Morphological and molecular characterization of two species of Neothada Khan, 1973 (Nematoda: Tylenchidae) from Iran, with notes on N. cancellata

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Khan (1973) proposed the genus Neothada to accommodate Thada cancellata Thorne, 1941 and T. tatra Thorne & Malek, 1968, two species with longitudinal incisures around the circumference of the body in addition to the lateral fields. Siddiqi (2000) accommodated the genera Neothada and Thada in the subfamily Thadinae Siddiqi, 1986, but Geraert (2008) placed them in the subfamily Boleodorinae Khan, 1964. According to Geraert (2008), the genus Neothada currently contains six valid species namely: N. tatra (Thorne and Malek, 1966) Khan, 1973 as the type species; N. cancellata (Thorne, 1941) Khan, 1973; N. costata (Geraert and Raski, 1986) Siddiqi, 2000; N. geraerti (Andrássy, 1982) Siddiqi, 1986; N. hades Heyns & van den Berg, 1996 and N. major Maqbool & Shahina, 1989. So far, only N. cancellata has been reported from Iran (Ghorbanzad et al., 2012; Yaghoubi et al., 2015). Neothada cancellata, N. hades and N. major were all collected in Khuzestan province, Iran and compared. The latter two species are illustrated and described by morphological, morphometric and molecular approaches.

Materials and methods

Morphological characterization

Three species of the genus Neothada, including N. cancellata, N. hades and N. major, collected from the rhizosphere of mosses in Khuzestan, southwestern Iran, are redescribed and illustrated. Neothada hades and N. major are new records from Iran. Neothada hades has 14 longitudinal incisures excluding the lateral field, body length of 586 (505–674) µm, stylet 10.5 (10.0–10.8) µm in length bearing distinct basal knobs, and an elongated-conical tail 70.4 (65–74) µm long with a finely to bluntly rounded terminus. N. major possesses 18–20 longitudinal incisures excluding the lateral field, body length of 657 (600–728) µm, stylet 10.9 (10.3–11.7) µm long with basal swellings but not distinct knobs, and an elongated-conical tail 78.2 (70–83) µm long ending to a finely to bluntly rounded terminus. Molecular phylogenetic studies of the two species (N. hades and N. major) with 664 bp of D2-D3 expansion segments of 28S rDNA revealed that they form a clade with N. cancellata.

Keywords
Neothada cancellata, Neothada hades, Neothada major, 28S rDNA, Khuzestan, phylogeny, Bayesian inference.
studied with a light microscope equipped with a Dino-eye microscope eye-piece camera and Dino Capture version 2.0 software. Specimens were identified to species with the identification key of Geraert (2008).

**DNA extraction, PCR and sequencing**

Nematode DNA was extracted from single individuals and DNA extracts were stored at −20°C until use as PCR template. Protocols for DNA extraction were followed as described by Tanha Maafi et al. (2003). Fragments of D2-D3 expansion segments of 28S rDNA were amplified using the forward D2A (5′–AC AAGTACCGTAGGAAAGT–3′) and reverse D3B (5′–TCGGAAGGAAACCAGCTACTA–3′) primers (Nunn, 1992). The 30 μl PCR contained 15 μl Taq DNA polymerase 2 × MasterMix (Ampliqon, Denmark), 1 μl (10 pmol μl⁻¹) each of forward and reverse primers, 2 μl of DNA template and 11 μl deionised water. This mixture was placed into a Hybaid Express thermal cycler (Hybaid, Ashford, Middlesex, UK). The thermal cycling profile was denaturation at 95°C for 4 minutes, then 33 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 90 seconds. A final extension was performed at 72°C for 10 minutes. The quality of PCR was checked by electrophoresis of 4 μl of the PCR reaction in 1% agarose gel containing ethidium bromide. Products were visualized and photographed under UV light. The length and concentration of each PCR product was measured by comparison with a low DNA mass ladder (Invitrogen, Carlsbad, CA). The PCR product was purified and sequenced directly for both strands using the same primers with an ABI 3730XL sequencer (Bioneer, Seoul, South Korea). The newly obtained sequences were submitted to GenBank database under accession numbers MN970001 and MN970002 for the D2-D3 expansion fragments of 28S sequences.

