RESEARCH/INVESTIGACIÓN

DISTRIBUTION AND DIVERSITY OF ROOT-KNOT NEMATODES IN AGRICULTURAL AREAS OF FIJI

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ABSTRACT

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A total of 675 soil samples were collected from 10 different agricultural areas around Viti Levu in Fiji during the period of April 2007- April 2008 to determine the distribution, host associations and species diversity of root-knot nematodes. Root-knot nematode species were identified using molecular analysis (SCAR and mt primers), and morphological examination of female, juvenile and male nematodes when available. *Meloidogyne incognita, M. javanica* and *M. arenaria* were found to be widely distributed in all localities throughout Viti Levu with a total incidence of 41% recorded from 675 farms. *Meloidogyne* spp. was found associated with 33 species of crop plants. *Meloidogyne incognita* was the most commonly occurring species on 56% of the infested farms followed by *M. javanica* on 28% and *M. arenaria* on 10%. Mixed populations of *M. incognita* and *M. javanica* were found on 5% of the infested farms. Three unidentified root-knot nematode species (1%) were also recorded and require further investigation.

Key words: Fiji, *M. arenaria, M. incognita, M. javanica,* molecular diagnostics, morphology, Root-gall, species identification.

RESUMEN

Singh, S. K., U. R. Khurma, and P. J. Lockhart. 2012. Distribución y diversidad de nematodos agalladores en áreas agrícolas de Fiji. Nematropica 42:16-25.

Se colectaron 675 muestras de suelo en 10 áreas agrícolas diferentes alrededor de Viti Levu en Fiji, de abril 2007 a abril 2008, con el fin de determinar la distribución, hospedantes y especies de nematodos agalladores. Se identificaron las especies de *Meloidogyne* utilizando técnicas moleculares (cebadores SCAR y mt) y observaciones morfológicas de las hembras, juveniles y machos. Se encontró que *Meloidogyne incognita, M. javanica* y *M. arenaria* están ampliamente distribuidas en todas las localidades con una incidencia total de 41% de las 675 fincas. Se encontró *Meloidogyne* spp. asociado a 33 especies de plantas cultivadas. *Meloidogyne incognita* fue la especie más común, presente en 56% de las muestras positivas para nematodo agallador, seguido de *M. javanica* con 28% y *M. arenaria* con 10%. Se encontraron poblaciones mixtas de *M. incognita* y *M. javanica* en 5% de las muestras positivas. También se encontraron tres especies de *Meloidogyne* no identificadas (1%) que requieren futura atención.

Palabras clave: identificación de especies, Fiji, *M. arenaria, M. incognita, M. javanica*, diagnóstico molecular, morfología. .

INTRODUCTION

The genus *Meloidogyne* is considered as one of the most important genera of plant parasitic nematodes worldwide (Siddiqi, 2000; Dong *et al.*, 2001; Trudgill and Blok, 2001; McK Bird and Kaloshian, 2003) and has 97 valid species as of June 2009 (Hunt and Handoo, 2009). In Fiji, root-knot nematodes have been reported to cause major damage to ginger production (Graham, 1971; Haynes *et al.*, 1973; Butler, 1974; Orton Williams, 1980) and most of the commonly grown crops can act as potential hosts of this pathogen (Gowen *et al.*, 2005), yet there is scarce documentation on the prevalence and losses caused by root-knot nematodes to crops in Fiji. Orton Williams (1980) reported the presence of root-knot nematodes from 31% of the soil samples from 9 islands of the Fiji group. Recently, a preliminary survey recorded the presence of root-knot nematodes on 29% of farms and gardens from Viti Levu, the largest and most agriculturally important island in the archipelago

(Khurma *et al.*, 2008). Subsequently, a more thorough and systematic survey was conducted, and this paper reports the results on the distribution, crop host range and species diversity of root-knot nematodes from agricultural areas of Viti Levu, Fiji. In addition weed hosts of *M. incognita*, *M. javanica* and *M. arenaria* were also observed during this survey and have been reported elsewhere (Singh *et al.*, 2010).

