Short Communication:
First record of *Hirschmanniella mucronata* (Nematoda: Pratylenchidae) in Yogyakarta, Indonesia

SIWI INDARTI[1,2,♥]**, ALAN SOFFAN**[1,♥♥]**, MUHAMMAD MAULANA FARDANI ANDRASMARA**[1]

[1]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.
Tel.: +62- 274)-563062, 901290, Fax.: 62-274-563062, 519717, ♥email: siwi.indarti@ugm.ac.id; ♥♥alan.soffan@mail.ugm.ac.id
[2]Agrotechnology Innovation Centre, Universitas Gadjah Mada. Jl. Tanjung Tirto, Kalitirto, Berbah, Sleman 55573, Yogyakarta, Indonesia

Abstract. *Indarti S, Soffan A, Andrasmara MMF. 2020. Short Communication: First record of* Hirschmanniella mucronata *(Nematoda: Pratylenchidae) in Yogyakarta, Indonesia. Biodiversitas 21: 2068-2073.* Hirschmanniella *spp. is one of the worldwide plant-parasitic nematodes affecting major losses in rice production and impact up to 25% yield losses especially on irrigated rice. Infection of* Hirschmanniella *spp. on the root system leading to the typical symptom of red color in the rice rooting system. In order to identify the species variation of* Hirschmanniella *spp. from the collected samples in Yogyakarta Province of Indonesia, a molecular-based identification using* Polymerase Chain Reaction *(PCR) method was conducted, complemented by morphological identification technique. PCR based identification was carried out by amplifying the area of 28S rRNA using universal nematode primer (D2A / D3B) which resulting about 766 bp of amplicon. Blastx analysis from Genbank showed that Cangkringan sample confirmed to be* H. mucronata *species, while other samples from Banguntapan and Imogiri were* H. oryzae *species. The Cangkringan samples of* H. mucronata *have 97.5 % similarities with Belgium sample, forming separate clades with other samples. While both Banguntapan and Imogiri samples have the 99 % similarity with* H. oryzae *and were located in the same clade, but separated from Cangkringan sample. Morphological identification confirmed both species were distinctly based on the unique characters of the tail tips.* H. mucronata *therefor is the first report nematode species in Indonesian rice field. Precaution should be designed to prevent the potential distribution of* H. mucronata *to other areas.*

Keywords: *Hirschmanniella mucronata*, *H. oryzae*, molecular identification, Yogyakarta

INTRODUCTION

Rice (*Oryza sativa sativa*) is one of the most important crops, and more than half of the world's population have been using rice as a staple food (Zin Oo and Hla Maw 2016). Indonesia is one of the biggest rice consumers due to its role as the main staple food. In order to meet the increasing rice demand, Indonesian government has implemented various technology such as through SRI (System of Rice Intensification). While intensification effort significantly increases rice production, however, at the same time, it deploys the potential risk, which becomes a significant threat to rice production. One of the potential risks is due to nematode infestation. The plant-parasitic nematodes play an important role and account for yield losses to the extent of 90% (Jain et al. 2012). There are several plant-parasitic nematode species known being able to infest rice plant, including white tip (*Aphelenchoides besseyi*), rice root-knot (*Meloidogyne graminicola*), rice root (*Hirschmanniella* spp.), rice stem (*Ditylenchus angustus*), rice cyst (*Heterodera oryzicol*a), root lesion (*Pratylenchus* spp.), *Hoplolaimus*, *Criconemoides,* and *Tylenchorhynchus* (Swe 1997; Than 2003).

The distribution studies of those species across the Indonesia rice fields are limited. Many studies reported that Indonesia rice fields mostly have severe damage due to *Meloidogyne* sp., and *H. oryzae* (Bridge et al. 2005). With the increasing movement of the rice grain and peoples across locations, Indonesian rice fields will be vulnerable for the infestation of other species in addition to *Meloidogyne* spp. and *H. oryzae*. Genus *Hirschmanniella* is known to be one of the most important nematode genera on rice (Khun et al. 2015), which can caused rice yield loss from 12.5% up to19 % (Bala and Khan, 2004). Two or more species of *Hirschmaniella* usually found in the same area and may lead to severe damage (Khan and Bala 2003). On the regular survey of nematode infestation across different areas in Indonesia, particularly in Yogyakarta, Java island, we finally reported the first occurrence of nematodes species *Hirchmaniella mucronata* which previously had not been reported yet. This report serves as an important alert for the potential infestation in the near future which might affect the decreasing of Indonesian rice production.

