Description and molecular phylogeny of *Mesocriconema abolafiai* n. sp. (Nematoda: Criconematidae) from Iran

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This paper was edited by Zafar Ahmad Handoo.

Received for publication October 15, 2019.

Abstract

*Mesocriconema abolafiai* n. sp. is described by morphological, morphometric, and molecular approaches. The new species is characterized by a body slightly curved with 402 to 612 μm length, 90 to 113 cuticular body annuli with smooth to irregular margins lacking of crenation with not more than one anastomoses, lip region not offset, small flattened submedian lobes, stylet robust (52.8-60.0 μm) with well-developed knobs, open vulva with simple anterior lip, straight vagina, filled spermatheca with globular sperms, presence of males, and conical-acute tail with last annulus bilobed or rounded. Discussions are made on the characterization of *M. abolafiai* n. sp. from the most closely related species, *M. ozarkiense*, and several other species having similar tail shape. Furthermore, results of phylogenetic analyses inferred from D2 to D3 expansion fragments of 28S rRNA, 18S rRNA, and ITS rRNA gene sequences revealed the phylogenetic position of the new species within representatives of Criconematidae and supported morphological justifications for considering this population from Iran as a new species in the genus *Mesocriconema*.

Keywords

Criconematidae, *Mesocriconema*, *M. abolafiai* n. sp., Phylogeny, Morphology, Morphometric, New species, 28S rRNA, 18S rRNA, ITS rRNA.

Ring nematodes of the genus *Mesocriconema* (Andrássy, 1965) are damaging root ectoparasites of many economical important crops (Cordero et al., 2012). This genus was first proposed for species of the genus *Criconemoides* (Taylor, 1936) sensu lato with crenated margins of annuli (Andrássy, 1965). Simultaneously and independently, De Grisse and Loof (1965) proposed to divide the large genus *Criconemoides* into several genera including *Macroposthonia* with type species *M. annulata* (De Man, 1880) being among them (De Grisse and Loof, 1965). Luc and Raski (1981) declared *Criconemoides* and *Macroposthonia* as *genera dubia* and placed most of their species in the genus *Criconemella* (De Grisse and Loof, 1965). Based upon SEM microscopy and discussing on plesiomorphic and apomorphic states of characters, Loof and De Grisse (1989) replaced the generic name *Macroposthonia* by the oldest available synonym *Mesocriconema* and revalidated *Criconemoides* based on the arguments of Loof and De Grisse (1967), but Siddiqi (2000) still considered *Macroposthonia* as a valid name. Brzeski et al. (2002) accepted this synonymy and provided a compendium of the genus *Mesocriconema* with 90 species (species having open vulva and submedian lobes arising from reduced pseudolips). Moreover, they considered that *Mesocriconema* differs from *Criconemoides* (species with closed vulva and pseudolips not reduced). Geraert (2010) replaced some species in the genus *Neobakernema* (Ebsary, 1981b) by validation of this genus and listed 90 valid species under *Mesocriconema* excluding *M. lamothei*. 

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from Mexico (Cid del Prado Vera, 2009) that was not included in the list. After that, three other species have not been identified. *Mesocriconema ozarkiense* (Cordero et al., 2012) was described from Ozark National Forest in Washington, USA (Cordero et al., 2012). *Mesocriconema ericaceum* (Powers et al., 2016) was differentiated from *M. xenoplax* (Raski, 1952; Loof and De Grisse, 1989) by morphological characters and mitochondrial genome (COI) analysis (Powers et al., 2016). *Mesocriconema nebraskense* (Olson et al., 2017) was described as a monosexual, cryptic species sympatrically distributed with its cryptic counterpart, *M. curvatum* (Raski, 1952; Loof and De Grisse, 1989; Olson et al., 2017). In this paper, we describe the new species *M. abolafiai* n. sp., based on morphological and molecular characteristics.

**Material and methods**

**Nematode populations and morphological characterization**

The specimens were recovered from two localities in Dehdasht and Basht (Kohgiluyeh and Boyer-Ahmad province, Southern Iran). The nematodes were extracted from the soil around roots of a grass (*Phragmites* sp.) using the combination of sieving and centrifugal-flotation method (Jenkins, 1964), killed and fixed by hot FPG (4:1:1, formaldehyde: propionic acid: glycerin), processed to anhydrous glycerin (De Grisse, 1969), and finally mounted in glycerin on permanent slides using paraffin wax. Specimens preserved in glycerin were selected for observation under SEM according to Abolafia (2015). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope (5kV) (Zeiss, Oberkochen, Germany).

Morphometric and morphological characters of the nematode populations were studied by a light microscope, equipped with a Dino-eye microscope eyepiece camera in conjunction with its Dino Capture version 2.0 software. The nematode species identified by using data documented by Brzeski et al. (2002) and Geraert (2010), as well as by comparison with recently published descriptions (Cid del Prado Vera, 2009; Cordero et al., 2012; Powers et al., 2016; Olson et al., 2017).

**DNA extraction**

For molecular analysis, DNA was extracted from a single specimen, and three amplifications were conducted on that single specimen. A single female nematode was transferred into a drop of distilled water on a microscopic slide and examined under a light microscope. The nematode specimen was transferred into deionized water, washed three times and then put into an Eppendorf tube with 8µl distilled water. Then, 12 µl lysis buffer (500mM KCl, 100mM Tris-HCl pH 8, 15mM MgCl₂, 10mM DTT, 4.5% Tween 20) and 2µl proteinase K were added to the Eppendorf tube. Nematode specimen was crushed with a microhomogenizer during 2 min. The tubes were incubated at 65°C for an hour and then at 95°C for 15 min (Tanha Maafi et al., 2003).

