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First Record of Free-Living Nematode *Ironus dentifurcatus* Argo & Heyns, 1972 (Ironidae: Enoplida) From Indonesia

Ankardiansyah Pandu Pradana[1*] & Toyoshi Yoshiga[2]

[1]Plant Protection Study Program, Faculty of Agriculture, University of Jember, Jember 68121, Indonesia.
[2]Department of Applied Biological Sciences, Saga University, Saga 840-8502, Japan.

*Corresponding Author e-mail: pandu@unej.ac.id

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Abstract. Free-living nematodes often serve as the bioindicators of environmental quality. These nematodes are highly sensitive to even minute changes in the environment. Studies related to the presence of free-living nematodes have flourished, but to date, only a few studies have investigated the morphological and molecular characterization of free-living nematodes from agroecosystems in Indonesia. This study aimed to characterize the free-living nematode *Ironus* spp. from the rhizosphere of Arabica coffee plants in Bondowoso Regency, Indonesia. Nematodes were extracted from the soil using the white-head tray method and then preserved in a DESS solution to maintain their morphology and DNA. The morphometric analysis of *Ironus* species obtained in this study showed that the nematode species is identical to *Ironus dentifurcatus*. The small subunit (SSU) region of rDNA of the nematode was successfully amplified using primers (SSU F07 and SSU R81). The results of phylogenetic analysis and sequence homology showed that the sequence in this study showed up to 95% homology to *Ironus* spp. In the database. This is the first study documenting *I. dentifurcatus* in Indonesia that integrates morphological and molecular characterization.

Keywords: 18S rDNA; agroecosystem; morphometry; rhizosphere; SSU (Small Subunit).

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INTRODUCTION

Research related to the role, importance, and identification of free-living nematodes in agroecosystems remains underexplored (di Montanara et al., 2022; Kimenju et al., 2009; Young & Unc, 2023). This subject has not received sufficient attention in scientific literature. Most reports originate from Europe and America, leaving many regions insufficiently studied. The distribution of nematodes in agroecosystems is still in the early stages of investigation, and researchers have only begun to explore the diversity within this group (Ridall & Ingels, 2021). There remains much to learn about the range of species present, and the lack of comprehensive surveys further complicates our understanding.

Few studies have examined free-living nematodes in detail (Khanum et al., 2021), and more work is required to fill this gap in knowledge. Nematodes serve as pivotal indicators of environmental quality (Hua et al., 2021; Neher, 2010), and their presence assists scientists in assessing the overall health of soil (Li et al., 2024; Melakeberhan et al., 2021). This research is essential for promoting sustainable agricultural practices, as a better understanding of these organisms could lead to improved soil management techniques. Further exploration of free-living nematodes will enhance the ability to monitor and improve environmental quality in diverse agroecosystems (Loranger-Merciris et al., 2022).

Soil free-living nematodes consist of several trophic groups, including bacterial-feeders, fungal-feeders, predators, and omnivores (Neher, 2010). Bacterial-feeding nematodes generally consume nitrogen from bacterial cells and other microflora (Trap et al., 2021). Under certain conditions, bacterial-feeding nematodes can release the excess nitrogen in their bodies into the surrounding environment. Nitrogen released can be utilized directly by plants (Rehman et al., 2018). Fungal-feeding nematodes can feed on mycelium, hyphae, conidia, and plant pathogenic fungi. Several studies point out that fungal-feeding nematodes can suppress the growth of plant pathogenic fungi (Haraguchi & Yoshiga, 2020; Javed & Khan, 2021). However, no further

field experiment has been underway to validate this finding. Predatory nematodes can feed on some invertebrates, such as protozoa, rotifers, and nematodes. Several studies suggest that predatory nematodes can also feed on plant parasitic nematodes (Biswal, 2022; Kanwar et al., 2021). In essence, free-living nematodes are crucial in maintaining the ecological balance, either directly or indirectly (Schratzberger et al., 2019).