Root-knot nematode species identification is fundamentally important to a wide range of scientific studies on virulence and nematode management. With reduced availability of broad spectrum chemical nematicides and increased reliance on non-chemical nematode management strategies, the precise identification of *Meloidogyne* species is becoming increasingly important for the design of effective nematode management and also for quarantine purposes (Hussey, 1990; Zijlstra, 2000; Zijlstra and van Hoof, 2006).

Traditionally, identification and description of *Meloidogyne* species was based mainly on differences in morphological and morphometrical characters of females, juveniles and males. A number of other methods such as North Carolina differential host test, reproductive and cytological characters, and enzyme and protein patterns have also been used to assist with *Meloidogyne* species identifications (Esbenshade and Triantaphyllou, 1985; Hussey, 1985); however, each of the methods has their own strengths and weaknesses, and a combination of methods are required for reliable species identification. More recently molecular methods based on DNA and PCR such as RAPD, RFLP, SCAR, Multiplex PCR, and AFLP have been developed and successfully used to aid in the identification of a number of *Meloidogyne* species (Harris *et al.*, 1990; Powers and Harris, 1993; Castagnone Sereno *et al.*, 1995; Zijlstra *et al.*, 1995; Petersen and Vrain, 1996; Petersen *et al.*, 1997; Randig *et al.*, 2002; Han *et al.*, 2004; Adam *et al.*, 2007). The combination of morphological and molecular analyses of nematodes has been reported as the most effective means of identifying nematodes (Evans, 1995; Thomas *et al.*, 1997). The objectives of this study were to determine the distribution, host associations and species diversity of root-knot nematodes (*Meloidogyne* spp.) on Viti Levu, Fiji.

MATERIALS AND METHODS

Sample collection and sites

Fiji Islands covers a land area of 18,333 km^2 and is located in the tropical region between 174° East and 178° West of Greenwich and latitudes 12° S and 22° South (Fiji Islands Bureau of Statistics, 2008). There are two major islands: Viti Levu, which covers an area of 10,429 sq km and Vanua Levu, which covers an area of 5,556 sq km. The climatic conditions are tropical with two seasons. The cool and dry season is from May to October with an average temperature range of 19°C to 22°C while the hot and wet season is from November to April with an average temperature range of 31°C to 34°C (Fiji Islands Bureau of Statistics, 2008). A total of 675 randomly chosen farms and gardens growing a variety of crops, representing all the 10 localities around Viti Levu, Fiji (Fig. 1) were sampled from April, 2007 - April, 2008.

On each of the farms sampled, roots of crop plants as well as any weed species present, were examined for the presence of root-galling and the observations were recorded. Composite soil samples consisting of 10 sub-samples were taken from the rhizosphere of the crop plant species at depths of 0-30 cm using a hand shovel while moving in a zigzag pattern. The soil sub-samples were mixed thoroughly and approximately 2 kg of the composite soil sample per farm was placed

Fig. 1. Map of localities sampled from Viti Levu Fiji.

in a polythene bag labeled with date, GPS coordinates, crop name, and sample number. The hand shovel and footwear were washed and cleaned after sampling each farm to avoid contamination of soil samples and prevent spread of nematodes between farms.

The presence of root-knot nematodes was determined by direct examination of root systems in the field and from field soil via bioassays. All soil samples were placed in plastic pots with a tomato (*Solanum lycopersicum* L.) cv. Moneymaker. The cv. Moneymaker was selected as bioassay host because of its susceptibility to *Meloidogyne* spp., determined from an earlier study (Singh and Khurma, 2007). Pot cultures were maintained for 8-10 weeks in a screen house on raised benches and separated at least 30 cm apart to avoid cross contamination. Nematode cultures were used for characterization studies of root-knot nematode populations and also to detect low numbers of *Meloidogyne* spp. in field soil where galling was not observed by direct examination of plant species in the field.