**Phylogenetic analyses**

For phylogenetic relationships, analyses were based on D2-D3 expansion fragment of 28S rDNA. The newly obtained sequences were edited and aligned with other sequences available in GenBank using the Muscle alignment tool implemented in MEGA7 (Kumar et al., 2016). The ambiguously aligned parts and divergent regions were identified using the online version of Gblocks 0.91b (Castresana, 2000) and were removed from the alignments with MEGA7. The best-fit model of nucleotide substitution used for the phylogenetic analysis was statistically selected using jModelTest 2.1.10 (Darriba et al., 2012). Phylogenetic tree was generated with a Bayesian inference method using MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2012). *Aphelenchus avenae* (Bastian, 1865 (KP527123) was chosen as outgroup for the tree according to (Bai et al., 2020; Hosseinivand et al., 2020b; Yaghoubi et al., 2015). The analysis under general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR+I+G) model was initiated with a random starting tree and run with the Markov Chain Monte Carlo (MCMC) for 1 x 10⁶ generations. The tree was visualized and saved with FigTree 1.4.3 (Rambaut, 2016) and edited with Adobe Acrobat® XI Pro 11.0.1.

**Results**

**Systematics**

*Neothada hades* Heyns & Van den Berg, 1996 (Figures 1 and 2; Table 1).

Figure 1: Iranian population of *Neothada hades*. Female (A-I): (A) Entire body; (B) Anterior end and pharyngeal region; (C) Lip region and stylet; (D) Stylet; (E) Reproductive system; (F) Amphidial aperture; (G) Cross section from mid-body; (H-I) Posterior end.
Description

Female

Body straight to slightly ventrally curved. Cuticular annules prominent, at neck 1.5–2.2µm and at mid-body 2.7–3.5µm in width. Lateral field with four incisures, delimiting three ridges, starting from middle of procorpus and continue to five or seven annules anterior to tail tip. In addition to the lateral lines, 14 evenly spaced longitudinal incisures around the circumference of the body. Lateral field 6.0–8.5µm wide occupying 32–42% of the corresponding body diameter, its ridges more pronounced and larger than other longitudinal incisures; annulation and longitudinal incisures produce rectangular tessellation. Cephalic region flatly rounded, with two annules, 7.2–7.5µm wide at base and 2.5–3.0µm high. Amphidial aperture a conspicuous longitudinal to slightly bent slit extending as far as the second neck annule. Cephalic framework inconspicuous, weakly sclerotized. Stylet delicate, conus length about one-third (3.2–3.9µm, or 31–36%) of the total stylet length, 7–8 annules from anterior end; knobs conspicuous, rounded, 1.7–2.2µm wide. Dorsal pharyngeal gland opening 2.6–4.0µm from stylet base. Corpus cylindroid with slightly swollen median bulb lacking valve; isthmus as wide as procorpus, nerve ring at

Figure 2: Iranian population of *Neothada hades*. Female (A-K): (A) Entire body; (B-D) Lip region and stylet; (E) Amphidial aperture; (F) Cross section from mid-body; (G) Basal pharyngeal bulb and secretory-excretory pore; (H) Deirid and lateral field; (I-K) Posterior end. (Scale bars: A = 100µm; B-K = 10µm).
Table 1. Morphometric data of *Neothada major*, *N. hades* and *N. cancellata* from Iran.

