MORPHOLOGICAL, ENZYMATIC AND MOLECULAR CHARACTERIZATION OF *MELOIDOGYNE ARENARIA* FROM *ANUBIAS BARTERI* VAR. *CALADIITOLIA* IN CHINA

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ABSTRACT

Huang, J., Y. Zeng, and X. Chen. 2013. Morphological, enzymatic and molecular characterization of *Meloidogyne arenaria* from *Anubias barteri* var. *caladiitolia* in China. Nematropica 43:261-270.

Studies were conducted to characterize morphological, enzymatic and molecular profiles of *Meloidogyne arenaria* from *Anubias barteri* var. *caladiitolia* in China. This nematode population can cause visible galls on the roots of the infected plants and the female vulval region was typically surrounded by an egg mass. Most morphometrics of the population fit within the ranges reported for *M. arenaria*. The isozyme phenotypes for esterase and malate dehydrogenase and a single 420 bp-band produced by sequencing characterized amplified regions marker with species-specific primers Far/Rar matched those reported for *M. arenaria*. The sequences of partial 18S small subunit rRNA gene and 28S D2/D3 expansion segment all share identities of 99% with other *M. arenaria* population (AY942623), *M. javanica* (AY942626) and *M. incognita* (JQ806343).

Key words: Anubias barteri, esterase, malate dehydrogenase, 18S small subunit rRNA, 28S large subunit rRNA, root-knot nematode, taxonomy.

RESUMEN

Huang, J., Y. Zeng, and X. Chen. 2013. Caracterización morfológica, enzimática y molecular de *Meloidogyne arenaria* procedente de *Anubias barteri* var. *caladiitolia* en China. Nematropica 43:261-270.

Se llevaron a cabo estudios para caracterizar morfológica, enzimática y molecularmente una población de *Meloidogyne arenaria* procedente de *Anubias barteri* var. *caladiitolia* en China. Este nematodo puede causar agallas visibles en la raíces de plantas infectadas y la región de la vulva en las hembras estaba típicamente cubierta por una masa de huevos. La mayoría de las medidas morfométricas de la población se encontraron dentro del rango citado para *M. arenaria*. Los fenotipos de isozimas para la esterasa y malato-deshidrogenasa y una banda sencilla de 420 bp producida al sequenciar las regiones amplificadas características del marcador con los cebadores específicos Far/Rar se ajustaron a aquellas citadas para *M. arenaria*. Las secuencias parciales de los genes de la subunidad pequeña 18S del rRNA y del segmento de expansión 28S D2/D3 compartieron la identidad en 99% con otras poblaciones de *M. arenaria* (AY942623), *M. javanica* (AY942626) and *M. incognita* (JQ806343).

Palabras clave: Anubias barteri, esterasa, malato deshidrogenasa, 18S subunidad pequeña rRNA 18S, subunidad grande rRNA 28S, nematodo de las agallas radiculares, taxonomóa.

INTRODUCTION

Anubias barteri Schott, found in rivers, streams, and marshes, is the most common species in the genus *Anubias* which originates from tropical central and western Africa. *Anubias barteri* contains several varieties differing in leaf forms and overall sizes. *Anubias barteri* var. *caladiitolia* Engler, a common aquatic ornamental plant, is a variety with relatively large size and heart-shaped leaves. Recently *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 infesting roots of *A. barteri* was reported by Xu *et al.* (2012), only second-stage juveniles (J2) were found when the plants were detected, and also no galls were observed. However, small galls (size of mung bean or smaller) were observed in *A. barteri* var. *caladiitolia* roots dwarfed plants with yellowing leaves during a diversity survey of nematodes associated with aquatic ornamental plants growing in an artificial soil mixture from 2012 to 2013 in Guangzhou, China. Galls were collected for isolation and identification of potential pathogen(s). A root-knot nematode (RKN) was isolated from the galls that were opened with a scalpel and placed in distilled water, and species identification was

performed in this study.

RKNs (*Meloidogyne* spp.) are major agricultural pests of a wide range of crops. Ninety five nominal species, including four major [*M. incognita* (Kofoid and White, 1919) Chitwood, 1949; *M. arenaria* (Neal, 1889) Chitwood, 1949, *M. javanica* (Treub, 1885) Chitwood, 1949; and *M. hapla* Chitwood, 1949], have so far been described (Skantar *et al*., 2008). Typically, morphological, isozymatic and molecular characters are collectively used for identification of RKN species. Perineal pattern of adult female of RKN is a morphologically important character. Isozyme analysis continues to be an effective mean to discriminate many RKN species (Esbenshade and Triantaphyllou, 1990; Carneiro, 2000). DNA sequencing has provided data useful for species identification and phylogenetic analysis. DNA markers that have been employed to identify *Meloidogyne* species include ribosomal DNA small subunit (SSU) (Powers, 2004), large subunit (LSU) D2/D3 expansion segments (Chen *et al*., 2003; Palomares Ruis *et al.*, 2007), internal transcribed spacer (Powers and Harris, 1993), mitochondrial DNA (Jeyaprakash *et al.*, 2006), and sequence characterized amplified regions (SCAR) (Zijlstra *et al*., 2000; Randig *et al*., 2002).

In this study, we presented symptoms, reproductive characterization, morphological and isozymatic features, SCAR marker analysis characteristics of the RKN from *Anubias barteri* var. *caladiitolia*, and its relationship to other RKNs based upon sequence analysis of the SSU and D2/D3.