The tomato plants in pots were carefully uprooted, washed with tap water, and examined for presence of root galls under a stereoscopic microscope. Root-galling for both direct examination of plant species in the field and tomato plants used in the bioassay was determined using a 0-5 scale (Taylor and Sasser, 1978). The presence or absence of root galls on the tomato plants used in the bioassay was considered as the final indicator for the presence or absence of root-knot nematodes in the soil samples.

Identification of Meloidogyne *species - Molecular characterization*

The field nematode populations present in composite soil samples maintained on tomato host was used for molecular identification. Since pure cultures were not established from the field populations, we included 5-10 egg masses, randomly handpicked from infected tomato plants for molecular evaluation. DNA was extracted from 5-10 egg masses per population using a modified salting out procedure (Miller *et al.*, 1988). The modifications included grinding of the egg mass into fine powder under liquid nitrogen followed by digestion in 600 µL of TNES solution (50 mM Tris HCl, 400 mM NaCl, 20 mM EDTA, 0.5% SDS) and 10 µL of Proteinase K for 3 hours at 55°C with occasional vortexing. The extracted DNA was suspended in 30 µL of TE buffer (10 mM Tris HCl, 1 mM EDTA) and stored at - 20°C for later use. The polymerase chain reactions (PCR) were carried out using 17.8 µl sterilized distilled water, 3.0 µl 10X reaction buffer, 3.0 µl 50mM $MgCl_2$, 3.0 µl 2 mM dNTPs, 1.0 µl each forward and reverse primer, 0.2 µl red hot Taq polymerase (Invitrogen) and 1.0 µl DNA template. The mitochondrial primers C2F3/1108 (Powers and Harris, 1993) and SCAR primers for *M. incognita* MI-F/MI-R (Meng *et al.*, 2004) *M. arenaria* Far/Rar and *M. javanica* Fjav/Rjav (Zijlstra *et al.*, 2000) were used for the identification of *Meloidogyne* species.

The PCR products were visualized by electrophoresis on 1.5% agarose gels stained with syber safe. The populations were categorized into five groups *M. incognita*, *M. arenaria*, *M. javanica*, mixed species and unidentified nematode species. Populations that showed amplification of a PCR product of the appropriate diagnostic size with only one species specific primer combination, and not with other combinations, were assigned to one of the appropriate three groups. DNA samples that consistently showed amplification with more than one species specific primer were considered as mixed nematode species. DNA samples extracted from nematode populations that did not amplify with any of the species specific primers but did amplify with the mitochondrial primers were categorized as unidentified *Meloidogyne* spp. The members of this latter group are being further investigated to determine their species identity.

Morphological characterization

Identified population groups based on molecular analyses were subjected to morphological examination. Thirty *M. incognita* (3 populations each from 10 localities), 20 *M. javanica* (2 populations each from the 10 localities) and 16 *M. arenaria* (2 populations each from seven localities and one each from Rakiraki and Korovou) were morphologically examined to verify the species identity and check for morphological variations. Female nematodes were dissected from infected tomato roots and processed for morphological examination as outlined in Shurtleff and Averre (2000). Juveniles were obtained by allowing eggs collected from a single egg mass to hatch in distilled water at room temperature (28-30°C) and male specimens when available were picked while dissecting roots to collect the females. Males were found only in some populations of *M. incognita* and *M. arenaria*. For morphometric examination female, juvenile, and male specimens were killed by adding hot (not boiling) FA fixative (Hooper *et al.*, 2005). The morphological features studied from the female specimens included body shape, the perineal pattern, stylet and measurements included body length, body width, a (body length/body width), stylet length, DGO (dorsal gland orifice), vulva length and vulva-anus distance. The juvenile body length, stylet length, tail length and hyline tail terminus and male body length, stylet length, distance to DGO, spicule length and gubernaculum length were measured to assist with the species identification.

The prepared slides were examined under a compound light microscope (Olympus BX51) attached with a digital camera (Q-imaging micropublisher 5.0 RTV) connected to a computer with Image-Pro Plus software (Version 6.1 Media cybernatics). The Image-Pro Plus software was used to take measurements from the image and take digital pictures from the slides of the

nematode specimen at 400x or 1000x magnification. All measurements were recorded and for each of the species, these data was summarized by calculating the average, range and standard error using Sigma plot Version 11. The molecular data, morphological observations and measurements were compared with the species descriptions (Cliff and Hirschmann, 1985; Eisenback, 1985; Karssen and Moens, 2006; Rammah and Hirschmann, 1990) to aid the species identifications.