MATERIALS AND METHODS

Nematode sampling and extraction

Samples were collected from the root system with nematode symptoms in 2018. Sampling locations were mainly conducted in the rice production areas of

Yogyakarta, Indonesia, namely Cangkringan, Banguntapan, dan Imogiri. Soil samples were extracted for nematode presence using the modified Whitehead Tray method (Southey, 1986). Before the nematode analysis, the root samples were scored to categorize the root necrotic damage. Modified scoring of root damaged was grouped into 10 classes according to the percentage of root cortex surface covered by necrotic surface, 0: no necrotic on the root cortex, 1: 1-10 % root cortex covered by necrotic, 2: 11-20 % root cortex covered by necrotic, 3: 21-30% root cortex covered by necrotic, 4: 31-40 % root cortex covered by necrotic, 5: 41-50 % root cortex covered by necrotic, 6: 51-60% root cortex covered by necrotic, 7: 61-70%' 8: 71-80%, 9: 81-90%, and 10: 91-100% (Coyne et al., 2007). Nematode extractions from root samples were done by cutting the root samples into 1 mm pieces then were laid on the filter paper on the nylon screen supported by the modified bucket allowing the water to attach the root samples. The dipping was at room temperature for 24 h. This method referred to Whitehead Tray Technique (Southey 1986) with modification. The nematode which was swimming out to the water was collected on the tubes for further both morphological and molecular-based identification. Morphometric measurement was incorporated to support identification in species level.

DNA extraction

Modified CTAB methods were used to extract the DNA from the nematode samples (Zhou et al., 2007). In brief, the four adults *Hirschmanniella* spp. were picked up and each nematode than chopped into 4-5 pieces by using tips of needle followed by the addition of 25μL buffer CTAB in a 1.5 mL tube. The samples were incubated in 65ºC temperature for 30 min with every 10 mins samples were up and down prior to centrifuge for 5min with 5000 rpm. Chloroform: Isoamylalkohol (CIAA) with ratio 24:1 were added to the sample followed by gentle shaking and 15 min centrifuge at 10.000 rpm, room temperature. Finally, the supernatant was collected and mixed with cold absolute alcohol with a ratio of 1:2 followed by 24 hours incubation at-20ºC. Sample washing was done by using 70% cold alcohol after 10.000 rpm for 15min after previous incubation. The final template of DNA was obtained by 10.000 rpm for 15min centrifuge followed by TE dilution for 20 μL. The DNA concentration was measured using Nanodrop spectrophotometer (Thermos, USA), and the samples were maintained at -20ºC until PCR and sequencing.

Amplification and sequencing

The D2A-D3B primers (D2A: 5'ACAAGTACCGTGA GGGAAAGTTG 3'; D3B:5'TCGGAAGGAACCAGCT ACTA 3') with 766 bp expected product were used to amplify 28S rRNA of nematodes which previously had been reported to distinguish until species level (Nunn 1992). PCR Kit (*Redmix BioLine*) was utilized with a total volume of 12.5 μL, including the DNA template, primers,

and aquabidest 2.5 μL, 2.5 μL and 5 μL, respectively. PCR program was performed with annealing, denaturation and extension temperature were 95°C, 54°C, and 72°C, respectively, with the time period of 30, 30, and 15 sec., respectively. The final extension was performed at 72°C for 10min, followed by 4°C. The PCR products were run on 2% agarose gels and visualized on UV transilluminator. The amplified PCR products within the expected size range were gel purified and sequenced on ABI 3500 genetic analyzer (Life Technologies). Sequences were analyzed using Bioedit ver. 7.1.9 followed by BLASTx searches conducted in the NCBI database, to confirm the species name. Finally, the phylogenetic tree of the 28S rRNA was constructed using the maximum likelihood statistics with the LG+G best fit model to look at the relationship with the available database.