In a survey we conducted on Arabica coffee plantations in Bondowoso regency, Indonesia, we found various types of free-living nematodes, one of which was a nematode of the genus *Ironus* spp. Worldwide, the genus *Ironus* spp. hardly receives sufficient research attention. Only a few studies have reported the morphometry of this nematode (Girgan et al., 2021). Molecular investigations are even more limited. Until July 2022, only 24 sequences of *Ironus* spp. were documented in GenBank. Most sequences come from the small subunit (SSU) (18S rDNA). A few others originate from the large subunit (LSU) (28S rDNA) and the cytochrome c oxidase subunit 1 (COX1) gene regions. This situation reveals a clear gap in our understanding of *Ironus* spp. More research is needed to explore its full diversity and molecular characteristics.

The genus *Ironus* spp. is a member of the family Ironidae de Man, 1876, and represents a distinctive group of aquatic nematodes. These organisms inhabit a range of environments including marine ecosystems, coastal shores, and brackish waters, with occasional occurrences in terrestrial settings (Lee et al., 2023). Their ecological versatility is complemented by a prolonged life cycle and an extended lifespan, traits that are relatively uncommon among free-living nematodes. In addition, *Ironus* species exhibit high sensitivity to subtle environmental fluctuations, making them potential indicators of ecosystem health (Waldo et al., 2024; Yeates, 1967).

Ironus spp. are predatory nematodes that can be readily identified under microscopic examination due to their unique morphological characteristics. They are characterized by a long and narrow body, which is an adaptation for their predatory lifestyle. The posterior end of the body is distinctly tapered, and the nematodes possess an elongated tail that further distinguishes them from other genera. Additionally, these nematodes have a similarly long and narrow pharyngeal cavity, a feature that is critical for their feeding mechanism. The vulva is typically located at or near the middle of the body, providing an important taxonomic marker for species identification. Generally, members of this genus move slowly, a behavior that may be linked to their ecological niche and predatory strategies (Tahseen & Mehdi, 2009).

Currently, no study has comprehensively documented the morphological and molecular characteristics of *Ironus* spp. in relation to agroecosystems in Indonesia. This gap in the literature is significant. Researchers have yet to explore the full potential of *Ironus* spp. in these environments. In response, this study aims to identify and characterize *Ironus* species found in the rhizosphere of Arabica coffee in Bondowoso, Indonesia. The investigation focuses on both morphological traits and molecular data. This study represents the first effort in Indonesia to integrate these two approaches for the characterization of *Ironus* spp. Detailed descriptions of the nematode's physical features will be provided alongside insights into its genetic makeup.

This study enhances knowledge of *Ironus* spp. in tropical agroecosystems. It improves taxonomic classification and phylogenetic resolution. As a predatory nematode, *Ironus* spp. may contribute to the natural regulation of plant-parasitic nematodes. Its sensitivity to environmental changes highlights its potential as a bioindicator of soil health. These findings support sustainable agricultural practices by promoting ecological pest control and reducing chemical inputs. This research also provides a foundation for future ecological monitoring and conservation in Indonesia.

METHODS

Nematode Extraction

Nematodes were extracted from soil taken at 20 cm deep around the roots of Arabica coffee trees in Bondowoso Regency, East Java, Indonesia. The sampling location is situated at 8° 0'17.4"S, 114° 4'1.3" E. Nematodes were extracted from 500 mL of soil using the White Head Tray method, followed by an incubation process of 48 hours (Bell & Watson, 2001). The nematodes contained in the extracted suspension were collected and filtered using a 400-mesh sieve. These nematodes were then stored in a DESS solution (containing 0.25 M EDTA and 20% DMSO, with pH 8), which was saturated with NaCl (Pradana & Yoshiga, 2023, 2024; Yoder et al., 2006).

Morphological Identification

Nematodes that were morphologically

identified as an *Ironus* species were taken using a nematode collection needle and placed on a glass slide that had been dripped with water. These nematodes were put under observation using an Olympus BH-2 compound microscope connected to a Toupup M20 camera that had been calibrated accordingly. Morphological observations were carried out by following the De Man formula (Ye & Hunt, 2021).

DNA Extraction

Nematodes were taken from the DESS solution and washed with distilled water 3 times to nullify the NaCl concentration on the nematode body. Afterward, a single nematode was then placed in a microtube containing 15 µL of nematodes dissolving solution (NDS) (ISOHAIR, Nippon Gene Japan). NDS is made by mixing enzyme solution, lysis solution, and TE Buffer in a ratio of 5:4:100 (v/v). The nematodes that had been put into the NDS were then heated at 60°C for 20 minutes (Tanaka et al., 2012).