RESULTS

Distribution and host associations

Meloidogyne incognita, *M. javanica*, and *M. arenaria* are widely distributed in agricultural areas throughout Viti Levu, Fiji. A total of 41% (n = 277) of the farms surveyed were infested with root-knot nematodes with 28% (n = 189) recorded after direct examination of plant roots on the farms and an additional 13% (n = 88) detected after bioassay of soil samples (Table 1). The highest incidence of the three *Meloidogyne* spp. identified in this study was recorded in Sigatoka, a major vegetable producing area in Fiji.

A total of 33 commonly grown crop plant species were found associated with root-knot nematodes in this study (Table 2). Based on direct examination, crops such as tomatoes (*Solanum lycopersicum* L.), okra (*Abelmoschus esculentus* (L.) Moench), beans (*Phaseolus vulgaris* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), pumpkin (*Cucurbita maxima* Duchesne), bele (*Abelmoschus manihot* (L.) Medik.), pawpaw (*Carica papaya* L.) and ginger (*Zingiber officinale* Roscoe) had greater frequency of severe root-knot nematode infections. Direct examination of infected root crops such as cassava (*Manihot esculenta* Crantz), yam (*Dioscorea esculenta* (Lour.) Burkill), dalo (*Colocasia esculenta* (L.) Schott) and sweetpotato (*Ipomoea batatas* (L.) Lam.) did not show evidence of distinct galls. Rather, infected plants exhibited signs of necrosis on storage tissues. Sugarcane, which is a major crop grown on the western side of Viti Levu (Sigatoka, Nadi, Lautoka, Ba, Tavua and Rakiraki) was also found infected with *M. incognita* and *M. javanica* (Table 2).

Nematode species identifications

Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949: A total of 154 populations collected from 10 different localities around Viti Levu (Table 1) were identified as *M. incognita* using the sequence characterized amplified region (SCAR) primers MIF/MIR (Meng *et al.*, 2004). These primers yielded a 1.0 kb PCR product (Fig. 2A). *M. incognita* was found associated with 27 species of crop plants (Table 2). The morphological identifications results were in agreement with the molecular species identification. The morphometric (data not shown) and morphological features of the Fijian *M. incognita* populations (females, males and juveniles) matched the descriptions given in literature (Eisenback, 1985; Karssen and Moens, 2006). Slight variations were observed in the perineal pattern morphology (Fig. 3A-G).

Meloidogyne javanica (Treub, 1885) Chitwood, 1949 was also distributed throughout Viti Levu and DNA extracted from 78 populations amplified with SCAR primers Fjav/Rjav yielding a characteristic 670 bp PCR product (Fig. 2B). *M. javanica* was found associated with 24 plant species (Table 2). Slight variations from the typical *M. javanica* perineal patterns included slightly higher than the typical dorsal arch which was more squarish than rounded (Fig. 3H-L). The morphological and morphometric characteristics (data not shown) of the Fijian *M. javanica* female and juvenile were in agreement with that of the species description (Eisenback, 1985; Rammah and Hirschmann, 1990;

Table 1. Distribution of root-knot nematodes in Fiji.[z]

Locality	No. of farms sampled	RKN incidence		Ma	Mj	Mi	Mi + Mj	M. spp.
		Direct exam	Bioassay					
1. Nausori	48	18	27	5	6	15	1	0
2. Suva-Nasinu	39	16	21	4	3	13	1	0
3. Navua	46	7	11	0	2	9	0	0
4. Sigatoka	92	43	62	6	17	36	3	0
5. Nadi	85	27	41	5	12	21	2	1
6. Lautoka	79	19	26	2	9	13	2	0
7. Ba	84	24	33	2	10	17	3	1
8. Tavua	73	11	20	2	8	9	0	1
9. Rakiraki	76	16	24	1	8	13	2	0
10. Korovou	53	8	12	1	3	8	0	0
Total	675	189	277	28	78	154	14	3

[z]Root-knot nematode species: Ma = *Meloidogyne arenaria*, Mi = *Meloidogyne incognita*, Mj = *Meloidogyne javanica,* M. spp. = Unidentified species. Nematodes used for species identifications were isolated from tomato cv. Moneymaker used in the field soil bioassay.

