               | 20                 | 1        |
| L                                             | 868.7           | 830.3±48.3 (767–893) | 600      |
| a                                             | 22.2            | 21.5±2.1 (17.9–24.5) | 18.0     |
| b                                             | 5.9             | 5.8±0.3 (5.4–6.5)  | 4.8      |
| c                                             | 10.7            | 10.1±1.2 (8.3–11.5) | 9.8      |
| c'                                            | 2.9             | 3.0±0.3 (2.5–3.5)  | 2.6      |
| o                                             | 11.5            | 12.2±0.9 (9.4–15.3) | 9.0      |
| DGO                                           | 7.9             | 8.1±0.9 (7.4–10.0) | 5.5      |
| V                                             | 85.5            | 84.1±0.9 (82.6–85.5) | –        |
| St                                            | 68.4            | 66.5±2.3 (63.3–71.0) | 60.8     |
| m                                             | 81.5            | 80.6±1.4 (77.4–83.7) | 80.7     |
| Stylet knob height                            | 4               | 4.1±0.5 (4–5)      | 3.8      |
| Stylet knob width                             | 7               | 6.6±0.7 (6–8)      | 6.6      |
| Excretory pore from anterior end              | 171             | 168.3±6.1 (159–180) | 168      |
| Diam. at mid-body                             | 39              | 38.4±3.6 (32–46)   | 33       |
| Diam. at anus (ABD)                           | 27              | 26.8±1.7 (24–29)   | 23       |
| Diam. at vulva                                | 38              | 38.4±2.1 (35–43)   | –        |
| Vulva-anterior body distance                  | 744             | 700±43 (653–751)   | –        |
| Vulva-tail terminus distance                  | 125             | 129.5±6.0 (113–142) | –        |
| Spermatheca-vulva distance                    | 89              | 87.6±13.2 (74–121) | –        |
| Lip diam.                                     | 15              | 15.7±0.9 (14–18)   | 14       |
| Lip height                                    | 7               | 6.7±0.7 (6–9)      | 6        |
| First body annulus diam.                     | 16              | 16.9±0.9 (15–19)   | 15       |
| Second body annulus diam.                    | 18              | 18.6±1.1 (16–21)   | 16       |
| Pharynx length                                | 145             | 142.6±4.8 (134–151) | 125      |
| Annulus width                                 | 4               | 4.1±0.3 (3.4–4.7)  | 2.8      |
| Tail length                                   | 81              | 83.3±7.9 (74–92)   | 61       |
| V-anus distance                               | 45              | 47.9±9.9 (32–64)   | –        |
| R                                             | 245             | 221.3±8.6 (212–247) | 216      |
| RSt                                           | 19              | 19.5±1.1 (18–21)   | 22       |
| Rph                                           | 41              | 41.1±3.2 (35–48)   | 46       |
| Rex                                           | 48              | 47.4±3.6 (42–50)   | 58       |
| RV(ant)                                       | 193             | 185.3±8.4 (167–198) | –        |
| RV                                            | 52              | 47.8±6.9 (38–59)   | –        |
| RVan                                          | 15              | 15.0±3.8 (10–22)   | –        |
| Ran                                           | 37              | 32.9±4.9 (25–47)   | –        |
| VL/VB                                         | 3.3             | 3.4±0.3 (2.8–3.9)  | –        |
| Spermatheca length                            | 29              | 19.8±5.4 (14–29)   | –        |
| Spermatheca diam.                             | 15              | 15.5±1.6 (12–22)   | –        |
| St%L                                          | 7.8             | 7.9±0.4 (7.5–8.4)  | 10       |

Note: All measurements are in μm and in the form: mean ± s.d. (range).
Van den Berg & Tiedt, 2001, a species with close phylogenetic affinities in both LSU and ITS phylogenies, by shorter stylet (63.3–71.0 μm) annuli and elevated labial disc. Stylet long and slender, knobs posteriorly directed. Pharynx typical of the genus. Nerve ring encircling isthmus. Excretory pore, four annuli posterior and opposite to pharynx base. Reproductive system monodelphic-prodelphic, outstretched, spermatheca rounded to ovate, filled with spheroid sperm cells, vulval lips modified, vulval sleeve absent. Tail conical, symmetrically narrowing at distal region, tip rounded.