Morphological identification

The morphological characters of *Hirschmanniella* spp. were evaluated using light microscope Olympus CX 31 with magnification 40-1000×. The identification of specimens was based on the related literature, i.e., Ebsary and Anderson (1982) and Loof (1991). The main characters for morphological identification are a general shape of nematode and their body length, cephalic framework, type and position of esophagus to intestine (Berliner et al., 2017). According to available literature, the identification up to species level can be distinguished from one species to another by the shape of the tail as in Figure 1.

Morphometric measurement

Morphometric characters were analyzed using De Mann formula (Southey 1986). Drawing and measurement of each specimen were conducted using a camera lucida which was connected to microscope Olympus BH2. Main notation of De Mann formulae includes n (number of specimens), L (total body length in nm or μm), a (body length: greatest body width), b (body length: distance from anterior end to junction of esophagus and intestine), V (distance of vulva from anterior end X 100: body length), and length of female tail (distance from anus to tip tail).

RESULTS AND DISCUSSION

Hirschmanniella spp. infect and multiply in the root system, through the air space across the radial lamella. An infected root system becomes necrotic, followed by a change in color to red and brown, indicating the decay process. This typical symptom is due to the injury of the cortex of the root system (Bridge and Starr 2007). The rice root systems (Figure 2) infected by *Hirschmaniella* spp. were stunted in growth, which could lead to rice production failure (Figure 3). Details of the *Hirschmaniella* spp. infestation is presented in Figure 4.
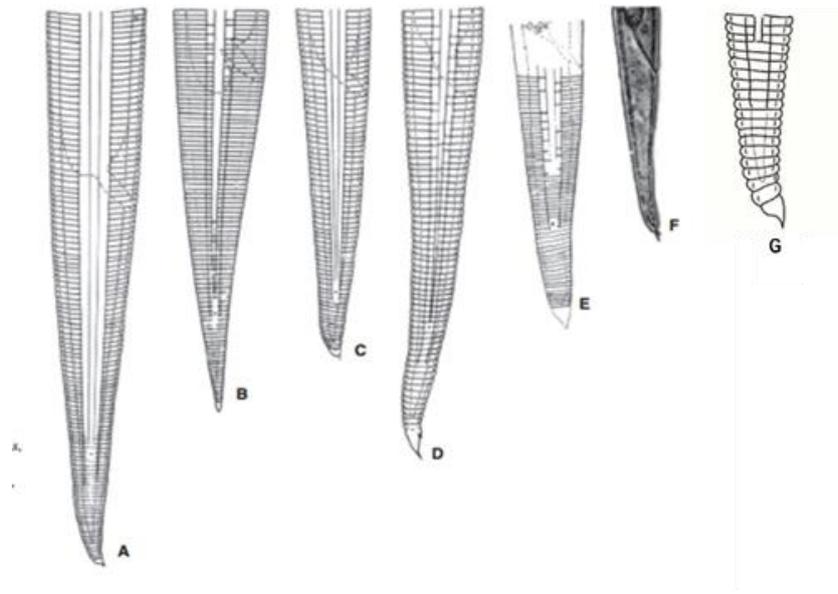
Figure 1. Tail shape of *Hirschmanniella* spp. A. *H. gracilis*, B. *H. spinicaudata*, C. *H. oryzae*, D. *H. caudacrena*, E. *H. miticausa,* F. *H. shamim* G. *H. mucronata* (Loof 1991)