MATERIAL AND METHODS

Morphological Characterization

Galls caused by the RKN were collected from infected roots of *A. barteri* var. *caladiitolia*. Adult females, egg masses, J2 and other juvenile stages, and males were kept in petri dishes at room temperature in a small amount of water. J2 and males were fixed in 3% formaldehyde and processed to glycerin by the formalin-glycerin method (Hooper, 1970; Golden, 1990). Specimen preparation and measurements were as described in Golden and Birchfield (1972). Perineal patterns of some females were cut and mounted in a clear lactophenol solution. Measurements of nematodes were performed with the aid of a camera lucida and a stage micrometer. The morphometric data were processed using Excel software (Ye, 1996). Photomicrographs were taken with a Leica video camera (DFC490) attached via a C-mount Adapter fitted on a Leica microscope (DM4000B), and edited using Adobe Photoshop 7.0.

Enzymatic Analysis

To determine the esterase (EST) and malate dehydrogenase (MDH) phenotypes, ten *Meloidogyne arenaria* young females from *A. barteri* var. *caladiitolia* were isolated from the galls under dissecting microscope. Each female was placed in sample well containing 0.5 µl of extraction buffer (20% sucrose, 2% Triton X-100, 0.01% Bromphenol blue) (Esbenshade and Triantaphyllou, 1985) and squashed to release body content, then subjected to 12% polyacrylamide gel electrophoresis. *M. javanica* was used as a reference species. Electrophoresis was performed using an electrophoresis apparatus (BioRad, Hercules, CA) (Brito *et al*., 2004). After electrophoresis, gel was stained for enzymatic activity with different staining solutions. Staining solution for MDH contained 0.05 g β-NAD, 0.03 g Nitro Blue Tetrazolium, 0.02 g Phenazine Methosulfate, 50.0 ml 0.5 M Tris pH 7.1 and 7.5 ml stock (10.6g Na_2CO_3 + 1.34 g L-malic acid in 100 ml water) dissolved in 70 ml of reagent-grade water. EST staining solution contained 100 ml 0.1 M Phosphate buffer pH 7.3, 0.06g Fast Blue RR salt, 0.03 g EDTA and 0.04 g α-Naphthyl acetate dissolved in 2 ml acetone (Karsen *et al*., 1995). After incubation in a Petri dish at 37ºC for 5 minutes for MDH, the gel was washed twice with distilled water and stained for EST activity for 30 min, then fixed for 5 minutes in a solution of 10% acetic acid, 10% glycerol and 80% distilled water (Muturi *et al*., 2010). Photographs of gels were taken with a digital camera (Nikon D300S) and edited using Adobe Photoshop 7.0.

Molecular Characterization

Five RKN females or males were handpicked into distilled water for DNA extraction, amplification, and sequencing. They were placed into 50 µl of worm lysis buffer (WLB) containing Proteinase K for DNA extraction (Williams *et al*., 1992). DNA samples were stored at –20°C until used as a PCR template.

Primers for LSU amplification were forward primer D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and reverse primer D3B (5'-TGCGAAGGAACCAGCTACTA-3') (Nunn, 1992). Primers for SSU amplification were forward primer 18S965 (5'-GGCGATCAGATACCGCCCTAGTT-3') and reverse primer 18S1573R (5'-TACAAAGGGCAGGGACGTAAT-3') (Mullin *et al*., 2005). Species-specific primers for *M. arenaria* identification were forward primer Far (5'-TCGGCGATAGAGGTAAATGAC-3') and reverse primer Rar (5'-TCGGCGATAGACACTACAAACT-3') (Zijlstra *et al*., 2000). The 25 µl PCR was performed using TaqMix DNA polymerase (Guangzhou Dongsheng Biotech Ltd., Guangzhou, China) according to the manufacturer's protocol. The thermal cycler program for PCR was as follows: denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94°C with 30 s; annealing at 55°C for 45 s, and extension at 72°C for 2 min. A final extension was performed at 72°C for 10 min (Ye *et al*., 2007). PCR products were cleaned using an EZ Spin Column DNA Gel Extraction Kit (Bio

Basic Inc., Markham, Ontario, Canada) according to the manufacturer's protocol before being sequenced by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China) using an ABI PRISM 3730 sequencing system.

New sequences were submitted to GenBank under accession numbers KF112872 and KF112873. DNA sequences were assembled using Clustal_X 1.83 (Genecodes, Ann Arbor, MI) and analyzed using the BLASTN megablast program optimized for highly similar sequences (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Similar sequences were downloaded from GenBank and aligned with Clustal_X software (Thompson *et al*., 1997). A phylogenetic tree was constructed using PHYLIP (version 3.573, J. Q. Felsenstein, Department of Genetics, University of Washington, Seattle, WA [http://evolution.genetics.washington.edu/phylip.html]) with *Tylenchus arcuatus* used as a SSU outgroup, and *Pratylenchus vulnus* as a D2/D3 outgroup in the dendrograms. The branching order of the neighbor-joining dendrograms was evaluated with 1000 bootstrap analyses by using the SEQBOOT program in the PHYLIP software package (Felsenstein, 1985).

RESULTS

Symptoms and Reproductive Characterization

RKN feeding caused small galls or "knots" and slight swellings in the roots of infected plants (Fig. 1). Galls were irregularly shaped, variable in size, but smaller than mung bean, and located at some distance from the root tip. Serious infections resulted in formation of large numbers of galls or merging of galls, and even root rot. While the severe damage occurs below ground, symptoms such as leaf yellowing and plant stunting similar to nutrient deficiency and plant stunting were also observed above ground (Fig. 1). Eggs were found in gelatinous masses on the surface

Table 1. Morphometrics of male, female and J2 of *Meloidogyne arenaria* mounted in formalin-glycerin. All measurements in µm and in the format: mean ± s.d. (Range).