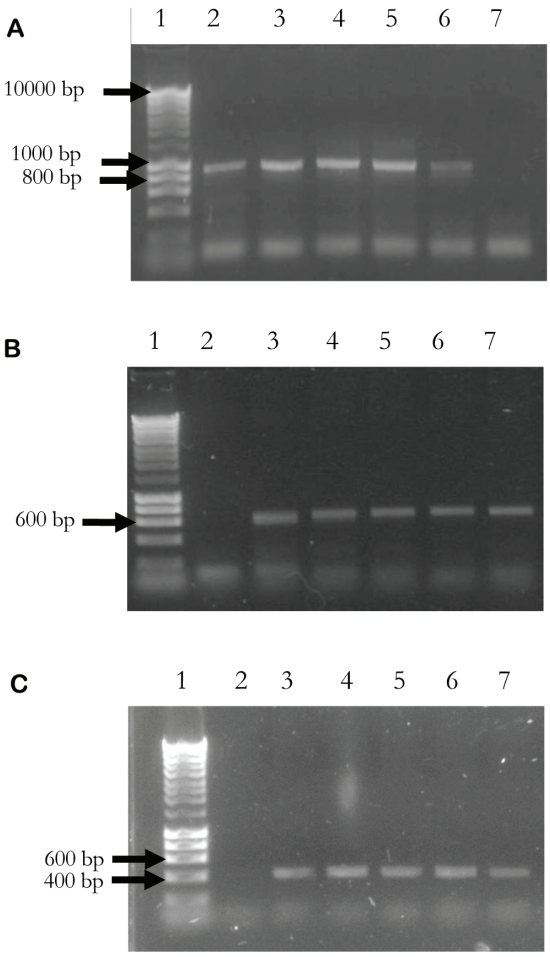

Fig. 2. Gel photographs of *M. incognita, M. javanica* and *M. arenaria* using SCAR primers. **A:** Lanes 1-10kb hyperladder, (2-6) *M. incognita* 1.0kb product using MIF/MIR, 7-Negative control; **B:** Lanes 1-10kb hyperladder, 2-Negative control, (3-7) *M. javanica* 670bp product using Fjav/Rjav; **C:** Lanes: 1-10kb hyperladder, 2-Negative control, (3-7) *M. arenaria* 420bp product using Far/Rar.

Karssen and Moens, 2006). Males of *M. javanica* were not found in this study but are reported in literature (Rammah and Hirschmann, 1990).

Mixed populations: Fourteen mixed populations consisting of *M. incognita* and *M. javanica* were recorded from seven localities. These populations were identified with both MIF/MIR and Fjav/Rjav SCAR primers. The populations were confirmed to have a mix of morphologically distinct females after examination of perineal patterns.

Meloidogyne arenaria (Neal, 1889) Chitwood, 1949 was identified in 9 of the 10 localities sampled but was less common than *M. incognita* and *M. javanica*. *Meloidogyne arenaria* was found associated with 12 plant species (Table 2). A total of 28 populations were identified using SCAR primers Far/Rar, yielding a PCR product of 420 bp characteristic of *M. arenaria* (Fig. 2C). The DNA isolated from *M. arenaria* populations did not amplify with MIF/MIR or Fjav/Rjav primers. The morphological and morphometric characteristics (data not shown) of *M. arenaria* were in agreement with those of the species descriptions (Cliff and Hirschmann, 1985; Eisenback, 1985; Karssen and Moens, 2006). Morphological variations observed in perineal patterns of some *M. arenaria* populations included less pronounced shoulders, a typical characteristic of *M. arenaria* and more coarse striations than the typical patterns. In some perineal patterns, there was slight wing formation (Fig. 3M-R).