An additional observation was conducted to correlate the injury level, *Hirschmanniella* spp. population, rice cultivar, and the location site, as presented in Table1. It was shown that the highest injury level occurred in the Cangkringan, Sleman on both Ciherang and Mentik Wangi cultivars, and it had a positive correlation with the number of *Hirschmanniella* spp. in the rooting system. While the IR 64 cultivar had less injury in the same location. Both of these cultivars, Ciherang and Mentik Wangi in the same area, were also as susceptible as IR64 in different locations. Based on this result, it was obvious that rice cultivar and site location seem to have a significant contribution to the injury level and *Hirschmanniella* spp. population. It was suggested that soil abiotic factors, especially soil humidity, C organic contents, and temperature have a significant effect on nematode abundance. Report of Mutala'liah et al. (2018) showed that cultivar host plants influenced the plant-parasitic nematodes abundance and Mutala'liah et al. (2017) also reported that C-Organic positively affects nematodes abundance, including on root-lesion nematode that belongs to the same family with *Hirschmanniela*.

Tabel 1. *Hirschmnniella* sp. population and the root damage index based on location and rice cultivar

Location	Local rice cultivar	Root damaged index	*Hirschmanniella* spp. population (per gram root)
Cangkringan, Sleman	IR64	2	14
	Ciherang,	6	134
	Mentik wangi	6	126
Banguntapan, Bantul	Ciherang	4	45
Imogiri, Bantul	IR64	5	69

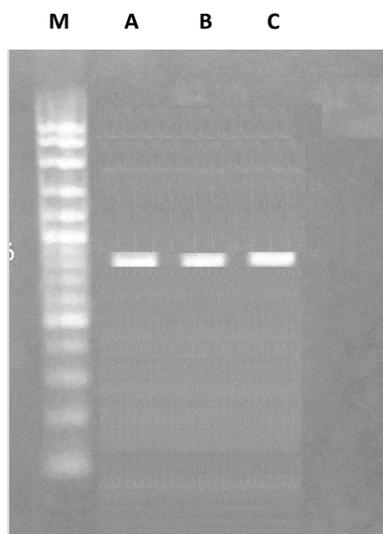**Figure 2**. Infected root of the rice by *Hirschmanniella* spp (A) and healthy root (B)

Figure 3. Field symptom of the *Hirschmnniella* spp. infestation. The rice plants in inside the circle with stunted growth and flowering were delayed

Figure 4. Root infected symptom by *Hirschmanniella* spp. in Imogiri (A), Banguntapan, (B), and Cangkringan (C)

Figure 5. Amplicons of ±766 bp were resulted from A. Cangkringan. B. Banguntapan. C. Imogiri)

Molecular detection of samples collected from Imogiri, Banguntapan, and Cangkringan was conducted by PCR using the D2A/D3B primer. All of the samples resulting in 766 bp amplicons (Figure 5). The sequencing results were submitted to GenBank (https://www.ncbi.nlm.nih.gov/) under Accession numbers of MK205364, MK2419389, and MK241938 for *H. oryzae* Imogiri, *H. oryzae* Banguntapan, and *H. mucronata* Cangkringan respectively,

To evaluate the similarity of the three tested samples with other reported *Hirschmanniella* spp. from the GenBank database, multiple alignment sequencing was conducted using the ClustalW program. The analysis showed that the Cangkringan sample had 84%-97% similarity with *H. mucronata* from Belgium. The rest of the samples from Imogiri and Banguntapan had 99% similarity with *H. oryzae*. A phylogenetic tree (Figure 6) was constructed using MEGA6 with the neighbor-joining method, the Kimura 2 parameter model, and 1,000 bootstraps. The phylogenic tree revealed that the Imogiri and Banguntapan samples were in the same clade together with *H. oryzae* from Myanmar, Iran, Vietnam, and the Philippines. The Cangkringan samples were grouped in another clade recognized as *H. mucronata*, together with *H. mucronata* from Belgium, the Philippines, and South China.

In general, Genus Hirschmanniella is a member of the family Pratylenchidae, which has monomorphism character, both either female and male are vermiform and slender and easily identified by their long body (1 to 4 mm). They have colorless body with well-developed cephalic framework (head) and consisted of one short feeding apparatus called stylet with rounded knobs. They have esophageal gland, which overlapping over the intestine in the ventral side (Berliner et al., 2017). All criteria were found on all specimens of Hirschmaniella from Cangkringan, Imogiri and also Banguntapan. However, there were differences in female tip shape of Cangkringan specimen to both Imogiri and Banguntapan (Figure 7).