**PCR amplification and sequencing**

For DNA amplification the protocol described by Tanha Maafi et al. (2003) was used. The D2 to D3 expansion regions of the 28S rRNA gene was amplified with the forward D2A (5’-ACAAGTACC GTGAGGGGAAGTTG-3’) and the reverse D3B (5’-TCGGAGAAACCTACTA-3’) primers (Nunn, 1992). The 18S rRNA was amplified as two partially overlapping fragments, using three universal and one nematode-specific primer (1912R). First 18S fragment forward primer 988F (5’-CTCAAAAGTT AAGCCATGC-3’) and reverse primer 1912R (5’-TTTA CGGTGAAAAGTTG-3’) and the second fragment forward primer 1813F (5’-CTCGCTGAGAAGGTTGAAA T-3’) and reverse 2646R (5’-GCTACCTTGTACCTTG TTTT-3’) were used in the PCR reactions for the amplification of the 18S rRNA gene (Holtermann et al., 2006). The ITS1-5.8S-ITS2 regions were amplified with the forward TW81 (5’-GTTTGCATGGTTGAAAC CTGC-3’) and reverse AB28 (5’-ATATGCTTTGAAGTT ACAGGG-3’) primers (Joyce et al., 1994).

The PCR products were purified using the QIAquick Gel Extraction Kit (Takapozist, Iran) according to the manufacturer’s instruction and used for direct sequencing. The PCR products were sequenced in both directions (BioNeer Inc., Korea). The newly obtained sequences of the new species were submitted to GenBank database under accession numbers MN334221 for the 18S, MN334222 for the 28S D2-D3, and MN334228 for the ITS sequences.

**Phylogenetic analysis**

The sequences of the studied specimens were compared with sequences of other taxa in GenBank, and then, the closest sequences were selected for phylogenetic analyses. The sequences of 18S rRNA and D2 to D3 segments of 28S rRNA were aligned with ClustalX 1.83 (Thompson et al., 1997), using default parameter values and were manually edited.
if necessary. The best fitted model of DNA evolution was obtained using jModelTest v. 2 (Darriba et al., 2012) with the Akaike information criterion (AIC). The best-fit nucleotide substitution models were considered to be GTR+I+G for 18S and 28S, and SYM+G for ITS. The phylogenetic tree of sequences was inferred by the Bayesian method using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Four MCMC chains for 1,000,000 generations were run. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given for appropriate clades. Pairwise divergences between taxa were computed as absolute distance values and as percentage mean distance values based on whole alignment, with adjustment for missing data with PAUP* 4.0b 10 (Swofford, 2002). Trees were visualized using TreeView (Page, 1996).

Results

Systematics

Mesocriconema abolafiai n. sp. (Figs 1-3; Table 1).

Description

Female

Body is slightly curved ventrally when relaxed by heat, assuming an open C-shaped. Cuticular annuli are retrorse, and margins are smooth to irregular across the entire body, without any hint of crenation. Not more than one anastomosis is observed. Cephalic region is not offset, tapering and slightly conical. Oral disc has rounded edges, and slit-like amphidial apertures are located laterally on the disc. Four small flattened submedian lobes are visible at the same level with a labial plate. Submedian lobes in SEM resemble a tongue with a central, longitudinal crease, not fused with labial plates. First body annulus is slightly smaller than the second annulus, not retrorse. Stylet is robust, with well-developed knobs that possess moderate anterior projections and 9 to 11 µm in width. Secretory–excretory pore is located at 27 to 30 annuli from the anterior end, almost at 4 to 5 annuli behind the pharyngeal basal bulb, which is small, pyriform, 10 to 13.5 µm in width and 18 to 20 µm in length. Female genital gonad is outstretched and spermatheca is slightly oval, offset from gonad, filled with globular sperm cells about 1 µm in diameter. Vagina is straight, occasionally slightly curved. Vulva is open with simple anterior lip. The post-vulval region of the body tapers gradually, ending in a pointed terminus or a small bilobed end annulus. SEM reveals that the anal opening is located usually three annuli posterior to the vulva.

Male

Body is vermiform and curved ventrally when fixed by heat treatment. Lateral field has four distinct longitudinal incisures. Lip region has distinct transverse striation, 8 to 9 µm wide and 5 to 7 µm high. Stylet and pharynx are degenerated. Spicules (33.6-35.0 µm in length) are slender and tylencoid, ending to a distinct penial tube (4-5 µm in length). Gubernaculum is simple and slightly curved. Bursa
**Mesocriconema abolafiai** n. sp. from Iran: Mirbabaei et al.

Figure 2: Light micrographs of *Mesocriconema abolafiai* n. sp. Female (A-J) and male (K-M). A: entire body; B, C and K: anterior end and pharyngeal region; D: cuticle at mid-body; E and F: cuticle at posterior end; G: vulval region and part of reproductive system; H-J and L: posterior end; M: spicule, gubernaculum and cloaca. (Scale bars: A = 50 µm; B-M = 10 µm).

not is observed. Tail is elongated-conical, ending to a pointed terminus.