Unidentified populations: Three species remain unidentified and further characterization of their morphology and genotypes is needed.

DISCUSSION

Occurrence of root-knot nematodes in Fiji has been reported previously (Orton Williams, 1980; Khurma *et al.*, 2008) but this is the first detailed report on their distribution and characterization. The current study reports more widespread incidence (41%) of *M. incognita*, *M. javanica M. arenaria* and at least three unidentified species compared to 29% incidence of *M. incognita*, *M. javanica* and *M. arenaria* from 185 farms on Viti Levu (Khurma *et al.*, 2008) and 31% incidence of *M. incognita*, *M. javanica* and *M. arenaria* from approximately 700 soil samples from nine islands (Orton Williams, 1980). The widespread distribution of *Meloidogyne* spp. in all of the 10 agricultural areas sampled in Fiji could have been due to sharing of farm implements and seedlings, common cultural practice in Fiji amongst farmers. Natural causes such as flooding and soil erosion also assist in the spread of root-knot nematodes which may partially explain the high incidence of root-knot nematodes in Sigatoka.

In this study, the incidence of root-knot nematodes determined upon direct examination (28%) was lower than the actual incidence of root-knot nematodes (41%) obtained after bioassay of soil samples. Although direct examination of plant roots for the presence of characteristic root galls is a relatively quick way of detecting root-knot nematodes in the soil, it may not totally reflect the presence or absence of root-knot nematodes especially when the plants do not exhibit root-galling, are resistant to root-knot nematodes, or when the population density in the soil is very low.

Nematode diagnostic services are not readily available to farmers in Fiji thus the soil bioassay method could be used as a simple, relatively inexpensive and effective method for general diagnosis of root-knot nematodes on the farms. The bioassay method can also be used to assess *Meloidogyne* infestations and

Table 2. *Meloidogyne* spp. found associated with crop plants and their distribution in Viti Levu, Fiji.[w]

Crop host	Common name	Root gall index range	*Meloidogyne* species [x]	Localities [y]
Abelmoschus esculentus (L.) Moench	Okra	2-5	Ma, Mj, Mi, Msp.3.	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Abelmoschus manihot (L.) Medik.	Sunset hibiscus or Bele	2-5	Ma, Mj, Mi	1, 2, 3, 4, 5, 6, 7, 8, 9
Amaranthus viridis L.	Slender amaranth [z]	2-5	Ma, Mj, Mi	2, 4, 6, 7, 8
Ananas comosus (L.) Merr.	Pineapple	2-3	Mj, Mi + Mj	1.4, 8
Arachis hypogaea L.	Peanut	2-3	Ma, Msp.1	1,5
Brassica oleracea L.	Cabbage	2-5	Mj, Mi	1, 2, 4, 5, 6, 7, 8, 10
Cajanus cajan (L.) Millsp.	Pigeon pea	2-3	Mj	6
Capsicum annuum L.	Chillies	2-5	Mj, Mi, Mi + Mj	1, 2, 4, 6, 7, 10
Carica papaya L.	Papaya	2-5	Ma, Mi, Mi + Mj	1, 2, 4, 6, 8, 9, 10
Citrullus lanatus (Thunb.) Matsum. & Nakai	Watermelon	2-5	Mi	4, 8, 9
Colocasia esculenta (L.) Schott.	Coco yam or Dalo	2-3	Mi, Mi + Mj	1, 7, 8
Coriandrum sativum L.	Coriander	2-5	Mi, Mi + Mj	2, 4, 6, 7, 8, 9
Cucumis sativus L.	Garden cucumber	2-5	Ma, Mj, Mi	1, 4, 6, 9
Cucurbita maxima Duchense.	Winter squash	2-5	Ma, Mj, Mi	1, 4, 5, 6, 7, 8, 9, 10
Dioscorea esculenta (Lour.) Burkill.	Yam	2-3	Mi	5, 8, 9
Ipomoea batatas (L.) Lam.	Sweetpotato	2-5	Mj, Mi	1, 3, 4, 10
Lactuca sativa L.	Garden lettuce	2-5	Mi	2, 6
Lagenaria siceraria (Molina) Standl.	Bottle gourd	2-5	Mi	6
Luffa acutangula (L.) Roxb.	Sinkwa towelsponge	2-5	Mj	5
Manihot esculenta Crantz	Cassava	2-5	Mj, Mi	4, 5, 7, 8, 9
Momordica charantia L.	Balsampear	2-3	Mj	5, 7
Musa acuminata Colla.	Edible banana	2-3	Mi	4, 8, 9
Nicotiana tabacum L.	Cultivated tobacco	2-5	Ma, Mi	4
Phaseolus vulgaris L.	Kidney bean	2-5	Ma, Mj, Mi,	1, 2, 4, 5, 6, 7, 8, 9, 10
Piper methysticum G. Forst.	Kava	2-5	Mi	9
Raphanus sativus L.	Cultivated radish	2-5	Ma	4, 7