The Amplification of 18S rDNA Gene and Sequencing

A total of 1 µL of DNA extract was added into a microtube and then mixed with PCR Mixture containing sterile MilliQ water (29.75 µL), 10 × EX Taq Buffer (5 µL), dNTP mixture (2.4 mM each) (4 µL), forward (SSU F07: 5' -AAA GAT TAA GCC ATG CAT G-3') and reverse (SSU R 81: 5' -TGA TCC WKC YGC AGG TTC AC-3') primers (5 µL each), and ExTaq (0.2 µL), which amplified the SSU rDNA region. PCR was performed using a Biometra Tprofessional 96 (Germany) thermal cycler with the following amplification program: initial denaturation at 94°C (10 min); followed by 35 cycles of denaturation at 94°C (30 s), annealing at 54°C (30 s), and extension at 72 °C (1 min); and the elongation process at 72° C (10 min) (De Ley et al., 2002; Pradana & Yoshiga, 2024). To ensure that the DNA was properly amplified, PCR product verification was carried out using agarose gel electrophoresis.

The PCR product was then purified using the ISOSPIN PCR Product (Nippon Gene, Japan). The purified DNA was then sent to Macrogen Japan Corp. to be sequenced.

Phylogenetic Analysis

The homology search was performed using the basic local alignment search tool (BLAST) program available on The National Center for Biotechnology Information (NCBI) website. The nucleotide sequences were then aligned using Clustal W multiple alignments. Phylogenetic tree and kinship analysis were determined using Molecular Evolutionary Genetic Analysis (MEGA) software version 11.0.10 with neighbor-joining tree method coupled with a bootstrap involving 1000 replicates (Bhat et al., 2021; Saswita et al., 2023).

RESULTS AND DISCUSSION

General Environmental Properties of Research Site

The *Ironus* spp. nematode found in this study originated from the rhizosphere of Arabica coffee in Bondowoso Regency, Indonesia. The research site is located near Mount Ijen, at an altitude of 1,260 meters above sea level. This area is an agroforestry site, where coffee plants grow alongside pine trees in a mixed-cropping system. The site is managed by smallholder coffee farmers using traditional methods. Organic material, including pine leaf litter and coffee leaves, is left to decompose on the ground without being cleaned. A small number of weeds are also found growing in the area. The region's elevation and land use practices contribute to the unique conditions of the agroecosystem. These features of the study site are depicted in Figure 1.

Figure 1. The Arabica coffee plantation where *Ironus* sp. was discovered

The Arabica coffee field where the *Ironus* species was discovered constituted an agroecosystem that resembled a natural ecosystem. Covered by leaf litter, the soil did not receive sufficient direct exposure to the sunlight, which made the area conducive to the *Ironus* nematode due to its high sensitivity to environmental changes. Notwithstanding, only little is documented on the bioecology of *Ironus* nematodes in agroecosystems. *Ironus* species belong to predatory nematodes which are generally found in fertile soils with high organic matter content (Hindersah & Asyiah, 2023). Predatory nematodes are more commonly found in natural ecosystems, rather than in agroecosystems that have received ample human interventions (Kanwar et al., 2021; Neher, 1999; Neher, 2010; Rueda-Ramirez et al., 2022).

Morphological and Morphometric Analysis

In this study, the morphometric data of the specimens were compared with the values reported by Tahseen & Mehdi (2009) for *Ironus dentifurcatus*. The measurements considered in the comparison include body length (L), ratio a, ratio b, ratio c, ratio c', V value, G1, G2, body diameter, lip diameter, lip height, stoma length, pharynx length, nerve ring position, vulva-anus distance, rectum length, anal body diameter, and tail length (Table 1). Each measurement was recorded as an average value with its standard deviation. The aim was to check if the range of values overlaps between the two studies. This comparison helps verify whether the specimens are indeed the same species.