**Juvenile**

Similar to female in general characteristics. Body is straight or slightly curved ventrally after fixation. Annuli are retrorse, lacking any crenation and ornamentation. Total number of annuli approximately equals to that of females, but annuli are narrower than of females (average 4.4 vs 5.5µm).

**Diagnosis**

*Mesocriconema abolafiai* n. sp. is characterized by 90 to 113 cuticular annuli with smooth to irregular margins lacking crenation, small and flattened submedian lobes, stylet 52.8 to 60.0 µm long, open
Figure 3: SEM micrographs of *Mesocriconema abolafiai* n. sp. Female (A-L). A: anterior end (arrow pointing the secretory–excretory pore); B-E: lip region in sublateral, left subventral, frontal and right subventral views, respectively (arrows pointing the amphids); F: annuli; G: cuticle at mid-body; H: entire body; I-K: posterior end in lateral, subventral and terminal views, respectively (white arrow pointing the vulva, black arrow pointing the anus); L: excretory pore (arrow).
Table 1 Morphometric characters of *Mesocriconema abolafiai* n. sp. (measurements are in μm and in the form of average ± s.d. (range)).

| Characters                          | Dehdasht population | Basht population |
|-------------------------------------|---------------------|------------------|
|                                     | Holotype            | 10 paratype females | 3 paratype males | 5 females |
| L                                   | 540                 | 540 ± 65 (402–612) | 471 ± 33.6 (435–502) | 519 ± 65 (425–605) |
| a                                   | 14.9                | 14 ± 1.5 (10.3–15.3) | 24.6 ± 3.2 (20.9–27.1) | 12.6 ± 1.2 (10.9–14.4) |
| b                                   | 4.8                 | 4.8 ± 0.6 (3.2–5.7) | 24.6 | 4.3 ± 0.5 (3.5–5.0) |
| c                                   | 14.5                | 15.8 ± 2.4 (12.1–19.5) | 10.5 ± 0.5 (9.9–11) | 19.1 ± 2.4 (16.3–21.9) |
| c’                                  | 1.4                 | 1.3 ± 0 (1.3–1.4) | 3.3 ± 0.5 (2.9–3.9) | 1.3 ± 0 (1.1–1.4) |
| V                                   | 90.5                | 90.3 ± 1.1 (87.8–91.9) | – | 90.1 ± 0.4 (89.8–90.8) |
| Stylet                              | 56.9                | 55.2 ± 1.3 (52.8–57.2) | – | 56.8 ± 2.3 (54–60) |
| Conus                               | 42.3                | 44 ± 6.3 (38.6–55.0) | – | 41.9 ± 1.6 (40.0–44.5) |
| m (conus/stylet %)                  | 74.4                | 80.1 ± 11.2 (73.1–100.0) | – | 73.7 ± 1.7 (71.9–76.3) |
| Pharynx                             | 110.7               | 113 ± 8 (103–124) | 59.3 | 119 ± 8 (105–126) |
| Post-vulval body length (VL)        | 50.7                | 51.8 ± 4.9 (44.6–58.9) | – | 51.2 ± 6.7 (43–60) |
| Secretory-excretory pore            | 109                 | 108 ± 7 (99–118) | 106 ± 12 (92–113) | 108 ± 8 (97–118) |
| Lip region-vulva                    | 485.2               | 488 ± 62 (353–553) | – | 468 ± 58.6 (382–545) |
| Lip region-anus                     | 498.9               | 506 ± 64.1 (372–575) | 426 ± 29.5 (396–455) | 492 ± 63.8 (399–577) |
| Vulva-anus                          | 24.0                | 22 ± 2.9 (17.1–26.0) | – | 24 ± 5.3 (17–32) |
| Tail length                         | 37.0                | 34.3 ± 3.6 (28.0–39.3) | 44.8 ± 4.5 (39.5–47.5) | 27.2 ± 2.9 (23–31) |
| Body width                          | 36.1                | 38.3 ± 2.4 (34.1–42.0) | 19.2 ± 1.6 (17.5–20.7) | 41.2 ± 4.3 (34–46) |
| Vulval body width (VB)              | 30.4                | 30.1 ± 1.6 (27.2–32.3) | – | 30.7 ± 1.9 (27.5–32.5) |
| VL/VB                               | 1.7                 | 1.7 ± 0.1 (1.6–1.8) | – | 1.6 ± 0.1 (1.5–1.8) |
| Annulus width                       | 5.2                 | 5.4 ± 0.7 (4.1–6.5) | 2.8 ± 0.4 (2.3–3.2) | 5.7 ± 0.6 (4.9–6.8) |
| R                                   | 104.0               | 104.2 ± 4 (97–113) | 132 | 97 ± 4.3 (90–101) |
| Rst                                 | 16.0                | 15.5 ± 0.8 (14–17) | – | 14.5 ± 0.5 (14–15) |
| Rph                                 | 26.0                | 25 ± 1.4 (23–28) | – | 24.8 ± 1.9 (23–28) |
| Rexp                                | 29                  | 23.2 ± 4.2 (19–30) | 49.6 ± 2 (48–52) | 27.1 ± 4.2 (24–29) |
| RV                                  | 12.0                | 12 ± 0.8 (11–14) | – | 12.8 ± 0.8 (12–14) |
| Ran                                 | 8.0                 | 9 ± 0.9 (8–10) | – | 7.4 ± 0.5 (7–8) |
| RVan                                | 3.0                 | 3.6 ± 0.4 (3–4) | – | 3.6 ± 0.8 (3–5) |
| St/L × 100                          | 10.6                | 10.3 ± 1.3 (9.2–13.6) | – | 11 ± 1.1 (9.4–12.7) |
| Spicules                            | –                   | – | 34.2 ± 0.7 (33.6–35.0) | – |
| Gubernaculum                        | –                   | – | 6.2 ± 0.9 (5.3–7.2) | – |
vulva with a simple anterior vulval lip, straight vagina, spermatheca filled with globular sperm, presence of males, and conical-acute tail with last annulus bilobed or rounded.

