sciendo

DOI 10.2478/helm-2022-0025

HELMINTHOLOGIA, 59, 3: 311 – 316, 2022

First report of *Tripylina zhejiangensis* associated with grassland in South Africa

E. SHOKOOHI

Department of Plant Production, Soil Science and Agricultural Engineering, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa. E-mail: *Ebrahim.Shokoohi@ul.ac.za*

Article info

Received March 5, 2022
Accepted August 9, 2022

Summary

Specimens of *Tripylina zhejiangensis,* collected from natural grass in the Free State Province of South Africa, were identified morphologically and molecularly. This species characterizes by body length (1037 – 1128 µm), a = 27.3 – 35.3, b = 5.1 – 5.6, c = 16.1 – 19.8, c' = 2.5 – 3.4, V = 62 – 65. Molecular analysis was also undertaken, based on the 28S rDNA regions, and confirmed this population as *T. zhejiangensis.* Phylogenetic analysis using the Bayesian inference method, places this population in a clade close to *T. zhejiangensis* with a 1.00 posterior probability value. According to the knowledge, this is the first report of the genus *Tripylina* and its species from South Africa. Besides, this is the second report of *T. zhejiangensis* worldwide after the original description from China.
Keywords: South Africa; grass; *Tripylina;* morphology; rDNA; phylogeny

Introduction

The genus *Tripylina* established by Brzeski, 1963 and it is represented by free-living, predacious species that inhabit soil, litter, moss, and other semi-wet biotopes (Andrássy, 2007; Zhao, 2009; Xu *et al*., 2013). This genus currently includes 22 valid species described from different regions of the world (Renčo *et al*., 2021). Besides, this genus members have been studied from all continents (Yeates, 1972; Tsalolikhin, 1983; Brzeski & Winiszewska-Ślipińska, 1993; Andrássy, 2008; Zhao, 2009; Cid del Prado-Vera *et al*., 2010; Tahseen & Nusrat, 2010; Cid del Prado-Vera *et al*., 2012, 2016; Renčo *et al*., 2021). *Tripylina* spp. are characterized by having six long and four short cephalic setae in a single whorl, a prodelphic and reflexed gonad without a post-vulval sac, and curved tails in both sexes (Zullini, 2006). Despite some species such as *T. bravoe* Cid del Prado-Vera, Ferris, Nadler and Lamothe-Argumedo, 2012; *T. iandrassyi* Cid del Prado-Vera, Ferris and Nadler, 2016, *T. longa* Brzeski and Winiszewska-Ślipińska, 1993 and re-described materials of an original description of *T. gorganensis* Asghari, Pourjam, Heydari, Zhao and Ramaji, 2012 studied by Renčo *et al*. (2021) have a post-vulval sac (Cid del Prado-Vera *et al*., 2016). *Tripylina* members were divided into two groups based on the position of the sub ventral teeth by Zhao (2009). Despite various research done on free-living nematodes, *Tripylina* species have not been reported yet from South Africa. *Tripylina zhejiangensis* Pham, Wang, Zhao and Zheng, 2013 has been described from China. Therefore, the aims of the present work were 1) to study the morphology of *T. zhejiangensis*, and 2) to study the molecular characters of *T. zhejiangensis* using 28S rDNA markers.

Materials and Methods

Nematode extraction, processing, and LM pictures

Specimens were collected at Parys, Free State Province, close to the river in association with the natural grass (S 26° 54' 37.336",

E 27°27' 24.582"). The specimens were extracted using the tray method and were fixed with a hot 4 % formaldehyde solution and transferred to anhydrous glycerin using the De Grisse (1969) method. The nematodes were processed and studied at Aquacultutre Research Unit, University of Limpopo. The classification provided by Zhao (2009) was used for the taxonomical study of *Tripylina* spp. Pictures were taken with a Nikon Eclipse 80i light microscope equipped with a digital camera (Nikon, Tokyo, Japan). The LM taken was used for the line illustration. The line illustration was edited using Adobe® Photoshop® CS.