Character	Male	Female	J2
n	10	10	10
L	1545.9 ± 189.4 (1388.6 - 1756.2)	112.8 ± 17.9 (78.0 - 131.0)	450.0 ± 26.5 (430.0 - 480.0)
a	38.3 ± 7.8 (31.7 - 47.1)		29.3 ± 2.3 (26.9 - 31.4)
b1	17.9 ± 3.1 (15.3 - 21.4)		7.3 ± 0.2 (7.0 - 7.5)
c	189.3 ± 41.5 (156.0 - 237.3)		8.3 ± 0.5 (8.0 - 8.6)
c'	0.3 ± 0.0 (0.3 - 0.3)		6.5 ± 0.4 (6.3 - 6.7)
Body diam. (greatest body diam.)	40.4 ± 3.3 (37.3 - 43.8)		15.3 ± 1.2 (14.0 - 16.0)
Stylet length	21.2 ± 2.5 (18.8 - 23.7)	16.1 ± 1.1 (15.1 - 17.2)	12.5 ± 0.8 (11.0 - 13.0)
DGO	5.0 ± 0.3 (4.7 - 5.3)	5.9 ± 0.5 (5.0 - 6.7)	4.3 ± 0.2 (4.1 - 4.5)
Pharynx length (head to metacorpus center)	86.3 ± 4.4 (81.9 - 90.7)		61.7 ± 2.1 (60.0 - 64.0)
Spicule length*	32.5 ± 1.2 (31.3 - 33.6)		-
Anal body diam. (males=cloacal body diam.)	25.3 ± 2.5 (23.0 - 28.0)		8.7 ± 0.6 (8.0 - 9.0)
Tail length	8.2 ± 0.8 (7.4 - 8.9)		54.1 ± 2.1 (50.0 - 60.0)
Excretory pore from anterior end			88.7 ± 1.2 (88.0 - 90.0)

* the distance between the condylus and the posterior-most point of the lamina measured in a straight line

of the galls. Newly produced egg masses were hyaline and turned brown with age. The average number of J2 that hatched from each egg mass was 210 per egg mass.

Morphological Description

Female vulval region was typically surrounded by an egg mass. Morphometrics of males, females and J2 of this population are presented in Table 1. Most measurements of this population lie within the ranges previously reported for *M. arenaria* (Eisenback *et al*., 1981).

Females

The body is pear-shaped, 78-131 µm in length and 32-67 µm in width. The stylet is 15-17 µm long and with large, posteriorly sloping, tear-drop-shaped or rounded knobs. The distance from the dorsal pharyngeal gland orifice to the base of the stylet (DGO) is 5.3-6.7 µm. The perineal pattern contains two wings that extend laterally and are marked by fusion of the striae in the dorsal and ventral arches. The perineal pattern is round to hexagonal, and has a relatively high, rounded dorsal arch, with striae that range from smooth to wavy, these striae become forked and the distance between them increases near the lateral areas which are often demarcated, but not delineated by distinct lateral incisures.

Males

Males are 1389-1756 µm long. The labial disc and medial lips form a smooth, posteriorly sloping head cap. The head annulus is smooth and is usually not marked with additional head annulations. Both the head annulus and the body annulations are in the same contour. The male head is low rounded. The stylet is 18.8-23.7 µm long and robust with a bluntly pointed tip. The wide, cylindrical shaft gradually merges with the large, rounded backwardly sloping knobs. The DGO is 4.7-5.3 µm.

Second-Stage Juveniles (J2)

J2s are 430-480 µm long. The tail length ranges between 50 and 60 µm. The poorly defined hyaline tail terminus is 10-13 µm and has a finely round tip. The stylet is 11-13 µm long, and the DGO is 4-5 µm.

Enzymatic Analysis

J2s are 430-480 µm long. The tail length ranges between 50 and 60 µm. The poorly defined hyaline tail terminus is 10-13 µm and has a finely round tip. The stylet is 11-13 µm long, and the DGO is 4-5 µm.

SCAR Marker Analysis

A single band of 420 bp was produced through PCR by *M. arenaria*-specific primers Far/Rar (Fig. 4).

Molecular Phylogenetic Relationships

Partial SSU and the LSU D2/D3 expansion segment were sequenced for relative phylogenetic placement of *Meloidogyne arenaria* from *Anubias barteri* var. *caladiitolia* population among other *Meloidogyne* species sequenced using the same loci. The dendrogram inferred from SSU (Fig. 5) using *Tylenchus arcuatus* as an outgroup suggested that: *i*) RKN population from *A. barteri* var. *caladiitolia* is in a highly supported clade with other two other populations of *M. arenaria* (*M.* cf. *arenaria* AY757832, *M.* cf. *arenaria* AY757833), *M. incognita* and *M. javanica* with 90% support; *ii*) RKN population from *A. barteri* var. *caladiitolia* is clearly different from *M. hapla*, *M. marylandi*, *M. graminis, M. chitwoodi*, *M. minor* and *M. naasi*.