Relationships

Mesocricinema abolafiai n. sp. is characterized by having flattened submedian lobes groups with M. antipolitanum (De Guiran, 1963); M. citricola (Siddiqi, 1966; Loof and De Grisse, 1989); M. juliae (Crozzoli and Lamberti, 2001); M. napoense (Talavera and Hunt, 1997); M. oostenbrinki (Loof, 1964); M. ozarkiense (Cordero et al., 2012); M. paralineolatum (Rashid et al., 1987); M. planilobatum (Talavera and Hunt, 1997); and M. rusticum (Khan et al., 1976) in the diagnostic compendium developed by Brzeski et al. (2002).

Mesocricinema abolafiai n. sp. can be distinguished from M. antipolitanum and M. rusticum by differences in the size of submedian lobes (small vs large), tail shape (conical vs rounded), spermatheca (filled vs empty), and occurring of males (present vs absent). It differs from M. citricola by a different shape of the anterior vulval lip (simple vs bilobed) and higher number of cuticular annuli (90-113 vs 73-78). It can be differentiated from M. juliae by stylet length (52.8-60.0 vs 79-86 µm) and shape of the anterior vulval lip (simple vs bilobed). Mesocricinema abolafiai n. sp. can be distinguished from M. napoense, M. paralineolatum, and M. planilobatum by the number of cuticular annuli (90-113 vs 73-79, 82-88, 75-84, respectively) and vagina direction (straight vs sigmoid). It differs from M. oostenbrinki by a different shape of the anterior vulval lip (simple vs bilobed), the number of cuticular annuli (90-113 vs 84-94), and vagina direction (straight vs sigmoid). Our new species can be distinguished from M. ozarkiense by differences in the posterior end of cuticular annuli on post-vulval region (smooth vs crenated), spermatheca (filled vs empty), occurring of males (presence vs absence), vagina direction (straight vs sigmoid), and VL/VB ratio (1.5-1.9 vs 1.0-1.4).

Regarding general morphometric characters and tail shape, our populations can also come similar to M. denoudeni (De Grisse, 1967; Loof and De Grisse, 1989); M. jessiense (Van den Berg, 1992, 1994); M. reedi (Diab and Jenkins, 1966; Loof and De Grisse, 1989); M. raskiense (De Grisse, 1964; Andrássy, 1965); M. vadense (Loof, 1964; Loof and De Grisse, 1989); M. kirjanovae (Andrássy, 1962; Loof and De Grisse, 1989); M. paradenoudeni (Rashid et al., 1987; Loof and De Grisse, 1989); and M. parareedi (Ebsary, 1981a; Loof and De Grisse, 1989). However, our populations can be differentiated from M. denoudeni by a different tail terminus shape (conical-acute vs conical-rounded), the number of post-vulval annuli (11-14 vs 8-11), VL/VB ratio (1.5-1.9 vs 1.0-1.3), and presence of males. The new species differs from M. jessiense and M. reedi by having more annuli at post-vulval region (11-14 vs 8-9 and 9-10) and higher value for the VL/VB ratio (1.5-1.9 vs 0.8-1.1 and 1.1-1.3), and differs from M. reedi by having a larger body size (402-612 vs 360-470 µm). In comparison with M. raskiense, it has more annuli throughout body (90-113 vs 62-72), and a different structure of cuticular annuli (smooth and without anastomoses vs crenated with anastomoses at midbody). It also differs from M. vadense by the number of cuticular annuli (90-113 vs 70-81), the number of post-vulval annuli (11-14 vs 7-10), VL/VB ratio (1.5-1.9 vs 0.8-1.3), and tail shape (conical-acute vs conical-rounded).

M. abolafiai n. sp. can be further distinguished from M. kirjanovae, M. citricola, M. paradenoudeni, and M. parareedi by a different shape of the anterior vulval lip (simple vs bilobed), and variations in the number of cuticular annuli (90-113 vs 79-89, 73-78, 102-130, and 111-121, respectively).

The males recovered in the type population have a unique elongated tail with pointed terminus, which only could be observed in M. raskiense and M. vadense. Spicules in our population are comparable with those of M. vadense (33-35 vs 30-34 µm) but shorter than those in M. raskiense (33.6-35.0 vs 38-43 µm). Males in some other species including M. brevicauda (Van den Berg and Spaull, 1985; Loof and De Grisse, 1989); M. curvatum, M. involutum (Loof, 1987, 1989); M. irregulare (De Grisse, 1964; Loof and De Grisse, 1989); M. juliae and M. oostenbrinki have more or less similar tails but shorter in size or with a different terminus shape.

Type host and locality

The type population was found from a canebrake in Dehdasht, Kohgiloyeh and Boyer-Ahmad province (30°49.42’N, 51°28.91’E). The other population was recovered from the rhizosphere of dog-rose shrubs (Rosa canina L.) in Basht, Kohgiloyeh and Boyer-Ahmad province (30°19.29’N, 51°15.04’E) during April 2017 by the first author.