Male

Cuticle annulation fine at midbody. Lateral fields marked by three longitudinal lines. Labial region distinctly trapezoid. Stylet and pharynx degenerated. Spicules semi-circular, tip slightly recurved. Gubernaculum linear, slightly thickened proximally. Bursa with crenate circular, tip slightly recurved. Gubernaculum linear, slightly thickened proximally. Bursa with crenate margin. Tail elongate, uniformly narrowing, annuli at distal region irregular.

Host and locality

This population was recovered from the rhizospheric soil of pomegranate (Punica granatum L.) collected from Rasht city, Gilan province, in north Iran. The geographical position of the sampling site is N36°54′1.687″, E49°28′37.923″.

Remarks

H. conida was originally described by Thorne (1955) from a sugar beet field in Ireland. It was later reported from several countries (Chitambar and Subbotin, 2014). In the report of Loof (1984), the species was recovered from East Azarbaijan province of Iran. Males, however, were not recovered in this study. Later, the species was again recovered from Azarbaijan province, but no morphometric or morphological data were provided (Barooti, 1998). The presently recovered population agreed well with other populations of the species that have been reported from different regions, based upon the morphometric data and morphology (Chitambar and Subbotin, 2014). The spicules length in The Netherlands populations was measured as 18–29 μm by Loof (1968) (Chitambar and Subbotin, 2014), but it was calculated about 55 μm after the drawings, which is in accordance with the presently studied population.
Figure 5: Light photomicrographs of *Hemicycliophora conida* Thorne, 1955 from Gilan province, Iran. A–G: Female. A: Anterior body region; B: Pharyngeal region; C: Lateral field at mid-body; D, E: Annuli ornamentation; F, G: Posterior body region; H, I: Male. H: Anterior body region; I: Posterior body region. (Scale bar = 20 μm).

**Molecular characterization and phylogenetic relationships**

Two 673 and 682 nt long D2-D3 expansion segments of LSU (MT901580, MT901581), one from each female specimen, were generated for the new species. A BLAST search using these sequences revealed they have 99.34% identity with *Hemicycliophora* sp. 9 and *Hemicycliophora* sp. 13 (KF430509 and KF430508, respectively). The efforts to get the LSU
Table 3. Morphometrics of *Hemicycliophora conida* Thorne, 1955 from Gilan province, Iran, and comparison with other population from East Azarbaijan province, Iran.