The tree inferred from D2/D3 of LSU (Fig. 6) using *Pratylenchus vulnus* suggested that: *i*) RKN population from *A. barteri* var. *caladiitolia* is in a highly supported clade with other five populations of *M. arenaria* (AF435803, U42342, EU363889, JQ317918 and JQ317919), *M. javanica* and *M. incognita* with 98% support; *ii*) The clade comprised of *M. arenaria*, *M. javanica* and *M. incognita* was closer to *M. marylandi*, *M. graminis* than to the other three species of *Meloidogyne* (*M. chitwoodi*, *M. minor* and *M. naasi*).

DISCUSSION

Morphological variation which makes species difficult to discriminate, is usually produced in several RKN species such as *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, including the variation of female perineal patterns that are thought as a valuble morphological feature for species identification (Karssen and van Aelst, 2001). García and Sánchez-Puerta (2012) also reported possessing a high dorsal arch in 38% of 100 female perineal patterns of *M. arenaria* race 2 from tomato plants. Female perineal pattern of the RKN population in this study has a relatively high rounded dorsal arch, this is a little similar to that of *M. incognita*. However, enzymatic analysis and SCAR marker analysis indicated that its EST (A2 type) and MDH (N1 type) isozyme patters are identical for *M. arenaria* and a single *M. arenaria*-specific band of 420 bp was produced through PCR by *M. arenaria*-specific primers Far/Rar. In addition, most measurements of this population fit well with the previous description for *M. arenaria* by Eisenback *et al.* (1981). Thus, this RKN population from the galls on roots of *A. barteri* var. *caladiitolia* was identified as *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949.

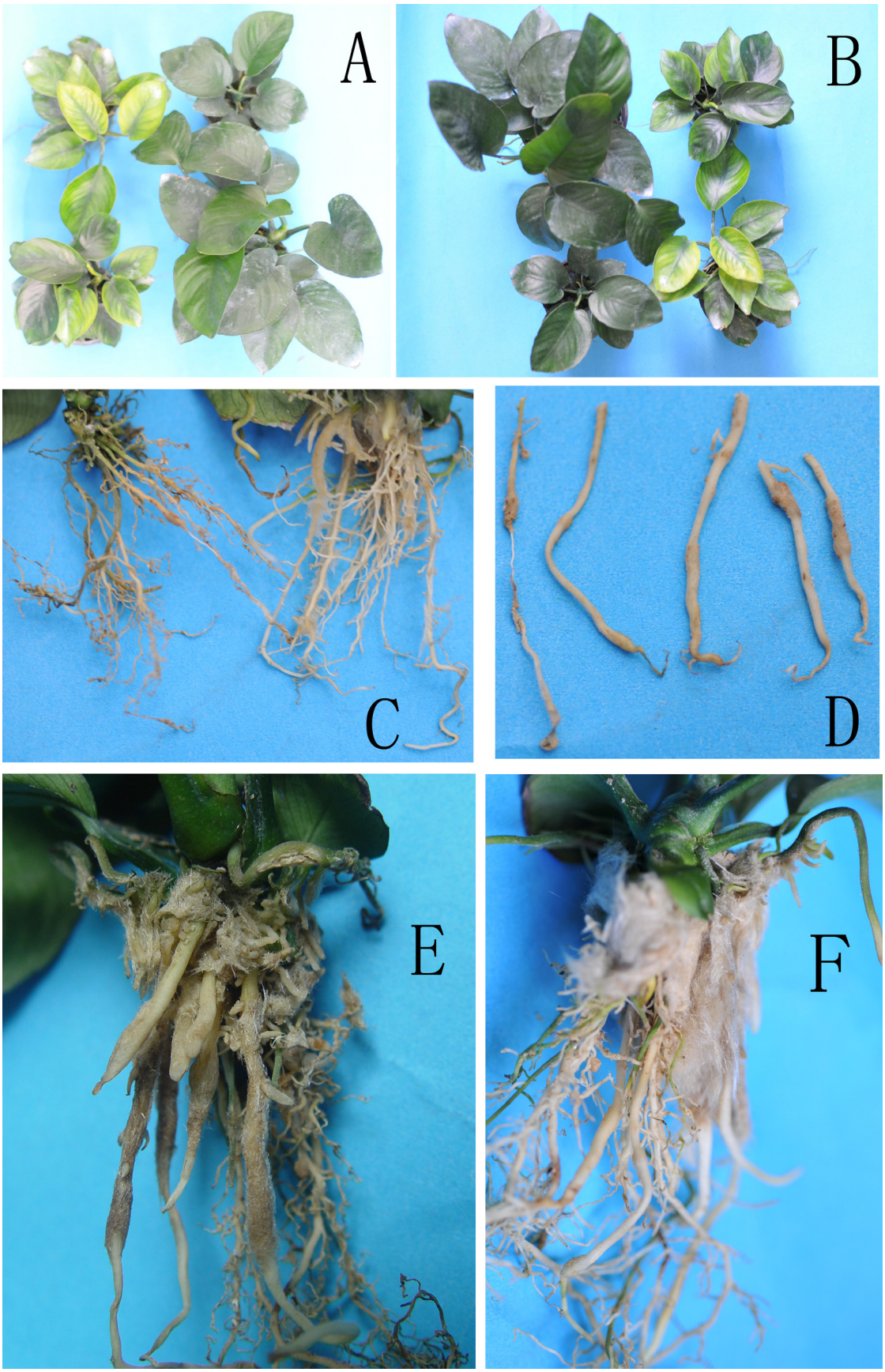

Fig. 1. Root-knot nematode damage to *Anubias barteri* var. *caladiitolia*. A and B: yellowing plants; C-F: diseased roots with galls.