Type specimens

Holotype, 10 paratype females and three paratype males, as well as five female specimens from the other recovered population were deposited in the nematode collection of the Department of Plant Protection, College of Agriculture, University of Zanjan, Zanjan, Iran.
Table 2. List of species, collection localities and GenBank accession numbers of individual specimens used in this study for phylogenetic analysis based on 28S rRNA gene.

| Species name                  | GeneBank accession no. | Locality     | Species name                  | GeneBank accession no. | Locality     |
|-------------------------------|------------------------|--------------|-------------------------------|------------------------|--------------|
| Aglenchus agricola            | AY780979               | Belgium      | Hemicycliophora typica        | KF430515               | South Africa|
| Caloosia longicaudata         | GU989627               | USA          | H. wyei                       | KC329574               | USA          |
| Criconema demani              | MH828126               | Russia       | H. wyei                       | KF430497               | USA          |
| C. demani                     | MH828128               | Russia       | Merlinius brevidens           | KP313844               | Iran         |
| C. mutabile                   | MK170079               | South Africa | Mesocriconema abolafiai n. sp.| MN334222               | Iran         |
| Criconema sp.                 | FN433874               | USA          | M. ornatum                    | AY780968               | Venezuela    |
| Criconemoides brevistylus     | JQ231183               | South Africa | M. solivagum                  | AY780969               | Russia       |
| C. brevistylus                | JQ231184               | South Africa | Mesocriconema sp.             | AY780967               | Italy        |
| C. brevistylus                | KC937033               | China        | M. sphaerocephalum            | AB933464               | Japan        |
| C. informis                   | KU722386               | Iran         | M. sphaerocephalum            | AB933465               | Japan        |
| C. myungsguage                | MH444641               | China        | M. sphaerocephalum            | AY780951               | Italy        |
| C. obtusicaudatus             | JQ231186               | South Africa | M. xenoplax                   | AB933468               | Japan        |
| C. obtusicaudatus             | JQ231187               | South Africa | M. xenoplax                   | AY780961               | Germany      |
| Eutylenchus excretorius       | AY780980               | Germany      | M. xenoplax                   | AY780963               | USA          |
| Hemicaloosia guangzhouensis   | KT381016               | China        | M. xenoplax                   | AY780965               | Italy        |
| H. guangzhouensis             | KT381017               | China        | M. xenoplax                   | FN433855               | USA          |
| H. vagisclera                 | JQ246422               | USA          | M. xenoplax                   | FN433858               | USA          |
| Hemicriconemoides gaddi       | MK050500               | China        | M. xenoplax                   | FN433859               | USA          |
| H. roae                       | MK371811               | India        | M. xenoplax                   | KC538862               | USA          |
| H. rosae                      | MK371813               | India        | M. xenoplax                   | MG680454               | Portugal     |
| H. silvaticus                 | KF856531               | Japan        | Ogma civellae                 | AY780955               | Venezuela    |
| H. strictathecatus            | MH142613               | China        | O. decalineatus               | MF683230               | South Africa |
| H. wessoni                    | KF856521               | USA          | Paratylenchus tenuicaudatus   | KU291239               | Iran         |
| Hemicycliophora conida        | FN433875               | Belgium      | Sphaeronema alni              | AY780978               | Germany      |
| H. epicharoides               | KF430512               | Italy        | Trophonema arenarium           | AY780971               | Italy        |
| H. gracilis                   | KF430482               | USA          | Tylenchulus semipenetrans     | KM598334               | Iran         |
| H. halophila                  | KF430444               | New Zealand  | T. semipenetrans              | KM598335               | Iran         |
| H. lutosa                     | GQ406240               | South Africa | T. semipenetrans              | MH156801               | China        |
| H. lutosa                     | GQ406241               | South Africa | T. semipenetrans              | MH156802               | China        |
| H. signata                    | MG019824               | Mozambique   | Xenocriconemella macrodora    | AY780960               | Italy        |
| H. subbotini                  | MG701275               | China        |                               |                        |              |
Figure 4: Bayesian 50% majority rule consensus tree as inferred from the D2 to D3 expansion segments of 28S rRNA gene dataset of Criconematoidea under the general time reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR + I + G). Posterior probabilities more than 50% are given for appropriate clades. The new obtained sequence in this study is indicated in bold. Scale bar=expected changes per site.
### Table 3. List of species, collection localities and GenBank accession numbers of individual specimens used in this study for phylogenetic analysis based on 18S rRNA gene.