|                | Neothada major | Maqbool and Shahina (1989) | N. hades | Heyns and Van den Berg (1996) | N. cancellata | Present study |
|----------------|----------------|----------------------------|----------|-------------------------------|---------------|---------------|
|                | Present study  | M. cv                     | Present study | M. cv | Present study | Present study |
| n              | 15             | 15                        | 10       | 10                            | 8             |               |
| L              | 657±40.6 (600–728) | 6.1                      | 640–800 | 586±50.3 (505–674) | 8.5 | 530–620 | 561±32.5 (501–596) |
| L'             | 579±37.8 (521–645) | 6.5                      | –        | 515±48.1 (440–600) | 9.3 | –         | 491±30.9 (431–527) |
| Head-Vulva     | 461±27.2 (420–508) | 5.9                      | –        | 414±35.5 (354–467) | 8.5 | –         | 396±24.4 (349–424) |
| R Head-Vulva   | 142±11.7 (120–160) | 8.2                      | 154–156  | 147±8.3 (127–157) | 5.6 | –         | 146±9.9 (125–156) |
| R Head-Anus    | 174±12.4 (149–194) | 7.1                      | –        | 175±9.2 (152–185) | 5.2 | –         | 173±11.1 (150–185) |
| Stylet         | 10.9±0.3 (10.3–11.7) | 3.3                      | 12–14.4  | 10.5±0.2 (10.0–10.8) | 2.1 | 9.0–10.5 | 10.8±0.2 (10.6–11.2) |
| a              | 35.9±4.1 (29–44) | 11.4                      | 34–39    | 30.3±2.0 (27.0–33.7) | 6.8 | 25–31   | 29.5±1.7 (27–32) |
| b              | 5.7±0.4 (5.2–6.5) | 6.9                      | 5.9–6.3  | 5.3±0.2 (4.8–5.7) | 5.4 | 5.3–6.4 | 5.2±0.2 (4.7–5.5) |
| c              | 8.3±0.3 (7.5–8.7) | 3.7                      | 9.0–10.2 | 8.3±0.4 (7.5–9.1) | 5.8 | 8.1–10.3 | 8.0±0.3 (7.5–8.6) |
| c'             | 6.4±0.3 (5.6–7.1) | 6.1                      | 5.5–6.0  | 6.0±0.3 (5.5–6.5) | 5.8 | 4.6–6.0 | 6.1±0.4 (5.5–6.8) |
| V              | 70.2±0.7 (68.8–71.2) | 1.0                      | 70–73    | 70.6±1.1 (68.5–72.7) | 1.6 | 70–74   | 70.9±0.6 (70.0–71.8) |
| V'             | 79.7±0.9 (78.0–81.1) | 1.1                      | –        | 80.3±1.3 (77.0–81.9) | 1.6 | –         | 80.8±0.5 (80.4–82.4) |
| R              | 202±12.5 (175–222) | 6.2                      | 215–245  | 204±10.1 (179–216) | 4.9 | 149–160 | 189±12.3 (175–191) |
| Excretory pore | 97.2±8.7 (75–110) | 9.0                      | –        | 94.7±5.8 (87–104) | 6.1 | –         | 92.5±6.7 (86–100) |
| Pharynx        | 114±7.6 (94–127) | 6.7                      | 108–133  | 109.5±8.4 (95–119) | 7.7 | –         | 107±9.3 (90–110) |
| R Pharynx      | 41±3.0 (36–50) | 7.2                      | 45–50    | 47±3.1 (43–52) | 6.6 | 30–38   | 45±2.5 (42–50) |
| Annulus width  | 3.8±0.7 (3.2–5.6) | 20.0                      | 3.2–4.0  | 2.9±0.2 (2.7–3.5) | 8.3 | 4.0–4.6 | 3.0±0.2 (2.6–3.6) |
| Body width     | 18.4±1.7 (15.6–21.0) | 9.6                      | –        | 19.2±0.8 (17.8–21.0) | 4.5 | –         | 18.8±0.6 (18–20) |
| Vulva body width | 17.5±1.5 (15–20) | 9.0                      | –        | 17.9±0.7 (16.5–19.0) | 4.0 | –         | 17.5±0.7 (15.5–18.8) |
| Vulva-Anus     | 117±11.8 (101–137) | 10.0                      | –        | 101.6±14.9 (86–138) | 14.7 | 95±7.1 | 95±7.1 (81–103) |

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mid-isthmus and located 56–73 μm from anterior end. Pharyngeal basal bulb short, pyriform, 7.5–9.0 μm wide, 19–24 μm in length. Pharyngo-intestinal valve hemispherical. Excretory pore slightly sclerotized, at middle of basal bulb, 38–45 annules from anterior end. Hemizonid one annule anterior to the excretory pore, 85–104 μm from anterior end. Deirids adjacent to the level of excretory pore, 87–107 μm from anterior end. Vulva a transverse slit, not protruding, without lateral flaps. Vagina width 6.7–8.0 μm, 37–46% of vulva body diameter. Post-vulval uterine sac length 9.0–12.8 μm or 57–63% of vulval body diameter. Spermatheca long, variable in shape, near-rectangular, 7.5– to 9.0 μm × 26 to 33 μm. Ovary outstretched, oocytes arranged in a single row. Rectum curved to slightly sigmoid, length half of anal body diameter. Tail elongate-conoid, tail tip finely to bluntly rounded, 27–33 annules on ventral side of the tail.

Male
Not found.

Voucher specimens
In all, 10 females are deposited in the nematode collection of the Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

Habitat and locality
Soil around of mosses in Dezful, Khuzestan Province, southwestern Iran, by Manouchehr Hosseinvand at February 2017 (GPS coordinates: 48°47'18"N, 26°36'32"E).