Table 1. Morphometric of *Ironus* spp. (all absolute measurements in µm)

Properties/ratios*	Ironus spp.	Ironus dentifurcatus
	This Study	Tahseen & Mehdi (2009)
n	5 (female)	7 (female)
L	1408 ± 92.81	1440.8 ± 104.9
	(1273.60 – 1503.20)	(1268-1568)
a	48.74 ± 3.12	29.3 ± 0.5
	(43.47 – 51.44)	(29-30)
b	5.54 ± 0.55	5.9 ± 0.4
	(4.80 – 6.20)	(5.0-6.2)
c	3.49 ± 0.14	3.8 ± 0.9
	(3.28 – 3.62)	(3.1-5.6)
c'	25.86 ± 0.46	25.8 ± 6.9
	(25.40 – 26.40)	(14.0-36.4)
V	45.96 ± 1.63	45.3 ± 6.1
	(43.10 – 47.20)	(38.9-46.4)
G_1	9.34 ± 0.79	9.6 ± 0.9
	(8.20 – 10.30)	(8.2-11.1)
G_2	10.14 ± 0.84	10.8 ± 0.8
	(9.30 – 11.20)	(9-12)
Body diameter	28.92 ± 0.94	29.3 ± 0.5
	(27.40 – 29.80)	(29-30)
Lip diameter	14.02 ± 0.69	14.3 ± 0.8
	(13.20 – 15.70)	(13-15)

Properties/ratios*	Ironus spp.	Ironus dentifurcatus
	This Study	Tahseen & Mehdi (2009)
Lip height	14.42 ± 1.09	7 ± 0.6
	(13.20 – 15.70)	(6-8)
Stoma length	90.98 ± 1.05	91.5 ± 4.9
	(89.70 – 92.30)	(85-100)
Pharynx length	242.40 ± 7.77	244 ± 12.5
	(233.00 – 252.00)	(218-253)
Nerve ring position	121.62 ± 4.90	118.3 ± 3.7
	(115.60 – 127.10)	(110-120)
Vulva-anus distance	442.56 ± 4.28	443.3 ± 13.8
	(437.20 – 447.30)	(424-463)
Rectum length	18.74 ± 0.84	18.6 ± 3.7
	(17.50 – 19.70)	(15-24)
Anal body diameter	14.16 ± 0.66	14 ± 1.16
	(13.50 – 15.20)	(13-16)
Tail length	403.28 ± 10.67	403.6 ± 91.8
	(388.70 – 415.20)	(225-430)

Note: For each parameter, the table lists the mean value followed by the standard deviation. The corresponding range, specifying the minimum and maximum values, is presented in parentheses below each mean and standard deviation.)

*__Abbreviations:__ n = number of specimens on which measurements are based; L = overall body length; a = body length ÷ greatest body diameter; c = body length ÷ tail length; c’ = tail length ÷ tail diameter at anus or cloaca; V = % distance of vulva from anterior; G1 = % length of anterior female gonad in relation to body length; G2 = % length of posterior female gonad in relation to body length.

In this study, the body length measured 1408 µm, which is about 2.3% shorter than the 1440.8 µm reported earlier. The ratio a was 48.74, approximately 66% higher than the value of 29.3. The ratio b was 5.54, about 6% lower than 5.9. The ratio c was 3.49, roughly 8% lower than 3.8, while the ratio c' was nearly identical at 25.86 versus 25.8. The V value measured 45.96, about 1.5% higher than 45.3. The G1 value was 9.34, roughly 2.7% lower than 9.6, and the G2 value was 10.14, about 6.1% lower than 10.8. The body diameter measured 28.92 µm, about 1.3% lower than 29.3 µm. The lip diameter was 14.02 µm, approximately 2% lower than 14.3 µm. The lip height was 14.42 µm, which is more than double the 7 µm reported, showing an increase of about 106%. The stoma length measured 90.98 µm, nearly identical to 91.5 µm with less than a 1% difference. The pharynx length was 242.40 µm, about 0.66% lower than 244 µm.

The nerve ring position was 121.62 µm, approximately 2.8% higher than 118.3 µm. The vulva-anus distance measured 442.56 µm, which nearly matches the 443.3 µm. The rectum length was 18.74 µm, about 0.75% higher than 18.6 µm, and the anal body diameter was 14.16 µm, roughly 1.14% higher than 14 µm. Finally, the tail length was 403.28 µm, almost identical to 403.6 µm. Although the average values show some differences, the overlapping minimum and maximum values for each property indicate that the specimens belong to the same species.