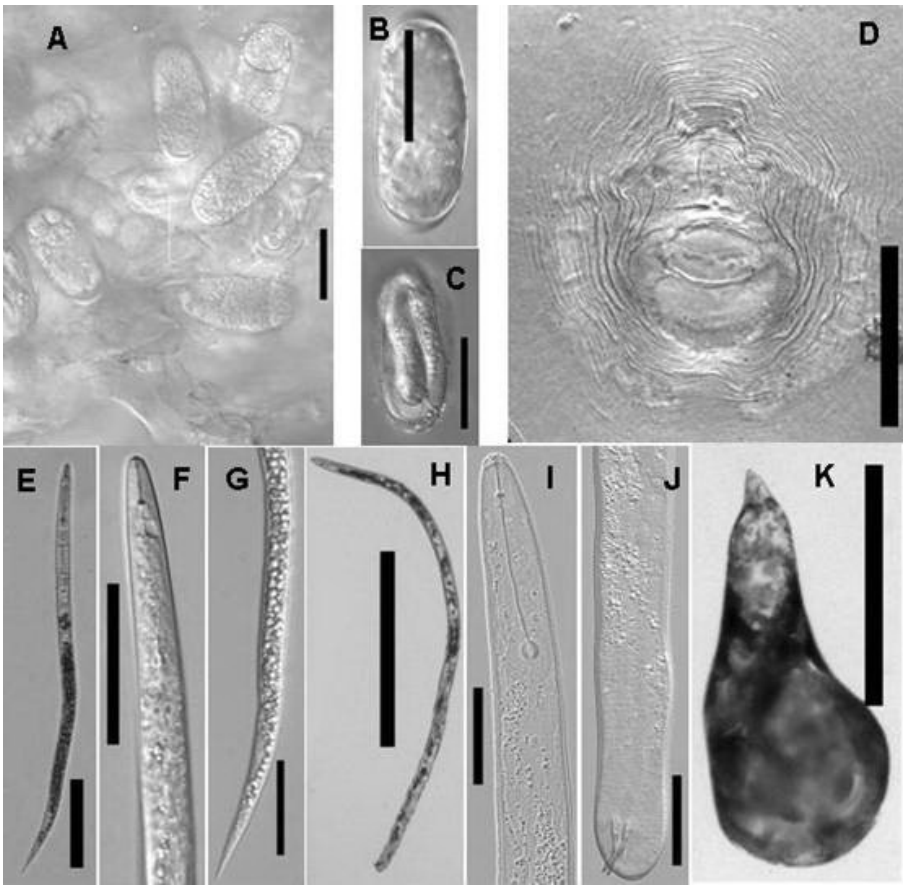

Fig. 2. Light micrographs of egg, J1, J2, male and female and perineal pattern of *Meloidogyne arenaria* from *Anubias barteri* var. *caladiitolia*. A: eeg mass; B: egg; C: J1 in egg; D: perineal pattern; E: Entire body of J2; F: Anterior body of J2; G: Tail of J2; H: Entire body of male; I: Anterior body of male; J: Tail of male; K: Entire body of female. Scale bars: A,B,C,F,G,I,J = 50 µm; D,E,K = 100µm; H = 500µm.

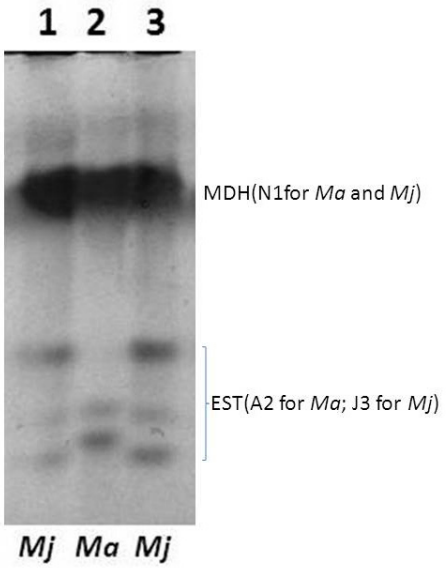

Fig. 3. Esterase and malate dehydrogenase isozyme patters for *Meloidogyne arenaria* from *Anubias barteri* var. *caladiitolia*. Lanes1 and 3: *M. javanica*; Lane 2: *M. arenaria* from *Anubias barteri* var. *caladiitolia*.

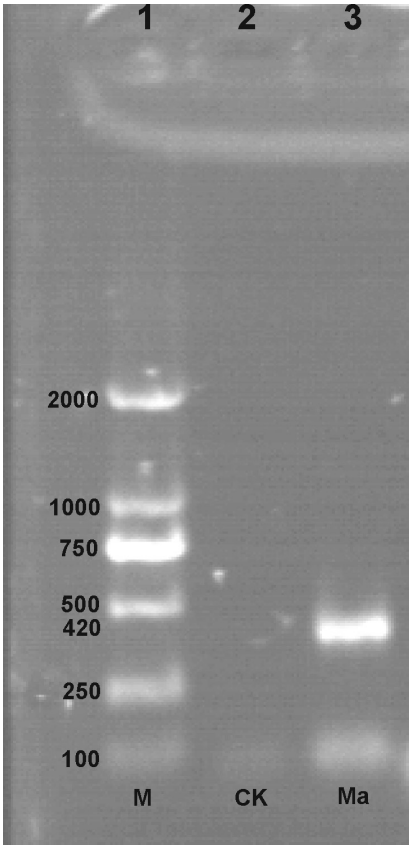

Fig. 4. PCR product with the *Meloidogyne arenaria* species-specific Far-Rar primers. M: DNA Ladder, Lane 2: CK (water); Lane 3: Study sample.