Morphological remarks
*Neothada hades* can be distinguished from all other known species of the genus by possession of distinct stylet knobs. The morphology and morphometrics of the Iranian population are coincident with the original species description of Heyns & Van den Berg (1996), except for the number and width of body annules (179–216 vs 149–160 and 2.7–3.9 vs 4.0–4.6 μm, respectively), tail length (65–74 vs 55–66 μm) and absence of males (vs presence).

*Neothada major* Maqbool & Shahina, 1989 (Figures 3 and 4; Table 1).

![Figure 3: Iranian population of *Neothada major*. Female (A-I): (A) Entire body; (B) Anterior end and pharynx; (C) Lip region; (D) Stylet; (E) Amphidial aperture; (F) Cross section from mid-body; (G-H) Posterior end; (I) Reproductive system.](image-url)
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Description

Female

Body straight to slightly ventrally curved in posterior half. Cuticular annules prominent, at neck 1.5–2.2 µm and at mid body 3.2–5.6 µm in width. Lateral field with four incisures, delimiting three ridges, starting at middle of isthmus and continue to seven or nine annules anterior to tail tip. In addition to the lateral lines, 19–20 evenly spaced longitudinal incisures around the circumference of the body. Lateral field 5.5–8.0 µm wide or occupying 30–39% of corresponding body diameter; annulation and longitudinal incisures produce rectangular tessellation. Cephalic region flatly rounded, with one or two annules, 6.8–8.1 µm wide at base and 2.5–3.6 µm high. Amphidial aperture a conspicuous longitudinal to slightly bent slit, extending as far as the second neck annule. Cephalic framework

Figure 4: Iranian population of Neothada major. Female (A-N): (A) Entire body; (B) Anterior end and pharyngeal region; (C-F) Lip region and stylet; (G) Amphidial aperture; (H) Annules at mid-body; (I) Vulval region; (J-L) Posterior end; (M, N) Cross section from mid-body. (Scale bars: A = 100 µm; B-N = 10 µm).
inconspicuous, weakly sclerotized. Stylet delicate, conus length about one-third, 3.4–3.9 µm or 32–35% of the total stylet length, without knobs but slightly swellings, 7–8 annules from anterior end. Dorsal pharyngeal gland opening 3.0–4.0 µm from stylet base. Corpus cyindroid, median bulb weakly developed, lacking valve; isthmus slender, slightly narrower than procorpus, nerve ring at posterior part of isthmus and located at 66–86 µm from anterior end. Basal bulb short, pyriform to slightly saccate, 7.7–10.0 µm wide and 18–29 µm in length. Pharyngo-intestinal valve hemispherical. Excretory pore at anterior part of basal bulb, 35–40 annules from anterior end. Hemizonid one to two annules anterior to the excretory pore, 88–104 µm from anterior end. Deirid at level of excretory pore, 92–113 µm from anterior end. Vulva a transverse slit, not protruding, without flaps. Vagina length less than half of vulva body diameter. Post-vulval uterine sac 10–13 µm. Spermatheca long, variable in shape. Ovary outstretched, oocytes in single row. Tail elongate-conoid, tail tip bluntly rounded, 26–33 annules on ventral side of the tail.

**Male**

Not found.

**Voucher specimens**

In all, 15 females are deposited in the nematode collection of the Department of Plant Protection, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

**Habitat and locality**

Soil around *Prosopis cineraria* (L.) (Jand) in Bostan, Khuzestan Province, southwestern Iran, by Manouchehr Hosseinvand, March 2019 (GPS coordinates: 48°05’19”N, 43°45’31”E).

**Morphological remarks**

The morphology and morphometrics of the Iranian population agree well with the type population of *N. major*, except for minor differences in stylet length (10.3–11.7 vs 12–14.4), c ratio (7.5–8.7 vs 9.0–10.2) and absence of males (vs presence).

*Neothada cancellata* (Thorne, 1941) Khan, 1973 (Figure 5; Table 1).