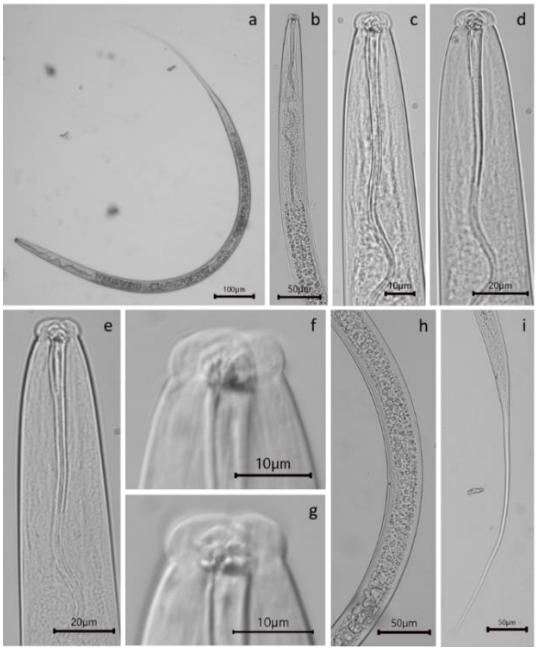

Figure 2. *Ironus* sp. found in this study, exhibiting morphological characteristics identical to *Ironus dentifurcatus*. (a) whole body of the nematode; (b-e) anterior region at different magnifications and focal planes; (f-g) lip structures at different focal planes; (h) vulva; (i) tail.

Most of the morphological features identified in this study matched in value to Tahseen & Mehdi (2009) findings. However, the value that contrasts body length with the greatest body diameter (a) differs significantly. The average value of (a) was 48.74 in this study, compared to 29.3 in Tahseen & Mehdi (2009) report. The value from the study we describe is more acceptable since the (a) value of 48.74 suggests a more proportionate body size.

When comparing the overall morphology of *I. dentifurcatus* in this study with the reference, the general shape and structural features are nearly identical. The body outline is robust and elongated. The cephalic region is well-defined and matches the reference description. The tail configuration is consistent with previous reports. The lip region is clear and shows the expected arrangement. The stoma has a similar shape to that described in the literature. The pharyngeal structure follows the same organization as in the reference. The cuticle texture appears smooth and uniform. The overall body proportions are alike. These observations indicate that the morphology observed in this study aligns very well with the established characteristics of *I. dentifurcatus*.

Molecular Characteristics and Phylogenetic Position

The sequences obtained in this study were registered with GenBank with accession number OP108811. These sequences were compared with other sequences that had a fairly high homology subsequent to BLAST analysis. The results of the phylogenetic analysis are presented in Figure 3.

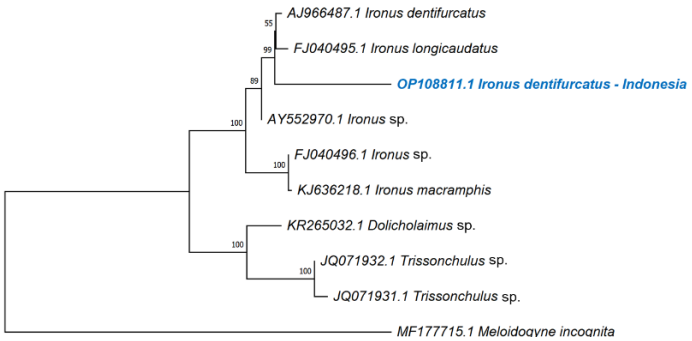

Figure 3. Phylogenetic tree of *Ironus dentifurcatus* from an agroecosystem in Bondowoso Regency, Indonesia, along with other *Ironus* species sequences from GenBank. The tree was constructed using MEGA v11.0.10 with the neighbor-joining method and 1,000 bootstrap replicates.