| Reference | Present study |  | Loof (1984) |
|-----------|---------------|---------------|--------------|
| Province  | Gilan province | East Azarbaijan province |
| Character | Female | Male | Female |
| n         | 10 | 5 | 11 |
| L         | 912.0 ± 21.4 (881–928) | 809.0 ± 13.6 (795–822) | 820–1020 |
| a         | 21.2 ± 2.5 (18.6–24.3) | 37.3 ± 5.7 (31.8–43.3) | 26–30 |
| b         | 5.3 ± 0.2 (5.2–5.6) | – | 5.0–5.6 |
| c         | 11.6 ± 2.0 (10.1–14.5) | 8.2 ± 0.4 (7.8–8.6) | 9.7–13.6 |
| c'        | 2.3 ± 0.4 (1.7–2.6) | 5.2 ± 0.5 (4.6–5.6) | – |
| V         | 86.7 ± 1.0 (85.5–87.6) | – | 86–89 |
| St        | 92.5 ± 2.1 (90–97) | – | 90–103 |
| m         | 78.3 ± 4.0 (75.3–84.2) | – | – |
| Stylet knob height | 5.0 ± 0.4 (4.3–5.6) | – | – |
| Stylet knob width | 7.8 ± 0.5 (6.9–8.6) | – | – |
| Excretory pore from anterior end | 178.0 ± 8.9 (169–192) | 140.2 ± 13.4 (127–164) | – |
| Diam. at mid-body | 43.5 ± 5.5 (38–50) | 22 ± 3 (19–25) | – |
| Diam. at anus/cloaca | 35.3 ± 4.3 (30–40) | 19 ± 1 (18–20) | – |
| Diam. at vulva | 46.4 ± 5.8 (40–55) | – | – |
| Vulva-anterior body distance | 791 ± 16 (770–809) | – | – |
| Vulva-tail terminus distance | 124.5 ± 7.8 (115–136) | – | – |
| Spermatheca-vulva distance | 82.2 ± 10.1 (72–96) | – | – |
| Lip diam. | 21.3 ± 2.2 (19–24) | 10.7 ± 1.5 (9–12) | – |
| Lip height | 7.5 ± 0.6 (7–8) | 6.2 ± 0.8 (6–7) | – |
| First body annulus diam. | 23.8 ± 1.9 (20–26) | – | – |
| Second body annulus diam. | 26.1 ± 2.7 (21–30) | – | – |
| Pharynx length | 171.0 ± 4.7 (167–176) | – | – |
| Annulus width | 4.1 ± 0.5 (3.6–5.1) | 1.9 ± 0.1 (1.8–2.0) | – |
| Tail length | 88.0 ± 6.5 (79–94) | 98.7 ± 6.1 (92–104) | – |
| V-anus distance | 42.0 ± 19.7 (28–71) | – | – |
| R         | 230.0 ± 9.2 (224–237) | – | 259–286 |
| RSt       | 21.0 ± 0.9 (18–23) | – | – |
| Rph       | 38.0 ± 0.2 (38–39) | – | – |
| Rex       | 41.4 ± 1.4 (39–43) | – | 48–52 |
| RV(ant)   | 187.0 ± 3.5 (185–190) | – | 207–226 |
| RV        | 46.0 ± 4.9 (37–54) | – | – |
sequences of *H. conida* failed. A total of 77 sequences of *Hemicycliophora* spp. and two sequences of *Paratylenchus nanus* Cobb, 1923 and *P. bukowinensis* Micoletzky, 1922 (AY780946 and AY780943, respectively), as outgroup taxa, were selected for a LSU phylogeny. This dataset comprised 750 total characters. The phylogenetic tree inferred using this dataset is presented in Figure 6. The major clade including the new species, also includes *Hemicycliophora* sp. 13 (KF430507, KF430508), the putative closest relative of it, based upon currently available data, *H. epicharoides* (KF430512), *H. labiata* (MK305971, MK305972) and *Helicycliophora* sp. 9 (KF430509, KF430511, KF430514, AY780973). *H. typica* (KF430515) is in a sister relation to the aforementioned major clade.

Two 904 and 907 nt long sequences of ITS rDNA (MT901582, MT901583) were generated for the new species. A single 683 nt long ITS rDNA sequence (MT901584) was obtained for the Iranian population of *H. conida*. A BLAST search using the ITS sequences of the new species revealed they have 98.93% identity with *Hemicycliophora* sp. 9 (KF430605). The BLAST search using ITS sequence of Iranian population of *H. conida* revealed it has 99.55% and 98.21% identity with two other ITS sequences of *H. conida* (KF430579 and KF430580, respectively).

A total of 70 sequences of *Hemicycliophora* spp. and three sequences of *Paratylenchus minutus* Linford in Linford, Oliveira & Ishii, 1949, *Trophoylenchulus floridensis* Raski, 1957 and *Gracilacus bilineata* Brzeski, 1995 as outgroup taxa (EF126180, JN112261 and EU247525, respectively) were selected for an ITS phylogeny. This dataset comprised 1164 total characters. The phylogenetic tree inferred using this dataset is presented in Figure 7. The major clade including the new species, also includes *Hemicycliophora* sp. 9 (KF430604, KF430605) that represents the putative closest relative of the new species, *H. epicharoides* (KF430606, KF430608) and *H. labiata* (MK305973, MK305974). The clade including two species *H. typica* (GQ406238, GQ406239, KF430603) and *H. dulli* (MT329671, MT329672) is in sister relation to the aforementioned clade. The ITS sequence of the Iranian isolate of *H. conida* formed a clade with two previously available sequences (KF430579, KF430580) of the species.