DNA extraction, PCR, and phylogenetic analysis

DNA extraction was done using the Chelex method (Straube & Juen, 2013). Five specimens of the species were hand-picked with a fine tip needle and transferred to a 1.5 ml Eppendorf tube containing 20 µl double distilled water. The nematodes in the tube were crushed with the tip of a fine needle and vortexed. Thirty microliters of 5 % Chelex® 50 and 2 µL of proteinase K was added to the microcentrifuge tube that contained the crushed nematodes and mixed. The microcentrifuge tube with the nematode lysate was incubated at 56 °C for two hours and then incubated at 95 °C for 10 minutes to deactivate the proteinase K and finally spun for 2 min at 16000 rpm (Shokoohi, 2021). The supernatant was then extracted from the tube and stored at –20 °C. Following this step, the forward and reverse primers, D2A (5'–ACAAGTACCGTGAGGGAAAGTTG–3'), D3B (5'–TCGGAAGGAACCAGCTACTA–3') (De Ley et al., 1999), were used in the PCR reactions for partial amplification of the D2/D3 region of 28S. PCR was conducted with 8 µl of the DNA template, 12.5 µl of 2X PCR Master Mix Red (Promega, USA), 1 µl of each primer (10 pmol μl^{-1}), and ddH_2O for a final volume of 30 µl. The amplification was processed using an Eppendorf master cycler gradient (Eppendorf, Hamburg, Germany), with the following program: initial denaturation for 3 min at 94 °C, 37 cycles of denaturation for 45 s at 94 °C; 56 °C annealing temperature for 45 s and 72 °C for 1 min, and finally an extension step of 6 min at 72 °C followed by a temperature on hold at 4 °C. After DNA amplification, 4 µl of PCR product was loaded on a 1 % agarose gel in TBE buffer (40 mM Tris, 40 mM boric acid, and one mM EDTA) for evaluation of the DNA bands. The band was stained with RedGel and visualized and photographed on a UV transilluminator. The PCR product was stored at –20 °C. Finally, Inqaba Biotech (South Africa) purified the PCR product for sequencing.

Table 1. Measurements of females of *Tripylina zhejiangensis* from South Africa.
All measurements are in µm and in the form: mean ± SD (range), except for the ratio.

	Present study	Pham *et al*., 2013
Character	South Africa	China
n	10 females	23 females
L	1075.0 ± 36.5 (1037 – 1128)	1152 – 1631
a	31.0 ± 3.3 (27.3 – 35.5)	23.3 – 36.4
b	5.4 ± 0.2 (5.1 – 5.6)	5.0 – 6.6
c	18.2 ± 1.8 (16.1 – 19.8)	13.4 – 19.7
c'	3.0 ± 0.4 (2.5 – 3.4)	2.6 – 3.6
V	64.0 ± 1.2 (62 – 65)	60 – 70
Lip region width	17.6 ± 1.0 (16 – 19)	19 – 30
Labial setae	11.4 ± 1.7 (10 – 15)	11 – 16
Dorsal tooth from the anterior end	14.5 ± 3.2 (10 – 20)	11 – 21
Amphid position from the anterior end	13.5 ± 0.7 (13 – 14)	12 – 17
Nerve ring from the anterior end	87.6 ± 2.1 (85 – 90)	99 – 139
Pharynx	170.1 ± 10.5 (155 – 186)	163 – 164*
Neck length	198.7 ± 6.1 (187 – 204)	183 – 186*
Cardia length	20.6 ± 3.1 (18 – 26)	20 – 27
Cardia diameter	7.3 ± 2.1 (5 – 9)	8.5 – 14
Body diameter at neck base	30.8 ± 4.0 (25 – 37)	34 – 36*
Body diameter at mid-body	33.7 ± 2.8 (25 – 38)	43*
Body diameter at anus	21.0 ± 2.4 (17 – 24)	23 – 29*
Tail length	61.2 ± 8.6 (53 – 72)	73 – 103
Anterior end to vulva	696.4 ± 49.5 (653 – 776)	757 – 1045

* = extracted from original drawing

The ribosomal DNA sequences were analyzed and edited with BioEdit (Hall, 1999) and aligned using CLUSTAL W (Thompson *et al*., 1994). Phylogenetic tree was generated using the Bayesian inference method as implemented in the program Mr Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The GTR+I+Γ model was selected using jModeltest 2.1.10 (Guindon & Gascuel, 2003; Darriba *et al*., 2012). Analysis was initiated with a random starting tree and ran with the Markov chain Monte Carlo (MCMC) for 10^6 generations for 28S rDNA. The tree was visualized with the TreeView program. Also, as outgroups, *Dorylaimus stagnalis* (MF125467, MF125468) were selected based on Pham *et al*. (2013). The original partial 28S rDNA sequence of *T. zhejiangensis* was deposited in GenBank under the accession number OM891776.