| Species name                      | GeneBank accession no. | Locality   | Species name                      | GeneBank accession no. | Locality   |
|-----------------------------------|------------------------|------------|-----------------------------------|------------------------|------------|
| Bakernema inaequale               | MF094908               | USA        | H. conida                         | AJ966471               | GenBank    |
| Criconema mutabile                | MF094914               | USA        | H. conida                         | KJ934172               | USA        |
| C. pernictum                      | MF094899               | USA        | H. conida                         | KJ934173               | USA        |
| C. petasum                        | MF094927               | USA        | H. subbotini                       | MG701280               | China      |
| C. sphagni                        | MF094968               | USA        | Lobocriconemella sp.              | MF094981               | USA        |
| Criconemoides annulatus           | MF095015               | USA        | L. thomei                         | MF094928               | USA        |
| C. annulatus                      | MF095024               | USA        | L. thomei                         | MF094996               | USA        |
| C. informis                       | MF094902               | USA        | Merlinius joctus                   | FJ969128               | GenBank    |
| C. informis                       | MF095025               | USA        | Mesocriconema abolafiai n. sp.    | MN334221               | Iran       |
| C. parvus                         | MF795587               | China      | M. curvatum                       | MF094891               | USA        |
| Crossonema fimbriatum             | MF095026               | USA        | M. discus                         | MF094892               | USA        |
| C. fimbriatum                     | MF094960               | USA        | M. inaratum                       | MF094903               | USA        |
| C. menzeli                        | MF094937               | USA        | M. onoense                        | MF094909               | USA        |
| Discocriconemella limitanea       | MF795591               | China      | M. ornatum                        | MF094893               | USA        |
| D. limitanea                      | MF095031               | Costa Rica | M. rusticum                       | MF094965               | USA        |
| Gracilacus paralatescens          | MH200615               | China      | Mesocriconema sp.                 | MF094967               | USA        |
| G. wuae                           | MF095028               | Canada     | Mesocriconema sp.                 | MF095012               | USA        |
| Hemicalosia graminis              | JQ446376               | USA        | M. sphaerocephalum                | KJ934182               | USA        |
| Hemicriconemoides. chitwoodi      | KJ934162               | USA        | M. xenoplax                       | KJ934180               | USA        |
| Hemicriconemoides. fujianensis    | MH444626               | China      | M. xenoplax                       | KJ934177               | USA        |
| H. kanayaensis                    | MG029558               | China      | M. xenoplax                       | MF095021               | USA        |
| H. kanayaensis                    | MG029559               | China      | M. xenoplax                       | MF094992               | USA        |
| H. parasinensis                   | MH444635               | China      | Ogma decalineatus                 | MF094952               | USA        |
| H. parataiwanensis                | MG029556               | China      | O. menzeli                        | EU669919               | GenBank    |
| H. parataiwanensis                | MG029557               | China      | O. seymouri                       | MF094933               | USA        |
| H. pseudobrachyurus               | AY284622               | GenBank    | Ogma sp.                          | KJ934175               | USA        |
| Hemicriconemoides sp.             | MF095013               | Thailand   | Paratylenchus straeleni           | AY284631               | GenBank    |
| H. wessoni                        | KJ934163               | USA        | Tylenchulus semipenetrans         | MH136626               | China      |
| H. wessoni                        | KJ934166               | USA        | T. semipenetrans                  | AJ966511               | UK         |
| Hemicyclophora aquatica           | MF094911               | USA        | Xenocriconemella macrodora         | MF095001               | USA        |
| H. conida                         | EU669914               | GenBank    |                                   |                        |            |
Etymology

The species epithet refers to the name of Dr. Joaquín Abolafia, the well-known nematologist from University of Jaén, Spain, who works on nematode systematics.

Phylogenetic relationships

The 28S alignment was 738 bp long and consisted of 58 sequences as ingroups and three sequences, including Aglenchus agricola (Andrássy, 1954; De Man 1884) (AY780979), Eutylenchus excretorius (Sher et al., 1966) (AY780980), and Merlinius brevidens (Allen, 1955; Siddiqi, 1970) (KP313844), as outgroups (Table 2). Phylogenetic relationships of M. abolafiai n. sp. with other representatives of Criconematidae (Taylor, 1936; Thorne, 1949) inferred from the analysis of D2 to D3 expansion fragments of 28S rRNA gene sequences with collapsed branches, with PP less than 50%, are given in Figure 4. In this tree, M. abolafiai...
Table 4. List of species, collection localities and GenBank accession numbers of individual specimens used in this study for phylogenetic analysis based on ITS rRNA gene.