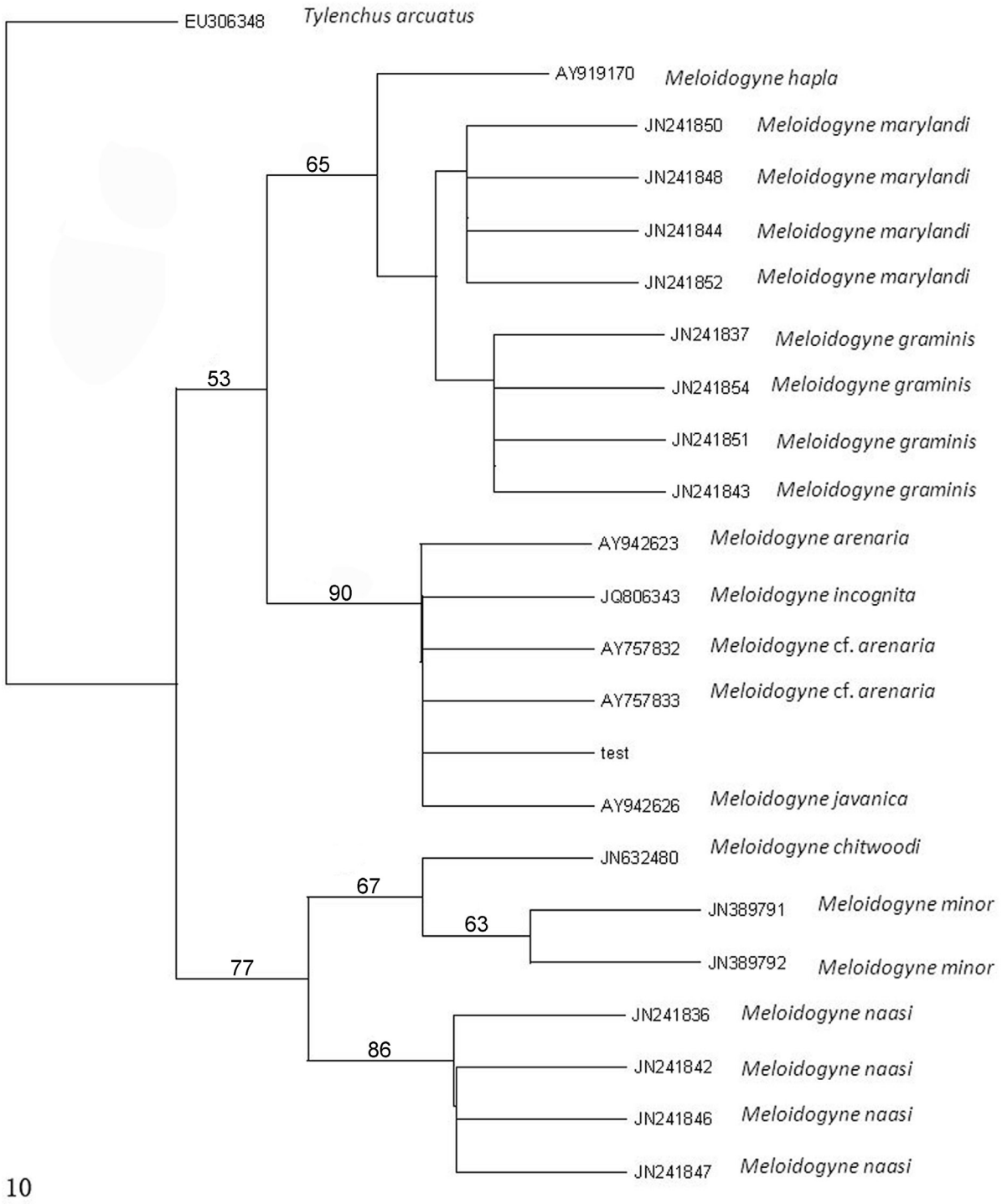

Fig. 5. A consensus tree was generated based on the 18S gene sequences of 22 taxa of *Meloidogyne*, rooted with *Tylenchus arcuatus* with Clustal W. The numbers at branches indicated the percentages from 1000 bootstrap replications in which the branch occurred.