Ethical Approval and/or Informed Consent

The author confirms that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered. The author confirms that the conducted research is neither related to human nor animal use.

Results

***Tripylina zhejiangensis* Pham, Wang, Zhao & Zheng, 2013**

Morphological characterization (ten females in a good state of preservation)

Fig. 1; Table 1

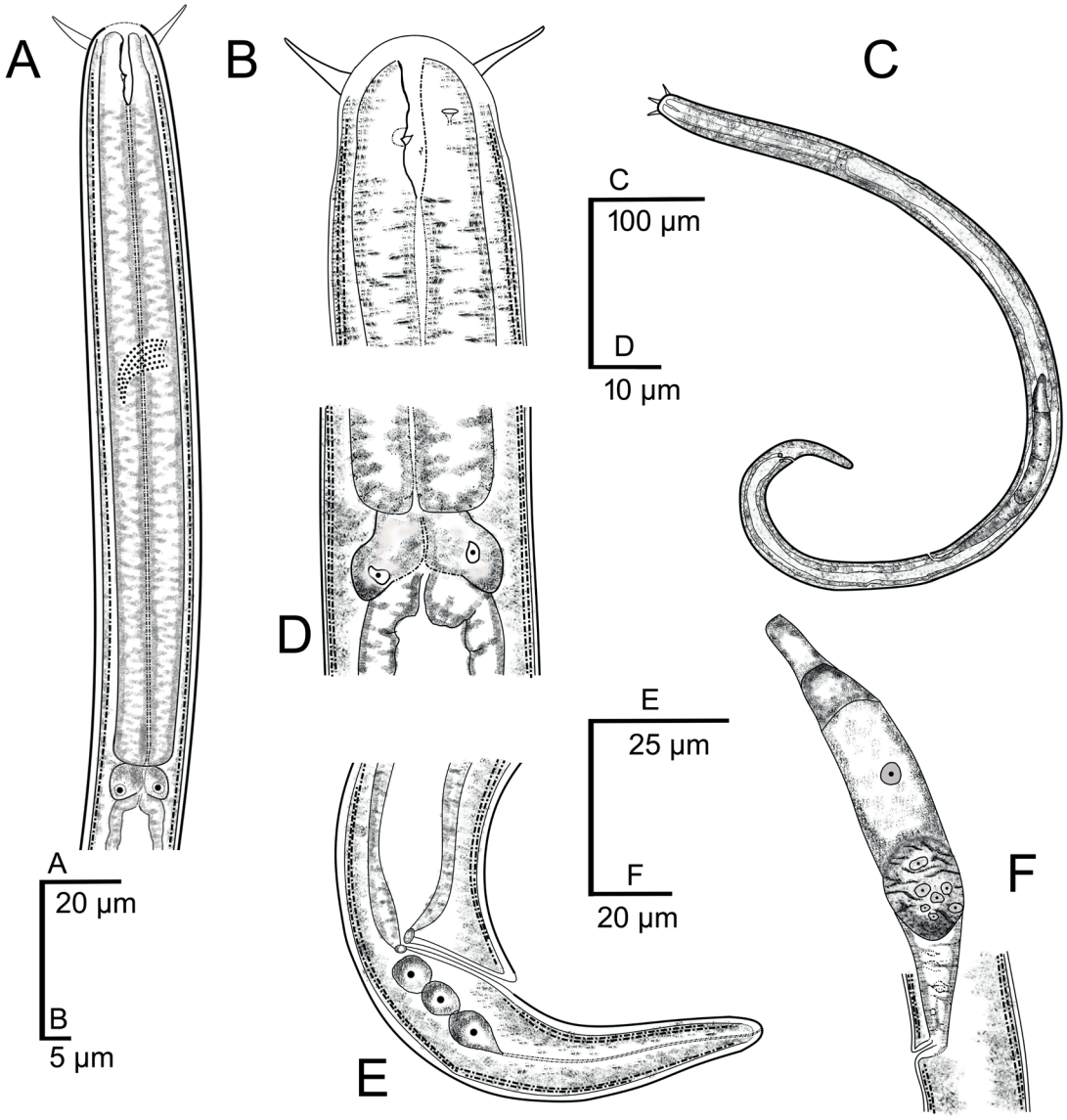

Fig. 1. *Tripylina zhejiangensis* Pham, Wang, Zhao and Zheng, 2013. (A) anterior end; (B) lip region; (C) entire body; (D) pharyngeal-intestinal junction; (E) female posterior end; (F) female reproductive system.