| Species name                          | GeneBank accession no. | Locality     | Species name                          | GeneBank accession no. | Locality     |
|---------------------------------------|------------------------|--------------|---------------------------------------|------------------------|--------------|
| Coslenchus rhombus                    | MK874505               | South Africa | Mesocriconema curvatum                | MF094891               | USA          |
| Criconema silvum                      | MF683236               | South Africa | M. inaratum                          | HM116070               | USA          |
| C. silvum                             | MF683237               | South Africa | M. inaratum                          | HM116069               | USA          |
| Criconemoides brevistylus             | KC937032               | China        | M. inaratum                          | HM116058               | USA          |
| C. brevistylus                        | JQ231188               | South Africa | M. inaratum                          | HM116055               | USA          |
| C. myungsugae                         | MH444640               | China        | M. inaratum                          | HM116052               | USA          |
| C. myungsugae                         | MH444639               | China        | M. inaratum                          | HM116051               | USA          |
| C. obtusicaudatus                     | JQ231189               | South Africa | M. nebraskense                        | MH013431               | USA          |
| C. obtusicaudatus                     | JQ231190               | South Africa | M. nebraskense                        | KY574844               | USA          |
| Crossonema sp.                        | MK292124               | USA          | M. nebraskense                        | KY574860               | USA          |
| Hemicriconemoides californianus       | KF856558               | USA          | M. nebraskense                        | KY574861               | USA          |
| H. californianus                      | KF856560               | USA          | M. nebraskense                        | KY574862               | USA          |
| H. chitwoodi                          | KF856543               | USA          | M. nebraskense                        | KY574863               | USA          |
| H. fujianensis                        | MH444616               | China        | M. nebraskense                        | KY574864               | USA          |
| H. kanayaensis                        | EF126179               | Taiwan       | M. nebraskense                        | KY574865               | USA          |
| H. kanayaensis                        | MG029566               | China        | M. onoenose                          | JQ708120               | USA          |
| H. kanayaensis                        | MG029568               | China        | M. ornatum                           | JQ708124               | USA          |
| H. ortonwilliamsi                     | KF856552               | Spain        | M. ozonikense                        | JQ708122               | USA          |
| H. paracamelliae                      | MG029560               | China        | Mesocriconema sp.                    | KY574858               | USA          |
| H. promissus                          | KF856555               | Spain        | Mesocriconema sp.                    | KY574857               | USA          |
| H. rosae                              | MK371815               | India        | Mesocriconema sp.                    | KY574856               | USA          |
| Hemicriconemoides sp.                 | KM516185               | USA          | M. xenoplax                          | JQ708112               | USA          |
| H. strictathecatus                     | KF856655               | South Africa | M. xenoplax                          | HM116073               | USA          |
| H. strictathecatus                     | MH142617               | China        | M. xenoplax                          | HM116057               | USA          |
| H. strictathecatus                     | KM516186               | USA          | M. xenoplax                          | MF095021               | USA          |
| H. strictathecatus                     | KM516190               | USA          | M. xenoplax                          | MF094992               | USA          |
| H. strictathecatus                     | KM516191               | USA          | M. xenoplax                          | MF094915               | USA          |
| Hemicyclophora californica             | KF430576               | USA          | M. xenoplax                          | MF094916               | USA          |
| H. gracilis                           | MG019827               | USA          | Neobakernema variabile                | MF683239               | USA          |
| H. raskii                             | KF430577               | USA          | N. variabile                         | MF683238               | USA          |
| H. thienemanni                         | KF430569               | Russia       | Ogma decalineatus                    | MF683235               | USA          |
| Mesocriconema abolafiai n. sp.        | MN334228               | Iran         | Parastylenchus hamatus                | KF242257               | USA          |
| M. curvatum                           | HM116062               | USA          | Tylenchulus semipenetrans            | JN112274               | USA          |
| M. curvatum                           | HM116064               | USA          | T. semipenetrans                    | FJ588909               | China        |
| M. curvatum                           | HM116066               | USA          | T. semipenetrans                    | MH124562               | China        |
| M. curvatum                           | HM116067               | USA          | T. semipenetrans                    | MH124561               | China        |
| M. curvatum                           | HM116068               | USA          |                                      |                        |              |
n. sp. formed a cluster with an isolate of *M. xenoplax* (MG680454) and an unnamed population (AY780967). Partial 28S rRNA sequences of the *M. abolafiai* n. sp. from Iran show about 23bp (3%) difference with the closest species according to 28S tree (*M. xenoplax*: MG680454), whereas two species distinguished with some characters such as tail (conical vs subcylindrical) and vagina (straight vs sigmoid) shape. There is not any record of partial 28S rRNA sequences of *M. ozarkience* that it is closest species to *M. abolafiai* n. sp. based on morphological characteristics.

The 18S alignment was 1538bp long and consisted of 60 sequences as ingroups and *Merlinius joctus* (Thome, 1949; Sher, 1974) as outgroup (Table 3). Phylogenetic relationships of *M. abolafiai* n. sp. with other representatives of Criconematidae inferred from the analysis of 18S RNA gene sequences with collapsed branches, with PP less than 50%, are given in Figure 5. The new species clustered with *M. rusticum* (MF094965) and *Mesocriconema* sp. (MF094967), all in a sister clade with *M. curvatum* (MF094891). The most important morphologically differences between *M. abolafiai* n. sp. and *M. rusticum* are related to lip region and post-vulval shape, respectively. The lip region in *M. rusticum* is set off with large submedian lobes, anteriorly flattened giving appearance of truncate

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**Figure 6:** Bayesian 50% majority rule consensus tree as inferred from the ITS rRNA gene dataset of Criconematoida under the symmetrical model of sequence evolution with a gamma-shaped distribution (SYM + G). Posterior probabilities more than 50% are given for appropriate clades. The new obtained sequence in this study is indicated in bold. Scale bar = expected changes per site.
Table 5. Summary of the diagnostic characteristics of 12 species of *Mesocriconema* recently described worldwide. For other species, see (Brzeski et al., 2002). (St measurements in µm, L measurements in mm).