The morphological observation of the female tail shape, especially on the tail tip confirmed that the Cangkringan sample was *H. mucronata*, whereas the Banguntapan and Imogiri samples were *H. oryzae*. *H. mucronata* is characterized by having the irregular tail edge sharpened into a projection (Khun et al. 2015), while *H. oryzae* has a regular tail shape with an outside projection (Luc and Goodey 1964) (Figs. 7-8).

Identification based on molecular, and morphological data confirmed the presence of two species of *Hirschmanniella*, which were *H. mucronata* (found on Cangkringan) and *H. oryzae* (found on Imogiri and Bantul). And based on the morphometric data, especially some characters on the length of stylet, and a and b values were supported that specimen from Cangkringan is *H. mucronata* (Table 2). Therefore, *H. mucronata* is the first report of this species infecting rice fields in Indonesia. This report should be followed up by anticipating the distribution of *H. mucronata* to other locations in Indonesia.
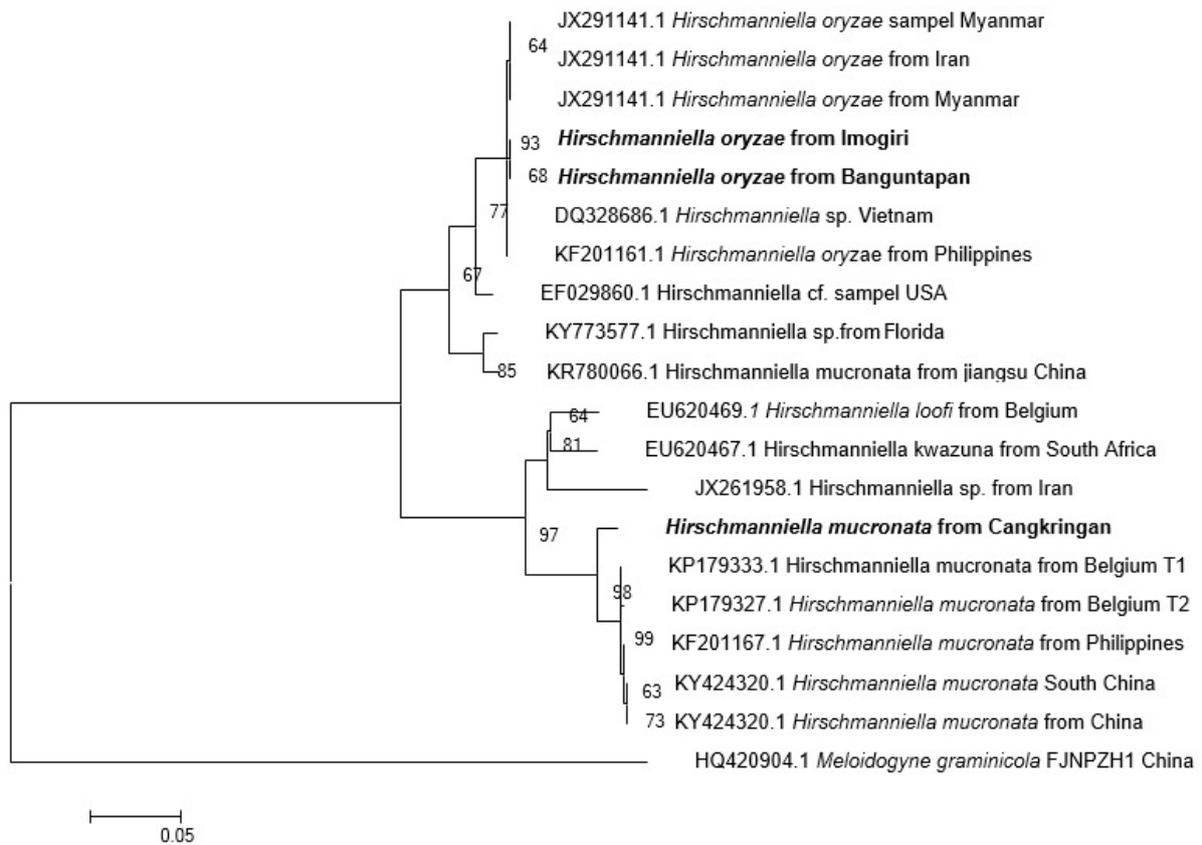
Figure 6. Neighbor-joining phylogenetic tree of 28 srRNA from nematode samples collected from rice fields in Cangkringan, Banguntapan, and Imogiri. The best-fit Kimura 2 parameter model with 1,000 bootstraps was used to construct the tree with other available *Hirschmanniella* spp. from the *Genbank* NCBI database
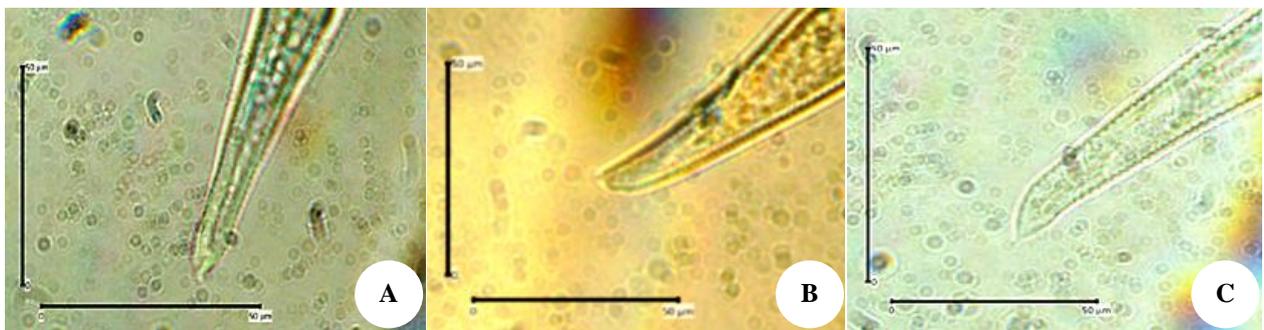
Figure 7. Female tail end of *Hirschmaniella mucronata* (from Cangkringan). (A) and *H. oryzae* from Imogiri (B) and Banguntapan (C)
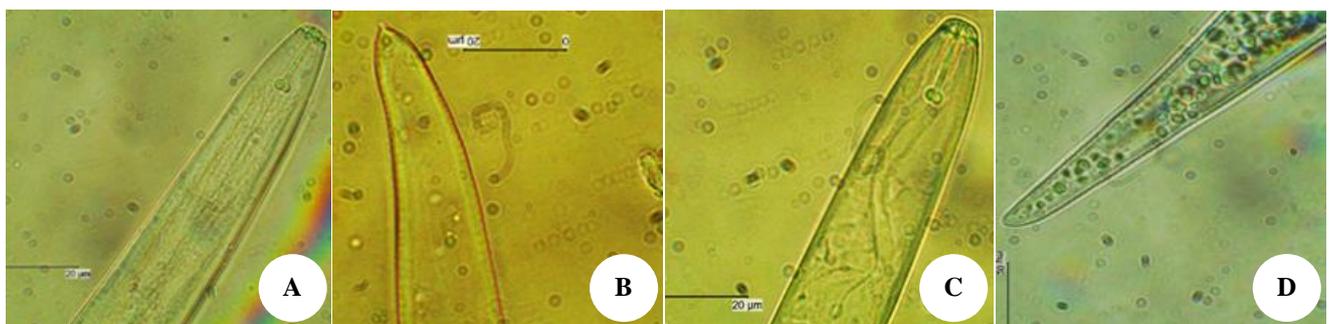
Figure 8. Anterior part showing lip, knob stylet, and female tail of *Hirschmanniella*. A-B. *H. mucronata* (from Cangkringan), C-D. *H. oryzae* (from Banguntapan)

Table 2. Morphometric data of the *Hirschmanniella* spp. females from Yogyakarta Indonesia (n = 5)