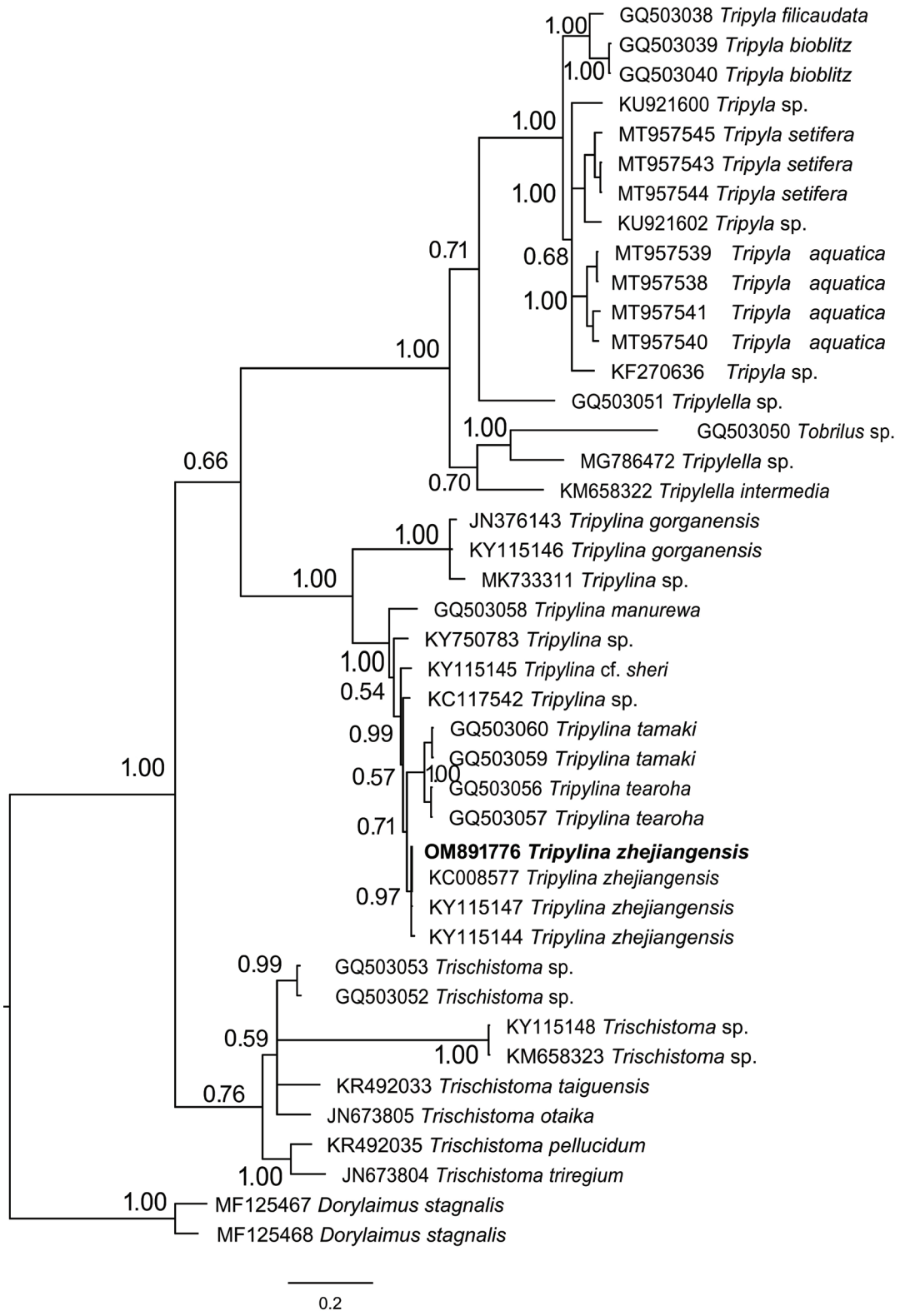

Fig. 2. 28S rDNA Bayesian tree inferred from known and newly sequenced *Tripylina zhejiangensis* from South Africa.