Table 2. *Meloidogyne* spp. found associated with crop plants and their distribution in Viti Levu, Fiji.[w] (cont.)

Crop host	Common name	Root gall index range	*Meloidogyne* species [x]	Localities [y]
Saccharum officinarum L.	Sugarcane	2-3	Mj, Mi, Mi + Mj	4, 5, 6, 7, 8, 9
Solanum melongena L.	Eggplant	2-5	Ma, Mj, Mi, Msp.2,	1, 2, 3, 4, 5, 6, 7, 8, 9
Solanum lycopersicum L.	Garden tomato	2-5	Ma, Mj, Mi	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Vigna radiata (L.) R. Wilczek.	Mung bean	2-5	Mj	5, 8
Vigna unguiculata (L) Walp	Yardlong bean	2-5	Mj, Mi, Mi + Mj	2, 3, 4, 5, 6, 7, 9
Zea mays L.	Corn	2-3	Mj, Mi	4, 5, 8
Zingiber officinale Roscoe.	Garden ginger	2-5	Mi	1, 3, 10

[w] The list only includes crops from fields found infected on direct examination.
[x] Root-knot nematode species: Ma = *Meloidogyne arenaria*, Mi = *Meloidogyne incognita*, Mj = *Meloidogyne javanica*; M. sp.1, M. sp.2, and M. sp.3 = Unidentified species; Mi + Mj = mixed populations. Nematodes used for species identifications were isolated from tomato cv. Moneymaker used in the field soil bioassay
[y] Localities number: refer to Table 1
[z] Slender amaranth is also a weed which is often allowed to grow on farms because of its alternative use as a vegetable.

as a decision management tool on farms (Gugino *et al.*, 2008). However, a possible limitation of bioassay method is that species existing in a mixture and which do not infect the bioassay host, could escape detection.

Our findings also confirm the trend of world-wide distribution of the three commonly occurring root-knot species. *Meloidogyne incognita* was the most prevalent, occurring on 56% of the infected farms followed by *M. javanica* on 28% and *M. arenaria* on 10%. Mixed populations on 5% of the infected farms consisted of *M. incognita* and *M. javanica*. Only 1% of sampled populations remains unidentified at species level and requires further investigation. This study highlights and supports the importance of an integrated approach combining molecular and morphological characteristics for accurate identification and diagnosis of *Meloidogyne* spp. A distinct advantage of a combined approach is that it allows making use of only key morphological features to identify the populations, particularly of known species.

After discussions with the farmers, during the farm visits, it came to light that a large number of farmers were not aware of nematode problems and the damage that nematodes can cause to crops. Further work is recommended on genus *Meloidogyne* to quantify the losses, create greater awareness and implement appropriate nematode management strategies in Fiji.