| Species            | St   | R    | Rex  | RV   | RVan | Ran  | V    | VL/VB | Anas. | A. M. | Vagina       | A. V. lip  | Tail shape | L   | S. M. L. | Original des. |
|--------------------|------|------|------|------|------|------|------|-------|-------|-------|--------------|------------|------------|-----|---------|----------------|
| *M. abolafiai* n. sp. | 52–60 | 90–113 | 19–30 | 11–14 | 3–5  | 7–10 | 87–90 | 1.5–1.8 | 1   | smo.–irr. | straight     | simple     | con–acute  | 0.4–0.61 | flat    | Present study |
| *M. apurense*      | 48–52 | 140–147 | 36–38 | 9–12 | 1–2  | 8–9  | 93–95 | –      | 0   | smo.     | straight     | lobulated, without projections | con.–rounded | 0.38–0.45 | rounded |    |
| *M. campbelli*     | 58–62 | 102–108 | 25–28 | 11–15 | –    | 5–7  | 89–93 | 1.2–1.8 | rare | smo.–ser. | straight     | serrated   | con.–pointed | 0.37–0.45 | –       | Wouts (2006) |
| *M. jilliae*       | 79–86 | 89–95  | 26   | 8–9  | 1–2  | 5–6  | 91–92 | –      | few  | smo.    | straight     | with two projections | conical     | 0.46–0.51 | flat    |    |
| *M. lamothei*      | 65–70 | 89–98  | 17–31 | 6–8  | 0–2  | 4–8  | 91–95 | –      | rare | smo.    | slightly curved | with two projections | con.–truncate | 0.40–0.49 | rounded |    |
| *M. lobellum*      | 51–60 | 85–92  | 25   | 6–7  | 2–3  | 3–4  | 92–93 | 0.9–1.2 | rare | –       | straight     | simple     | rounded   | 0.41–0.53 | rounded | Pramodini et al. (2007) |
| *M. malagutii*     | 45–49 | 108–114 | 33–35 | 8–9  | 2    | 4–6  | 93–94 | –      | many | smo.    | straight     | lobulated, without projections | con.–truncate | 0.34–0.40 | rounded |    |
| *M. nebraskense*   | 45–59 | 84–113 | 24–31 | 6–11 | 2–3  | 3–7  | 90–96 | 0.7–1.6 | 1–4  | smo.    | straight     | with two projections | rounded   | 0.39–0.60 | rounded |    |
| *M. ovospermatum*  | 82   | 109    | 38   | 9    | 3    | 6    | 93   | –      | 0   | finely crenate | straight     | ?         | conical   | 0.22  | ?       | Mohilal and Dhanachand (1998) |
| *M. ozarkiense*    | 49–61 | 107–119 | 27–34 | 10–14 | 2–4  | 6–10 | 89–93 | 1.0–1.4 | 0–1  | som.–irr. | straight     | simple     | conical   | 0.38–0.51 | flat    | Cordero et al. (2012) |
| *M. theobromae*    | 47–51 | 73–74  | 24–26 | 7    | 1    | 5    | 93–94 | –      | many | som.    | straight     | lobulated, without projections | conical     | 0.27–0.32 | rounded |    |
| *M. waitha*        | 66–78 | 117–140 | 26–31 | 9–10 | 4–5  | 4–6  | 92–94 | 0.9–1.0 | many | som.–irr. | sigmoid     | simple     | rounded   | 0.43–0.53 | flat?   | Pramodini et al. (2006) |

Notes: St, stylet; Anas., Anastomoses; A. M., Annuli margin; A. V. lip, Anterior vulval lip; S. M. L, Submedian lobes; smo., smooth; ser., serrate; irr., irregular; con, conical; Original des., Original description. Note1: *Mesocriconema bakeri* (Wu, 1965; Loof and De Grisse, 1989); *M. calvatum* (Eroshenko, 1982; Loof and De Grisse, 1989); *M. hymenophorum* (Wouts and Sturhan, 1999); *M. longistyletum* (De Grisse and Maas, 1970; Loof and De Grisse, 1989); *M. paramonovi* (Razzhivin, 1974; Loof and De Grisse, 1989); *M. variabile* (Raski and Golden, 1966; Brzeski et al., 2002) and *M. yukonense* (Ebsary, 1982, 1991) that have been listed in Brzeski et al. (2002), transferred to *Neobakernema* (Ebsary, 1981b) by Geraert (2010). Note2: *Mesocriconema incrassatum* (Raski and Golden, 1966; Loof and De Grisse, 1989) that has been listed in Brzeski et al. (2002), transferred to *Lobocriconema* (De Grisse and Loof, 1965) by Geraert (2010).
The genus *Mesocriconema* has a large number of species, which are morphologically very close to each other. Powers et al. (2016) distinguished 24 COI haplotype groups; only five of them corresponded to morphologically characterized species. The authors further noticed that it is not unexpected that additional sampling of *Mesocriconema* will continue to reveal cryptic species within Linnaean morphospecies; as these species have been described in the recent works (Powers et al., 2016; Olson et al., 2017).

*Mesocriconema abolafiai* n. sp. comes close to *M. ozarkiense* and some other species bearing conical tails with narrow tails (Table 5); however, detailed morphological observations made by light microscopy and SEM, as well as molecular phylogenetic analysis using different genes allowed us to consider *M. abolafiai* n. sp. as a new species. The new species can be differentiated from the most closely related species, *M. ozarkiense*, by some morphological and morphometric characters, as well as a different phylogenetic position in the ITS rRNA tree which resulted from a 129 bp (32%) nucleotide divergence. Unfortunately, there is no molecular information on 28S rRNA and 18S rRNA genes of *M. ozarkiense* so the above results can be further supported by additional analyses of these gene sequences.

**Acknowledgments**

The authors thank anonymous reviewers and the editor of this MS for their valuable suggestions to improve the manuscript. SEM pictures were obtained with the assistance of Dr Joaquín Abolafia and the technical staff (Amparo Martínez-Morales) and equipment of ‘Centro de Instrumentación Científico-Técnica (CICT)’ from University of Jaén, Spain, and the financial support of the research activities ‘PAIUJA 2017/2018: EI_RNM02_2017’ and ‘PAIUJA 2019/2020: EI_RNM02_2019’ of the University of Jaén.

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