Description

Female: Body ventrally arcuate after heat relaxation, moderately large, robust, posterior region more curved than anterior. Cuticle smooth, not striated, with numerous body pores along entire body. Six long (10 – 15 µm) and four short cephalic setae (5 – 7 µm) in a single whorl. Lip region rounded, continuous with body contour, 16 – 19 µm width. Lip region dome-shaped, with three triangular lips, inner labial papillae conical, outer labial setae. Thick stomatal walls, with a large triangular dorsal tooth in a stomatal chamber, pointing towards ventral side. Stoma 18 – 30 µm long, 1 – 3 µm width. Two triangular subventral teeth located posterior to dorsal tooth (Fig 1B). Tooth length 1 – 2 µm, tooth width 0.5 – 0.8 µm. Amphids cup-like, located at 13 – 14 µm from anterior end. Amphidal aperture diameter 2.8 – 3.3 µm. Excretory pore not observed. Nerve ring 85 – 90 µm or 43 – 47 % of neck length from anterior end of body. A prominent cardia separates the pharynx and intestine, 18 – 26 µm long and 5-9 µm wide. Pharyngo-intestinal valve composed of three glands around anterior portion of intestine. An ingested nematode was observed within the intestine of one specimen of this species. Female genital system mono-prodelphic without post-vulval sac. Vulva simple, lacking protuberant lips, vagina 5 – 9 µm long. Vulva-anus distance 278 – 319 µm. Rectum 16 – 23 µm long. Tail bent ventrad, a pair of subdorsal caudal setae on anterior part of tail, three tandem caudal glands, a terminal spinneret, 3 – 4 µm long.

Male: not found

Remarks: The South African population of *T. zhejiangensis* resembles the original description studied in China (Pham *et al*., 2013). However, compared with the Chinese population, they differ in body length (1037 – 1128 vs 1152 – 1631 µm), amphid from anterior end (13 – 17 vs 12 – 17 µm), and tail length (53 – 72 vs 73 – 103 µm).

Discussion

The forward D2 and reverse D3 primers of 28S rDNA for *T. zhejiangensis* isolated 722 base pairs long. The nBlast test of 28S rDNA showed 99 % similarity of the test population with the Chinese population of *T. zhejiangensis* (KC008577) with only one base pair difference. Compared with the Iranian sequences of *T. zhejiangensis* (KY115144; KY115147), it showed 99 % similarity with one and three base pairs differences, respectively.

Our phylogenetic analysis using 28S rDNA, placed the South African *T. zhejiangensis* population in a clade together with other *T. zhejiangensis* populations with 1.00 posterior probability values (Fig. 2). Findings in the current study were in agreement with the phylogenies of *Tripylina* species studied (Zhao, 2009; Pham *et al*., 2013; Renčo *et al*., 2021). Two permanent microscope slides containing the females of *T. zhejiangensis* were deposited in the Aquaculture Research Unit of the University of Limpopo, South Africa. According to the literature, this is the first record of *T. zhejiangensis* in South Africa. In conclusion, the morphometrical variation that exists between the *T. zhejiangensis* (e.g., vulva position, tail length, body length) is due to the geographical location of the population.

References

Andrássy, I. (2008): Two new and a known species of the family Tripylidae (Nematoda: Enoplida) from the tropics. *Opusc Zool Budapest*, 37: 3 – 9

Andrássy, I. (2007): Free-living nematodes of Hungary, II (Nematoda errantia). In: Csuzdi, C., Mahunka, S. (Eds) *Pedozoologica Hungarica*, 4. Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Sciences, Budapest.

Asghari, R., Pourjam, E., Heydari, R., Zhao, Z.Q., Ramaji, F.A. (2012): *Tripylina gorganensis* n. sp. (Triplonchida: Tripylidae) from northern Iran. *Nematology*, 14: 613 – 621. DOI: 10.1163/156854112X624609

Brzeski, M.W. (1963): Nematode genera of the family Tripylidae (Nematoda, Enoplida). *Acta Zool Cracoviensia*, 8: 295 – 308.