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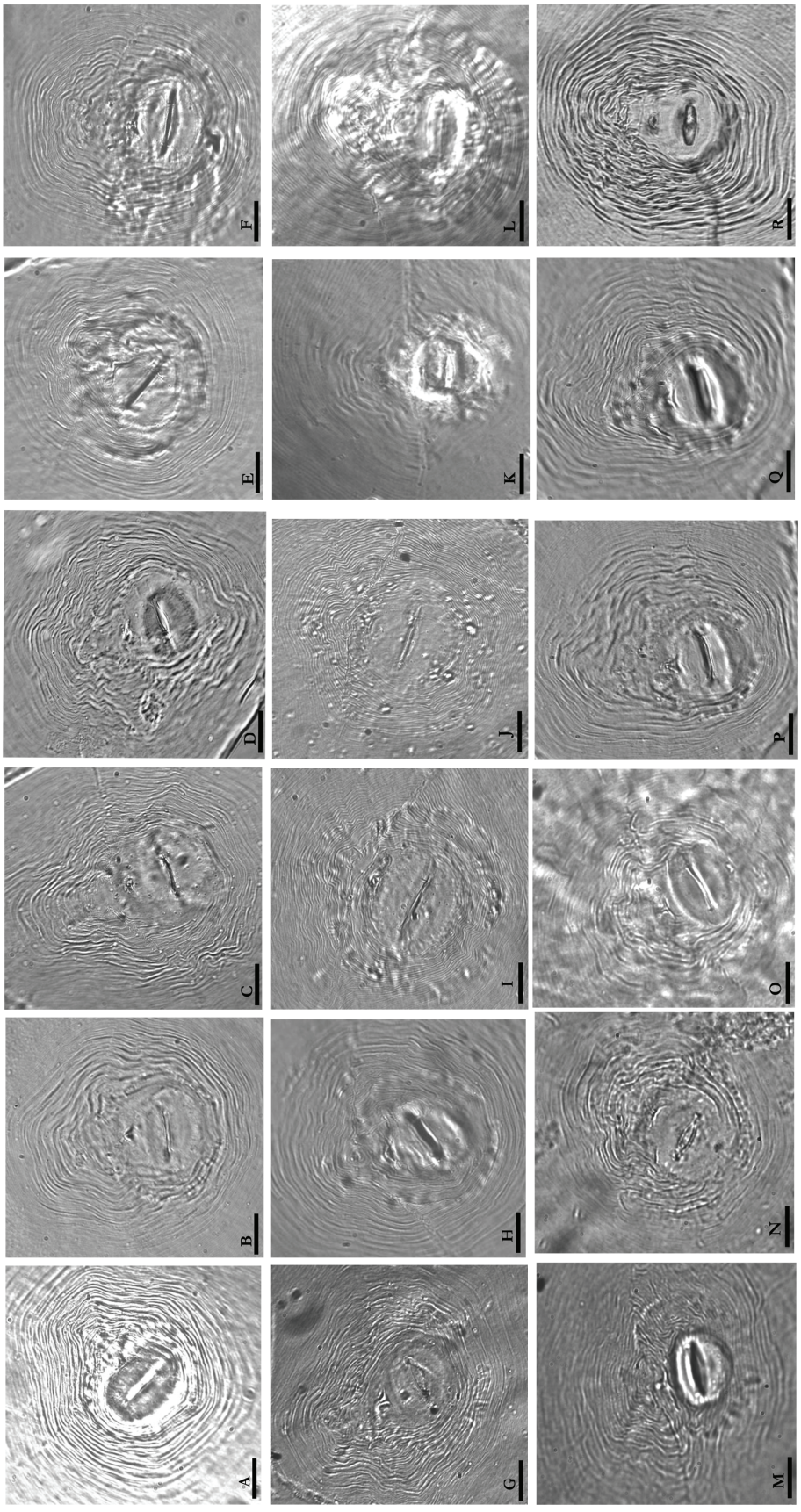

Fig. 3. LM photographs of *M. incognita*, *M. javanica* and *M. arenaria* perineal patterns A- C: typical *M. incognita*, D - G: *M. incognita* variations, H: typical *M. javanica*, I-J: typical *M. javanica,* K-L: *M. javanica* variations, M-O: typical *M. arenaria*, P-R: *M. arenaria* variations, Scale bar = 24 µm

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