Characters	*H. oryzae*	*H. mucronata*	Reference (Khun et al. 2015)	
			H. oryzae	*H. mucronata*
Length of body (µm)[*]	550-1,150	760-1,050	1,200-2,700	900-1,850
Length of stylet [*]	15-20	22-23	13-21 µm	20-29 µm
a	29.33-42	33-52.27	29-80.6	44.8-84
b	6.9-13.8	9.2-15.8	6.8-14	9.3-16.6
V	40-65	55-60	47.2-63.1	47.2-63.1
Length of tail[*]	80-85	60-90	45-123 µm	60-160 µm

Note: *) Measurements in µm

REFERENCES

Bala S, Khan M. 2004. Observation on *Hirschmanniella* species associated with rice in West Bengal. Environ Ecol 22: 449-453.

Berliner J, Pokhare S, Adak T, Guruprasannapandi. 2017. Rice Root Nematode an emerging threat to irrigated rice. Indian Farming 67 (3): 31-32

Bridge J, Plowright RA, Peng D. 2005. Nematode parasites of rice. In: Luc M, Sikoro RA, Bridge J (eds). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CTA Postbus, AJ, Wageningen, The Netherlands

Bridge J, Starr J. 2007. Plant Nematodes of Agricultural Importance: A Colour Handbook. CRC Press, Boca Raton.

Coyne DL, Nicol JM, Claudius-Cole B. 2007. Practical Plant Nematology: A Field and Laboratory Guide. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin

Ebsary B, Anderson R. 1982. Two new species of *Hirschmanniella* Luc and Goodey, 1963 (Nematoda: *Pratylenchidae*) with a key to the nominal species. Can J Zool 60: 530-535.

Jain RK, Khan MR, Kumar V. 2012. Rice root-knot nematode (*Meloidogyne graminicola*) infestation in rice. Arch. Phytopathol Plant Protect 45: 635-645.

Khan M, Bala S. 2003. Morphometric variations in *Hirschmanniella mucronata* and *H. oryzae* from West Bengal. Intl J Nematol 13: 225-228.

Khun K, Decraemer W, Couvreur M, Karssen G, Steel H, Bert W. 2015. Deceptive morphological variation in *Hirschmanniella mucronata* (Nematoda: Pratylenchidae) and a polytomous key to the genus. Nematology 17: 377-400.

Loof, P. 1991. The family *Pratylenchidae* Thorne, 1949. In: Nickle WR (ed). Manual of Agricultural Nematology'). Marcel Dekker, New York.

Luc M Goodey JB. 1964. *Hirschmanniella* nom. nov. for *Hirschmannia*. Nematologica 9: 471

Mutala'liah, Indarti S, Putra, NS. 2017. Relationship of soil abiotic factors with population abundance and vertical distribution of root-lesion nematode in robusta coffee plantation. Pakistan J Nematol 35:183-196.

Mutala'liah, Indarti S, Putra, NS. 2018 Abundance and diversity of plant-parasitic nematodes associated with BP 308 and BP 42 clones of robusta coffee in Java, Indonesia, Biodiversitas 19 (1): 67-70

Nunn G. 1992. Nematode Molecular Evolution. [Dissertation]. University of Nottingham, UK.

Southey JF. 1986. Laboratory methods for work with plant and soil nematodes. Her Majesty's Stationary Office. London.

Swe A. 1997. Status of nematode problems and research in Myanmar *In* Sharma, SB. 1997. Diagnosis of key nematode pests of chickpea and pigeon pea and their management: Proceedings of a Regional Training Course, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India.

Than PP. 2003. Survey of Plant Parasitic Nematodes on Some Economic Crops and Study on Rice Root rot Disease Caused by *Hirschmanniella oryzae*. [Thesis]. Yezin Agricultural University, Myanmar.

Zhou X, Li Q, Zhao J, Thang K. 2007. Comparison of rapid DNA extraction methods applied to PCR identification of medicinal mushroom *Ganoderma* spp. Prep Biochem Biotechnol 37: 369-380