### Table

| Trait                  | Value          |
|------------------------|----------------|
| RVan                   | 16.0 ± 8.5     |
| Ran                    | 27.0 ± 2.8     |
| VL/VB                  | 2.7 ± 0.3      |
| Spermapheca length     | 22.7 ± 5.9     |
| Spermapheca diam.      | 31.8 ± 8.1     |
| Spicules length        | 54.3 ± 2.1     |
| Gubernaculum length    | 20.3 ± 0.6     |
| Bursa length           | 41.7 ± 6.4     |

Note: All measurements are in μm and in the form: mean ± s.d. (range).

### Discussion

The objectives of this study were to characterize one new and one known species of the genus *Hemicycliophora* from Iran. As common in reliable identifications of *Hemicycliophora* spp., the new species was studied using an integrative approach exploiting both morphological (including SEM) and molecular data (Subbotin et al., 2014).

In both inferred LSU and ITS phylogenies, *Hemicycliophora ahvasesiensis* n. sp. belonged to a clade including *Hemicycliophora* sp. 9, *H. labiata*, *H. epicharoides*, *H. typica* and *H. dulli*. The close affinity of the aforementioned species was already observed (Subbotin et al., 2014; Van den Berg et al., 2018; Maria et al., 2018; Mwamula et al., 2020).

The newly described species in present study appeared similar to *H. typica* under LM, however, the SEM and molecular data revealed they differ. Sequences of LSU D2-D3, and ITS rDNA sequences of *H. ahvasesiensis* n. sp. differed from those of *H. typica* by 6 bp (1.4%) and 31 bp (1.5%), respectively. In the inferred phylogenies, it formed a subclade separate from *H. typica* and other *Hemicycliophora* species.

The new species was isolated from the rhizosphere of date palm tree, that is a major food source for local populations in the Middle East, and plays important roles in their culture and economy (Chao and Krueger, 2007). Additional study is required to clarify if the parasitism of high nematode populations of *H. ahvasesiensis* n. sp. can cause damages to this plant.
Hemicycliophora ahvasiensis n. sp. from Iran: Azimi et al.

Figure 6: Bayesian 50% majority rule consensus tree inferred from analysis of the D2-D3 domains of the LSU rDNA sequences of *Hemicycliophora ahvasiensis* n. sp. under the GTR + G + I model. (lnL = 6023.6660; freqA = 0.2165; freqC = 0.2342; freqG = 0.3064; freqT = 0.2429; R(a) = 0.4542; R(b) = 1.5000; R(c) = 1.0798; R(d) = 0.6155; R(e) = 4.2300; R(f) = 1; Pinvar = 0.3122; Shape = 0.7157). Bayesian posterior probability values more than 0.50 are given for appropriate clades. New sequences are indicated in bold.
Figure 7: Bayesian 50% majority rule consensus tree inferred from analysis of the ITS rRNA gene of Hemicycliophora ahvasiensis n. sp. and Iranian population of H. conida under the GTR + G + I model. (lnL = 13525.0293; freqA = 0.2348; freqC = 0.2548; freqG = 0.2520; freqT = 0.2583; \( R(a) = 1.6876; R(b) = 2.3417; R(c) = 1.8416; R(d) = 0.8470; R(e) = 3.6304; R(f) = 1; \) Pinvar = 0.1024; Shape = 0.4967). Bayesian posterior probability values more than 0.50 are given for appropriate clades. New sequences are indicated in bold.
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