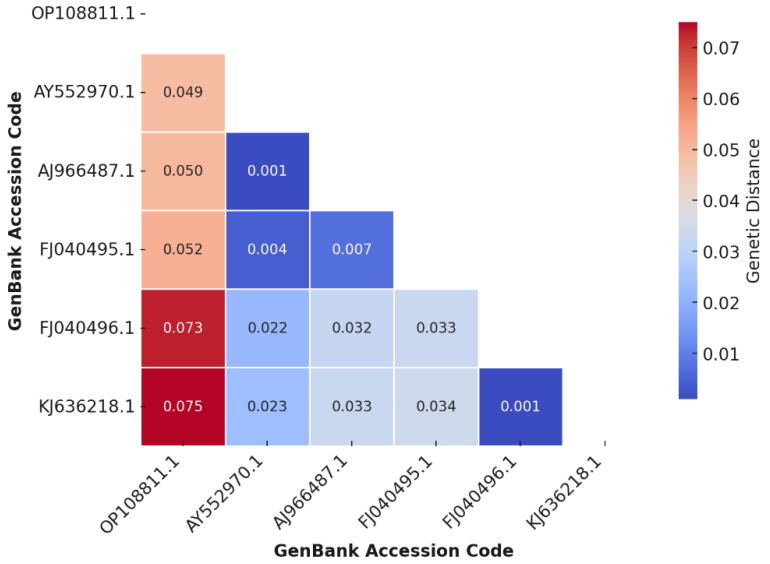

Figure 4. Genetic distance heatmap of 18S rDNA from an *Ironus* nematode isolated in Indonesia, compared to similar sequences registered in GenBank

Based on the genetic distance analysis between sequences, the sequence reported in this study has the highest homology with *Ironus* sp. (AY552970.1) (0.049), followed by *I. dentifurcatus* (AJ966487.1) (0.050), and *I. longicaudatus* (FJ040495.1) (0.052). The sequence data of *Ironus* spp. at GenBank is very limited, so the comparative data in this study is limited as well. The results of the genetic distance analysis are presented in Figure 4.

Based on the morphometric characteristics, the nematode in this study was identified to be *I. dentifurcatus*. However, molecular analysis did not clearly support the results of our morphological identification clearly. There could be several reasons. The original paper that describes *I. dentifurcatus* has no sequence data of the nematode. The sequences of *Ironus* spp. Including *I. dentifurcatus* and *I. longicaudatus* in the database did not provide the morphological evidence for their identification, and thus there is a possibility that species identification was mistaken. In the present study, we report both morphological and molecular data for the identification of *I. dentifurcatus* from Indonesia for the first time.

Although the nematode in this study is not a novel species, this research is the first to report its morphological and molecular characterization in Indonesia. The morphometric data aligns with previous studies. The molecular analysis further clarifies its taxonomic position despite limited sequence data. This study confirms the presence of *Ironus dentifurcatus* in an Indonesian agroecosystem and highlights the importance of integrating morphological and molecular approaches.

Comparative data improves the precision of molecular analysis. When employing specific primers, molecular analysis will be more accurate (Bogale et al., 2020; Seesao et al., 2017). Due to the lack of sufficient GenBank data, morphometric confirmation is needed. Molecular and morphometric traits help identify nematode species (Oliveira et al., 2011).

The novelty of this research lies in its integrated approach. It combines morphological and molecular characterizations of *I. dentifurcatus* in Indonesia. Previous studies relied solely on morphological identification. They lacked strong data for species-level confirmation. This study provides robust evidence. It clarifies the nematode's phylogenetic placement. It fills a gap in the literature with a dual-method approach.

The benefits and contributions of this research extend to both science and society. It improves the taxonomic resolution of free-living nematodes. It supports their use as reliable bioindicators. This aids in monitoring soil health. It promotes sustainable agricultural practices. The findings lay a strong foundation for future ecological studies.

CONCLUSION

The morphological analysis identified the nematode from the rhizosphere of Arabica coffee plants in Bondowoso regency, Indonesia as *I.*

dentifurcatus and the SSU rDNA sequence of the nematode was determined. Further studies including both morphological and molecular analyses are necessary to infer the phylogenetic relationship among the *Ironus* species.

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REFERENCES

Bell, N., & Watson, R. (2001). Optimising the Whitehead and Hemming tray method to extract plant parasitic and other nematodes from two soils under pasture. *Nematology*, *3*(2), 179-185.

Bhat, A. H., Chaubey, A. K., Shokoohi, E., & Machado, R. A. (2021). Molecular and phenotypic characterization of Heterorhabditis indica (Nematoda: Rhabditida) nematodes isolated during a survey of agricultural soils in Western Uttar Pradesh, India. *Acta Parasitologica*, *66*, 236-252.