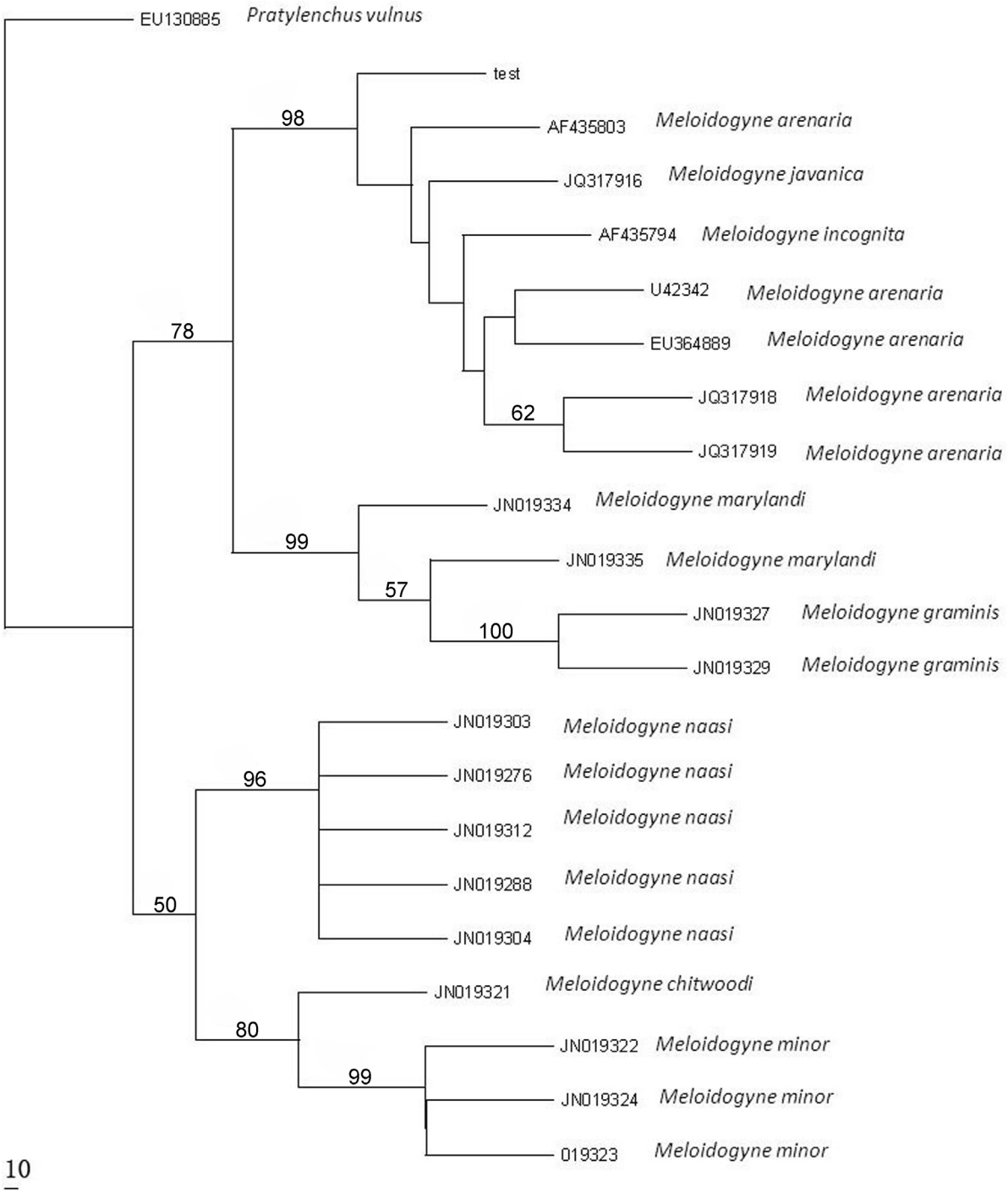

Fig. 6. A consensus tree was generated based on the D2/D3 gene sequences of 21 taxa of *Meloidogyne*, rooted with *Pratylenchus vulnus* with Clustal W. The numbers at branches indicated the percentages from 1000 bootstrap replications in which the branch occurred.

Meloidogyne arenaria has an extensive host range. It can infect peanut, vegetables, grasses, fruit ornamentals and tobacco (Ferris, 1999). While most frequent hosts of this nematode species are vegetables, fruit trees, tobacco, grapevine, and weeds (López-Pérez *et al*., 2011). Xu *et al.* (2012) confirmed that *Anubias barteri* is a host of *M. arenaria* by artificial inoculation. In this study we observed that *M. arenaria* naturally infesting *A. barteri* var. *caladiitolia* can lay eggs that formed gelatinous masses on the surface of the galls, and numerous J2 were hatched out. This population of *M. arenaria* can cause galls and swellings even root rot in the roots of infected plants, and leaf yellowing above ground while severe damage occurs, indicating *M. arenaria* is of economic importance to *A. barteri* production and related trade.

Identification of *Meloidogyne* species is challenging due to high intraspecific and interspecific variations (Skantar *et al*., 2008). DNA sequence analysis of the population of *M. arenaria* from *A. barteri* var. *caladiitolia* indicated that the SSU rRNA, even LSU D2/D3 expansion segments are relatively conserved. This increases difficulty for species demarcation, especially for new *Meloidogyne* species and populations. Accurate diagnosis requires a wide range of morphological and molecular characters such as scanning electron microscope (SEM) for detailed morphology as well as species-specific primer detection or SCAR marker, and enzymatic analysis as presented in this study. These additional characters and molecular markers will help to discriminate *Meloidogyne* species as well as examine their evolutionary relationships.

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LITERATURE CITED

Brito, J., T. O. Powers, P. G. Mullin, R. N. Inserra, and D. W. Dickson. 2004. Morphological and molecular characterization of *Meloidogyne mayaguensis* isolates from Florida. Journal of Nematology 36:232-240.

Carneiro, R. M. D. G. 2000. Enzyme phenotypes of *Meloidogyne* spp. populations. Nematology 2:645-654.

Chen, P., P. A. Roberts, A. E. Metcalf, and B. C. Hyman. 2003. Nucleotide substitution patterning within the *Meloidogyn*e rDNA D3 region and its evolutionary implications. Journal of Nematology 35:404-410.