Brzeski, M.W., Winiszewska-Ślipińska, G. (1993): Taxonomy of Tripylidae (Nematoda: Enoplia). *Nematologica*, 39: 12 – 52

Cid del Prado Vera, I., Ferris, H., Nadler, S. A., Lamothe-Argumedo, R. (2012): Four new species of *Tripylina* Brzeski, 1963 (Enoplida: Tripylidae) from Mexico, with an emended diagnosis of the genus. *J Nematode Morphol System*, 15: 71 – 86

Cid del Prado-Vera, I., Ferris, H., Nadler, S.A. (2010): Soil inhabiting nematodes of the genera *Trischistoma, Tripylina* and *Tripyla* from Mexico and the USA with descriptions of new species. *J. Nematode Morphol System.*, 13: 29 – 49

Cid del Prado-Vera, I., Ferris, H., Nadler, S. A. (2016): Five new species of the family Trischistomatidae (Nematoda: Enoplida) from North and Central America, with keys to the species of *Trischistoma* and *Tripylina*. *Zootaxa*, 4109: 173 – 197. DOI: 10.11646/zootaxa.4109.2.4

Darriba, D., Taboada, G.L., Doallo, R., Posada, D. (2012): jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods*, 9: 772. DOI: 10.1038/nmeth.2109

De Grisse, A. (1969): Redescription ou modifications de quelques techniques utililisés dans l'étude des nématodes phytoparasitaires [Redescription or modifications of some techniques used in the study of plant parasitic nematodes]. *Mededelingen van de Rijksfaculteit Landbouwetenschappen Gent*, 34: 351 – 369 (In French)

De Ley, P., Felix, M.A., Frisse, L.M., Nadler, S.A., Sternberg, P.W., Thomas, W.K. (1999): Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology*, 2: 591 – 612. DOI: 10.1163/156854199508559

Guindon, S., Gascuel, O. (2003): A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Sys Biol*,

52: 696 – 704. DOI: 10.1080/10635150390235520
Hall, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser*., 41: 95 – 98
Pham, T.H., Wang, H.T., Zhao, Z.Q., Zheng, J.W. (2013): A new species of the genus *Tripylina* Brzeski, 1963 (Nematoda: Enoplida: Trischistomatidae) from Zhejiang Province, eastern China. *Zootaxa*, 3717: 158 – 168. DOI: 10.11646/zootaxa.3717.2.2
Renčo, M., Rybarczyk-Mydłowska, K., Flis, Ł., Kubicz, M., Winiszewska, G. (2021): Morphological and molecular characterisation of *Tripylina gorganensis* from the Slovak Republic as a contribution to the redescription of the species. *J Nematol*, 53: 1 – 10. DOI: 10.21307/jofnem-2021-048
Ronquist, F., Huelsenbeck, J. (2003): MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572-1574. DOI: 10.1093/bioinformatics/btg180
Shokoohi, E. (2021): First report of *Bitylenchus ventrosignatus* (Tobar Jiménez, 1969) Siddiqi, 1986 associated with wild grass in Botswana. *J Nematol*, 53: 1-9. DOI: 10.21307/jofnem-2021-037
Straube, D., Juen, A. (2013): Storage and shipping of tissue samples for DNA analyses: A case study on earthworms. *Eur J Soil Biol*, 57: 13-18. DOI: 10.1016/j.ejsobi.2013.04.001
Tahseen, Q., Nusrat, T. (2010): Some new and known species of genera *Tripylina* Brzeski and *Trischistoma* Cobb, 1913 (Nematoda) with a discussion on their relationships. *J Nematol*, 42: 128 – 138
Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, 22: 4673 – 4680. DOI: 10.1093/nar/22.22.4673
Tsalolikhin, S.J. (1983): *Nematodes of the families Tobrilidae and Tripylidae of the World Fauna*. Leningrad, Russia, Nauka, 232 pp. (In Russian)
Xu, Y.M., Zhao, Z.Q., Wang, J.M., Zheng, J.W. (2013): A new species of the genus *Tripylina* Brzeski, 1963 (Nematoda: Enoplida: Trischistomatidae) from Shanxi province, China. *Zootaxa*, 3630: 561 – 570. DOI: 10.11646/zootaxa.3630.3.10
Yeates, G.W. (1972): *Trischistoma stramenti* n. sp. (Nematoda: Enoplida) from leaf litter. *New Zeal J Sci*, 14: 578 – 900
Zhao, Z.Q. (2009): A review of the genus *Tripylina* Brzeski, 1963 (Nematoda: Triplonchida), with descriptions of five new species from New Zealand. *Zootaxa*, 2238: 1 – 24. DOI: 10.11646/zootaxa.2238.1.1
Zullini, A. (2006) Order Triplonchida. In: Eyualem, A., Andrássy, I., Traunspurger, W. (Eds) *Freshwater Nematodes: Ecology and Taxonomy*. CABI Publishing, Wallingford, UK, pp. 293 – 325