Biswal, D. (2022). Nematodes as ghosts of land use past: elucidating the roles of soil nematode community studies as indicators of soil health and land management practices. *Applied Biochemistry and Biotechnology*, *194*(5), 2357-2417.

Bogale, M., Baniya, A., & DiGennaro, P. (2020). Nematode identification techniques and recent advances. *Plants*, *9*(10), 1260.

De Ley, I. T., De Ley, P., Vierstraete, A., Karssen, G., Moens, M., & Vanfleteren, J. (2002). Phylogenetic analyses of *Meloidogyne* small subunit rDNA. *Journal of Nematology*, *34*(4), 319.

di Montanara, A. C., Baldrighi, E., Franzo, A., Catani, L., Grassi, E., Sandulli, R., & Semprucci, F. (2022). Free-living nematodes research: State of the art, prospects, and future directions. A bibliometric analysis approach. *Ecological Informatics*, *72*, 101891.

Girgan, C., Shokoohi, E., Marais, M., Fourie, H., Tiedt, L., & Swart, A. (2021). Description of *Ironus telperionensis* n. sp.(Nematoda: Ironidae) and two known species of *Chronogaster* (Nematoda: Chronogastridae) and *Paraphanolaimus* (Nematoda: Aphanolaimidae) from the Telperion Nature Reserve (Mpumalanga, South Africa). *Nematology*, *23*(8), 939-961.

Haraguchi, S., & Yoshiga, T. (2020). Potential of the fungal feeding nematode *Aphelenchus avenae* to control fungi and the plant parasitic nematode *Ditylenchus destructor* associated with garlic. *Biological Control*, *143*, 104203.

Hindersah, R., & Asyiah, I. N. (2023). Abundance of beneficial soil microbes in the rhiosphere of coffee plants infected by *Pratylenchus coffeae*. *Biosaintifika: Journal of Biology & Biology Education*, *15*(2), 220-229.

Hua, E., Zhu, Y., Huang, D., & Liu, X. (2021). Are free-living nematodes effective environmental quality indicators? Insights from Bohai Bay, China. *Ecological Indicators*, *127*, 107756.

Javed, S., & Khan, S. (2021). Mass culturing of mycetophagous nematode *Aphelenchus avenae* (Nematoda: Aphelenchidae) in vitro system by feeding on pathogenic fungus. *Sarhad Journal of Agriculture*, *37*(2), 675-682.

Kanwar, R., Patil, J., & Yadav, S. (2021). Prospects of using predatory nematodes in biological control for plant parasitic nematodes–a review. *Biological Control*, *160*, 104668.

Khanum, T. A., Mehmood, N., & Khatoon, N. (2021). Nematodes as Biological Indicators of Soil Quality in the Agroecosystems. In *Nematodes-Recent Advances, Management and New Perspectives*. IntechOpen.

Kimenju, J., Karanja, N., Mutua, G. K., Rimberia, B., & Wachira, P. (2009). Nematode community structure as influenced by land use and intensity of cultivation. *Tropical and Subtropical Agroecosystems*, *11*(2), 353-360.

Lee, H. J., Lee, H., & Rho, H. S. (2023). Two new records of free-living marine nematodes of the family Ironidae de Man, 1876 (Nematoda: Enoplida) from Korea. *Journal of Species Research*, *12*(1), 55-67.

Li, G., Liu, T., Whalen, J. K., & Wei, Z. (2024). Nematodes: an overlooked tiny engineer of plant health. *Trends in Plant Science*, *29*(1), 52-63.

Loranger-Merciris, G., Ozier-Lafontaine, H., Diman, J.-L., Sierra, J., & Lavelle, P. (2022). Fast improvement of macrofauna communities

and soil quality in plantain crops converted to agroecological practices. *Pedobiologia*, *93*, 150823.

Melakeberhan, H., Bonito, G., & Kravchenko, A. N. (2021). Application of nematode community analyses-based models towards identifying sustainable soil health management Outcomes: a review of the concepts. *Soil Systems*, *5*(2), 32.

Neher, D. (1999). Soil community composition and ecosystem processes: comparing agricultural ecosystems with natural ecosystems. *Agroforestry Systems*, *45*(1), 159-185.