**Description**

**Female**

Body straight to slightly ventrally curved. Cuticle annules prominent at mid body 2.6–3.6 µm. Lateral field with four incisures, delimiting three ridges, starting at mid of procorpus to five or seven annules anterior to tail tip. In addition to the lateral lines, 14–16 evenly spaced longitudinal incisures around the circumference of the body. Head flatly rounded, with two annules. Amphidial aperture a conspicuous longitudinal to slightly bent slit, extending as far as the second neck annule. Cephalic framework inconspicuous, weakly sclerotized. Stylet delicate, conus length about one-third of the total stylet length. Dorsal pharyngeal gland opening 2.5–4.0 µm from stylet base. Median bulb lacking valve, isthmus as wide as procorpus, nerve ring at mid of isthmus, basal bulb short, pyriform. Excretory pore slightly sclerotized, at mid of basal bulb. Deirids at level of excretory pore. Vulva with transverse slit. Vagina length less than half of vulva body diameter. Post-vulval uterine sac 10–13 µm. Spermatheca long, variable in shape. Ovary outstretched, oocytes in single row. Tail elongate-conoid, tail tip bluntly rounded, 26–33 annules on ventral side of tail.

**Male**

Not found.

**Habitat and locality**

Soil around roots of *Cynodon dactylon* (L.) Pers. and mosses in Dezful, Khuzestan Province, southwestern Iran, by Manouchehr Hosseinvand at February 2017 (GPS coordinates: 48°47’21”N, 26°35’06”E).

**Molecular phylogenetic status**

Two new D2-D3 28 S rDNA gene sequences were obtained in the present study (MN970001 and
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Figure 5: Iranian population of Neothada cancellata. Female (A-N): (A) Entire body; (B-F) Anterior end and stylet; (G) Vulval region; (H) Cross section from mid-body; (I-N) Posterior end.

MN970002). These sequences showed a 96–97% similarity values to N. cancellata (KP730046), and 80–82% similarity with Basiria spp. using BlastN search in NCBI. The partial D2-D3 of 28S rDNA gene sequences alignment contained 38 taxa including Aphelenchus avenae (KP527123) as outgroup taxon and was 664 bp in length after removing ambiguously aligned regions. The 50% majority rule consensus phylogenetic tree generated from the partial D2-D3 region of 28S rDNA alignment by Bayesian inference (BI) analysis under GTR + I + G model is presented in Figure 6.

N. hades is closely related to N. cancellata (PP = 100) and both species are related in the clade with N. major (PP = 100). N. hades differs from N. cancellata by 16 bp (2.1%) and from N. major by 11 bp (1.6%), and N. major differs from N. cancellata by 20 bp (1.9%).

Discussion

In our 28S rDNA phylogeny, Neothada is highly supported as a valid genus (Figure 6), similar to previously published works (Yaghubi et al., 2015; Hosseinvand et al., 2020a), and as a member of the subfamily Boleodorinae as suggested by Geraert (2008). In this tree, Neothada formed a sister clade with species of Basiria Siddiqi, 1959, the other representative of the subfamily Boleodorinae (PP = 100); Boleodorus and two sequences of Neopsilenchus magnidens (MK639379, MK639380) formed a basal clade with them. Discopersicus iranicus (KM502982) and two sequences of Neopsilenchus (KP313832, JQ005018), other likely members of the subfamily Boleodorinae, are placed in other clades in the tree. According to Hosseinvand et al. (2020c), Thada forms a clade with Filenchus Andrásy, 1954 in the 18S rDNA tree, which is not in agreement with the position of Thada along with Neothada in the subfamily Boleodorinae. We believe that in the subfamily Boleodorinae, the distance of dorsal esophageal gland orifice (DGO) from the stylet base and development of median bulb and stylet knobs are also important diagnostic characters as much as the shape of amphidial apertures. In the genus Neothada, the number of longitudinal incisures, degree of stylet knobs development and tail tip shape...
are important diagnostic characters for identification of the known species.

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References
Andrássy, I. 1954. Revision der Gattung *Tylenchus* Bastian, 1865 (Tylenchidae, Nematoda). Acta Zoologica Hungaricae 1:5–42.

Andrássy, I. 1982. Újabb huszonöt Nematoda faj a magyar faunában (Further twenty-five nematode species new to the fauna of Hungary). Állattani Közlemények 69:139–46.

Bai, M., Qing, X., Qiao, K., Ning, X., Xiao, S., Cheng, X. and Liu, G. 2020. Mitochondrial COI gene is valid to delimitate Tylenchidae (Nematoda: Tylenchomorpha) species. Journal of Nematology 52:1–12, doi: 10.21307/jofnem-2020-038.