Eisenback, J. D., H. Hirschmann, J. N. Sasser, and A. C. Triantaphyllou. 1981. A Guide to the Four Most Common Species of Root-Knot Nematodes, (*Meloidogyne* species) with a pictorial key. A Coop. Publ. Depts. Plant Pathol. and Genetics and U.S. Agency for International Development, Raleigh, NC.

Esbenshade, P. R., and A. C. Triantaphyllou. 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. Journal of Nematology 17:6-20.

Esbenshade, P. R., and A. C. Triantaphyllou. 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. Journal of Nematology 22:10-15.

Eisenback, J. D., and H. H. Triantaphyllou. 1991. Root-knot nematodes: *Meloidogyne* species and races. Pp 191- 274. *in*: W. R. Nickle, ed. Manual of Agricultural Nematology, Marcel Dekker, Inc. New York.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Ferris, H. 1999. http://plpnemweb.ucdavis.edu/nemaplex/taxadata/G076S1.htm.

García, L. E., and M. V. Sánchez-Puerta. 2012. Characterization of a root-knot nematode population of *Meloidogyne arenaria* from Tupungato (Mendoza, Argentina). Journal of Nematology 44:291-301.

Golden, A. M., and W. Birchfield. 1972. *Heterodera graminophila* n. sp. (Nematoda: Heteroderidae) from grass with key to closely related species. Journal of Nematology 4:147-154.

Golden, A. M. 1990. Preparation and mounting nematodes for microscopic observation. Pp. 197-205. *in*: Zuckerman, B. M., Mai, W. F., Krusberg, L. R., editors. Plant Nematology Laboratory Manual. Amherst, MA: University of Massachusetts Agricultural Experiment Station.

Hooper, D. J. 1970. Handling, fixing, staining and mounting nematodes. Pp. 39-54. *in*: Southey J. F., editor. Laboratory methods for work with plant and soil nematodes. 5th edition. London: Her Majesty's Stationery Office.

Jeyaprakash, A., M. S. Tigano, J. Brito, R. M. D. G. Carneiro, and D. W. Dickson. 2006. Differentiation of *Meloidogyne floridensis* from *M. arenaria* using high-fidelity PCR amplified mitochondrial AT-rich sequences. Nematropica 36:1-12.

Karssen G., and A. van Aelst. 2001. Root-knot nematode perineal pattern development: a reconsideration. Nemalology 3:95-111.

Karssen, G., T. van Hoenselaar, B. Verkerk-Bakker, and R. Janssen. 1995. Species identification of cyst and root-knot nematodes from potato by electrophoresis of individual females. Electrophoresis 16:105-9.

López-Pérez, J. A., M. Escuer, M. A. Díez-Rojo, L. Robertson, A. P. Buena, J. López-Cepero, and A. Bello. 2011. Host range of *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Nematoda: Meloidogynidae) in Spain. Nematropica 41:130-140.

Mullin, P. G., T. S. Harris, and T. O. Powers. 2005.

Phylogenetic relationships of Nygolaimina and Dorylaimina (Nematoda: Dorylaimida) inferred from small subunit ribosomal DNA sequences. Nematology 7:59-79.

Muturi, J., C. Gichuki, J. W. Waceke, and S. M. Runo. 2010. Use of isoenzyme phenotypes to characterise the major rootknot nematodes (*Meloidogyne* spp.) parasiting indigenous leafy vegetables in Kisii. *In*: Transforming agriculture for improved livelihoods through agricultural value Proceedings of the 12th Kari biennial scientific conference, 8 – 12 November 2010, Kari Headquarters, Kaptagat Road, Loresho, Nairobi, Kenya. P605-612.

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

Palomares, R. J. E., N. Vovlas, A. Troccoli, G. LieBanas, B. B. Landa, and P. Castillo. 2007. A new root-knot nematode parasitizing sea rocket from Spanish Mediterranean coastal sand dunes: *Meloidogyne dunesis* n. sp. (Nematoda: Meloidogynidae). Journal of Nematology 39:190-202.

Powers, T. 2004. Nematode molecular diagnostics: From bands to barcodes. Annual Review of Phytopathology 42:367-383.

Powers, T. O., and T. S. Harris. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. Journal of Nematology 25:1-6.

Randig, O., M. Bongiovanni, R. M. D. G. Carneiro, and P. Castagnone-Sereno. 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR marker specific for the coffee-damaging species. Genome 45:862-870.

Skantar, A. M., L. K. Carta, and Z. A. Handoo. 2008. Molecular and morphological characterization of an unusual *Meloidogyne arenaria* population from traveler's tree, *Ravenala madagascariensis*. Journal of Nematology 40:179-189.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876-4882.

Williams, B. D., B. Schrank, C. Huynh, R. Shownkeen, and R. H. Waterston. 1992. A genetic mapping system in Caenorhabditis elegans based on polymorphic sequence-tagged sites. Genetics 131:609-624.

Xu, C. L., C. B. Zhao, S. Ding, J. F. Zhang, H. Xie, and C. X. Huang. 2012. First report of root-knot nematode *Meloidogyne arenaria* infesting roots of *Anubias barteri* in Guangdong, China. Plant Disease 96:773.

Ye, W. 1996. Applying Microsoft Works spreadsheet in statistics for morphometric data of nematode identification. Afro-Asian Journal of Nematology 6:203-211.

Ye, W., R. M. Giblin-Davis, H. Braasch, K. Morris, and W. K. Thomas. 2007. Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution 43:1185-1197.

Zijlstra, C., D. T. H. M. Donkers-Venne, and M. Fargette. 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assays. Nematology 2:847-853.

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