Neher, D. A. (2010). Ecology of plant and free-living nematodes in natural and agricultural soil. *Annual Review of Phytopathology*, *48*(1), 371-394.

Oliveira, C. M. G. d., Monteiro, A. R., & Blok, V. C. (2011). Morphological and molecular diagnostics for plant-parasitic nematodes: working together to get the identification done. *Tropical Plant Pathology*, *36*, 65-73.

Pradana, A. P., & Yoshiga, T. (2023). First record of free-living nematode *Mylonchulus hawaiiensis* from Bondowoso Regency-Indonesia. *Jurnal Sumberdaya Hayati*, *9*(1), 17-23.

Pradana, A. P., & Yoshiga, T. (2024). Molecular identification to verify the existence of *Helicotylenchus dihystera* in coffee plants at East Java, Indonesia. *ASEAN Journal on Science and Technology for Development*, *41*(3), 217-224.

Rehman, P., Nazir, R., Naqvi, T. A., Pervez, A., & Irshad, U. (2018). Bacterial feeder Nematodes: Facilitator or competitor for Plant Phosphorus in soil. *Journal of Soil Science and Plant Nutrition*, *18*(4), 1173-1186.

Ridall, A., & Ingels, J. (2021). Suitability of free-living marine nematodes as bioindicators: status and future considerations. *Frontiers in Marine Science*, *8*, 685327.

Rueda-Ramirez, D., Palevsky, E., & Ruess, L. (2022). Soil Nematodes as a means of conservation of soil predatory mites for Biocontrol. *Agronomy*, *13*(1), 32.

Saswita, H. M., Syamsuardi, S., Nurainas, N., Suwardi, A. B., & Taufiq, A. (2023). Phylogenetic analysis of *Baccaurea* spp. in West Sumatra using MatK molecular markers. *Biosaintifika: Journal of Biology & Biology Education*, *15*(3), 362-369.

Schratzberger, M., Holterman, M., van Oevelen, D., & Helder, J. (2019). A worm's world: Ecological flexibility pays off for free-living nematodes in sediments and soils. *BioScience*, *69*(11), 867-876.

Seesao, Y., Gay, M., Merlin, S., Viscogliosi, E., Aliouat-Denis, C., & Audebert, C. (2017). A review of methods for nematode identification. *Journal of Microbiological Methods*, *138*, 37-49.

Tahseen, Q., & Mehdi, S. (2009). Taxonomy and relationships of a new and the first continental species of *Trissonchulus* Cobb, 1920 along with two species of *Ironus* (Nematoda: Ironidae) collected from coal mines. *Nematologia Mediterranea*, *37*, 117-132.

Tanaka, R., Kikuchi, T., Aikawa, T., & Kanzaki, N. (2012). Simple and quick methods for nematode DNA preparation. *Applied Entomology and Zoology*, *47*(3), 291-294.

Trap, J., Ranoarisoa, M. P., Raharijaona, S., Rabeharisoa, L., Plassard, C., Mayad, E. H., Bernard, L., Becquer, T., & Blanchart, E. (2021). Agricultural practices modulate the beneficial activity of bacterial-feeding nematodes for plant growth and nutrition: evidence from an original intact soil core technique. *Sustainability*, *13*(13), 7181.

Waldo, B. D., Shahoveisi, F., & Carroll, M. J. (2024). Long-term fertilization and cultivation impacts on nematode abundance and community structure in tall fescue turfgrass. *Ecology and Evolution*, *14*(2), e10905.

Ye, W.-m., & Hunt, D. J. (2021). Measuring nematodes and preparation of figures. In *Techniques for work with plant and soil nematodes* (pp. 132-151). CABI Wallingford UK.

Yeates, G. (1967). Studies on nematodes from dune sands. 3. Oncholaimidae, Ironidae, Alaimidae and Mononchidae. *New Zealand Journal of Science*, *10*(1), 299-321.

Yoder, M., De Ley, I. T., King, I. W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L., & De Ley, P. (2006). DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology*, *8*(3), 367-376.

Young, E. H., & Unc, A. (2023). A review of nematodes as biological indicators of sustainable functioning for northern soils undergoing land-use conversion. *Applied Soil Ecology*, *183*, 104762.