Bastian, H. C. 1865. Monograph on the Anguillulidae, or free nematoids, marine, land and freshwater; with descriptions of 100 new species. Transactions of the Linnean Society 25:73–184.

Castresana, J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic
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analysis. Molecular Biology and Evolution 17:540–52. Available at: http://molevol.cmima.csic.es/castresana/Gblocks_server.html.

Darriba, D., Taboada, G. L., Doallo, R. and Posada, D. 2012. jModel Test 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

De Grisse, A. 1969. Redescription ou modification de quelques techniques utilisees dans l'étude des nématodes phytoparasitaires. Mededelingen Rijksfaculteit Landbouwwetenschappen Gent 34:351–69.

Geraert, E. 2008. The Tylenchidae of the World, Identification of the Family Tylenchidae (Nematoda: Tylenchida). Ghent: Academia Press.

Geraert, E. and Raski, D. J. 1986. Three new species of Basirienchus g. n. from Southern Chile compared with Campbellenchus, Neothada and Basiria (Nematoda: Tylenchida). Nematologica 31:266–88.

Ghorbanzad, H., Heydari, R., Pourjam, E. and Atighi, M. R. 2012. First report of genus Neothada from Iran. 20th Iranian Plant Protection Congress, Shiraz, August 25-28, p. 655.

Heyns, J. and Van den berg, E. 1996. Neothada hades n.sp. from South Africa, with notes on the genus and a key to the species (Nematoda: Tylenchida). South African Journal of Zoology 31:165–9.

Hosseinvand, M., Eskandari, A., Castillo, P., Palomares-Rius, J. E. and Ghaderi, R. 2020a. Systematic position of the genus Atetylenchus Khan, 1973 (Nematoda: Tylenchidae) with description of two new species. Nematology 22:1155–67, doi: 10.1163/15685411-bja10019.

Hosseinvand, M., Eskandari, A. and Ghaderi, R. 2020b. Morphological and molecular characterization of Coslenchus persicus n. sp. (Nematoda: Tylenchidae) from Iran. Nematology 1–10, doi: 10.1163/15685411-bja10045.

Hosseinvand, M., Eskandari, A., Ghaderi, R. and Karegar, A. 2020c. Morphological and molecular data of two species of the rare genera Thada Thorne, 1941 and Tenunemellus Siddiqi, 1986 (Nematoda: Tylenchidae) from Iran. Journal of Helminthology 94:1–10, doi: 10.1017/S0022149X20000279.

Huelsenbeck, J. P. and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–5.

Khan, S. H. 1973. On the proposal for Neothada n. gen. (Nematoda: Tylenchinae). Proceedings of the National Academy of Sciences, Biological Sciences 43:17–8.

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–4.

Maqbool, M. A. and Shahin, A. F. 1989. Nematodes of northern areas in Pakistan. Description of Neothada major n.sp. and Pratylenchoides maqsoodi n. sp. (Nematoda: Tylenchina), Revue de Nematologie 12:211–6.

Nunn, G. B. 1992. Nematode molecular evolution. Ph.D. thesis, University of Nottingham.

Rambaut, A. 2016. Figtree, a graphical viewer of phylogenetic trees [Internet]. Available at: http://tree.bio.ed.ac.uk/software/figtree.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. and Huelsenbeck, J. P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a largemodel space. Systematic Biology 61:539–42.

Siddiqi, M. R. 1986. Tylenchida: Parasites of Plants and Insects Commonwealth Agricultural Bureaux, Farnham Royal, Slough.

Siddiqi, M. R. 2000. Tylenchida: Parasites of Plants and Insects 2nd ed., Wallingford: CABI Publishing.

Tanha Maafi, Z., Subbotin, S. A. and Moens, M. 2003. Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. Nematology 5:99–111.

Thorne, G. 1941. Some nematodes of the family Tylenchidae, which do not possess a valvar median oesophageal bulb. Great Basin Naturalist 2:37–85.

Thorne, G. and Malek, R. B. 1968. Nematodes of the Northern Great Plains. Part 1. Tylenchida (Nematata: Secernentea). South Dakota Agricultural Experiment Station Technical Bulletin 31, 111 pp.

Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods for extracting small veriform nematodes from soil. Annals of Applied Biology 55:25–38.

Yaghoubi, A., Pourjam, E., Atighi, M. R. and Pedram, M. 2015. Description of Atetylenchus minor n. sp. (Tylenchina: Tylenchidae) and data on two other species of the family. Nematology 17:981–94.