On the species status of the root-knot nematode
Meloidogyne mayaguensis Rammah & Hirschmann, 1988

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Academic editor: S. Subbotin | Received 30 January 2012 | Accepted 28 March 2012 | Published 6 April 2012

Citation: Karssen G, Liao J, Kan Z, van Heese EYJ, den Nijs LJMF (2012) On the species status of the root-knot nematode Meloidogyne mayaguensis Rammah & Hirschmann, 1988. ZooKeys 181: 67–77. doi: 10.3897/zookeys.181.2787

Abstract
Holo- and paratypes of the root-knot nematodes Meloidogyne mayaguensis Rammah & Hirschmann, 1988 and M. enterolobii Yang & Eisenback, 1983 were morphometrically and morphologically compared. All observed female, male and second-stage juvenile morphometrical and morphological characters are identical for the two studied species. Additionally, contradictions between the original species descriptions were unravelled.

The present study of holo- and paratypes confirms the taxonomical status of Meloidogyne mayaguensis as a junior synonym for M. enterolobii.

Keywords
Junior synonym, Meloidogyne, M. enterolobii, M. mayaguensis, Nematoda, root-knot nematode, synonymisation

Introduction
In 1983 Yang and Eisenback described the root-knot nematode Meloidogyne enterolobii from roots of pacara earpod trees (Enterolobium contortisiliquum (Vell.) Morong), on Hainan Island in China. The authors reported severe damage on these pacara earpod trees. In 1988 Rammah and Hirschmann described the root-knot nematode M. maya-
guensis from eggplant (Solanum melongena L.) roots, from Puerto Rico. Meloidogyne mayaguensis was described by the authors as: “superficially resembles M. enterolobii”, and reported at the same time “several distinct morphologically features and a unique malate dehydrogenase pattern (N3c)”. It was Fargette and Braaksma (1990) and Fargette et al. (1996) who reported for the first time on the resistance-breaking behaviour of M. mayaguensis in Africa and concluded that it is present in both continents of Africa and America. The authors reported (1996) on M. enterolobii: “M. enterolobii from China has been described as having the same esterase phenotype as M. mayaguensis. However it is not known whether their DNA are closely related”. In 2000 Carneiro et al. published esterase and malate dehydrogenase patterns for a Brazilian population of M. mayaguensis, and detected a different (N1a) malate dehydrogenase pattern. Additionally Blok et al. (2002) published mtDNA results from different M. mayaguensis populations, including type material from Puerto Rico.

In their comprehensive studies on the characterisation of Meloidogyne species from China, with isozymes and mtDNA, Meng et al. (2004) and Xu et al. (2004) included two M. enterolobii populations from Hainan Island, isolated from the fruit tree Guava (Psidium guajava L.). They proved for the first time that M. enterolobii esterase (VS1-S1) and malate dehydrogenase (N1a) patterns and mtDNA results are identical to reported M. mayaguensis data, and concluded carefully: “the mtDNA sequence evidence presented here, suggests that M. mayaguensis could be conspecific with M. enterolobii”.

In 2005–2006 we compared the available holo- and paratypes of M. enterolobii and M. mayaguensis. Meanwhile our Chinese co-authors collected live M. enterolobii material on Hainan Island at the type locality from the type host and we kindly received live M. mayaguensis type material from Dr. V. Blok (originating from Dr. M. Fargette). The preliminary isozyme and morphological results were presented by the first author during a Pest Risk Analysis meeting on M. enterolobii at EPPO in Paris (Anonymous, 2008). Additionally this type material of both species was compared at DNA level to Meloidogyne sp. from Switzerland and we identified the Swiss population as M. enterolobii (Kiewnick et al. 2008).

Finally, as again at DNA level no differences were found, the two species were synonymised: “The species M. enterolobii (syn. M. mayaguensis)” and “…of M. mayaguensis (junior synonym of M. enterolobii)” (Kiewnick et al. 2009).

Although taxonomical not strictly necessary, we present herein a morphological and morphometrical comparison between the holo- and paratype slides of M. mayaguensis and M. enterolobii. Additionally we discuss anomalies between the descriptions of M. mayaguensis and M. enterolobii.

Material and methods

Holo- and paratype slides (Table 1) originating from USDA Nematode Collection (USDANC), Beltsville, USA were kindly provided by Dr. Z. Handoo. The type slides
Table 1. Meloidogyne mayaguensis and M. enterolobii holo-, allo- and paratype slides studied, including USDANC codes.

|                | M. mayaguensis | M. enterolobii |
|----------------|----------------|----------------|
| Holotype       | 1 female       | 1 female       |
| Allotype*      | 1 male         | 1 male         |
| Paratype       | 10 perineal patterns | 8 perineal patterns |
| Paratypes      | 25 J2's        | 25 J2's        |

*M. mayaguensis* and *M. enterolobii* holo-, allo- and paratype slides studied, including USDANC codes.

*According to the ICZN rules (4th edition) the allotype concept is no longer valid, and treated herein as a paratype.

are in good condition and includes female holotypes, male allotypes, perineal patterns and second-stage juvenile paratypes. These slides were observed by compound light microscopy (Olympus BH-2 and Zeiss Axio Imager), including Differential Interference Contrast and photographed by Leica DMC-50 digital camera. For the overall morphological and morphometrical comparison between the types we focussed on the most differential and supplementary *Meloidogyne* characters, as described by Jepson (1987) and as previously applied by Karssen (2002). Live type material of both species was propagated and maintained on tomato at the greenhouse of the PPS the Netherlands. This material was studied morphologically (females, males and second-stage juveniles) and used for isozyme electrophoresis (Mdh; EC 1.1.1.37 and Est; EC 3.1.1.1). For details on the preparation of slides and applied electrophoresis method we respectively refer to Karssen (1996) and Karssen et al. (1995).

Results and discussion

See Figure 1 and 2 for LM photographs of female and second-stage juvenile morphological characteristics.

See Table 2–5 for respectively female, male and second-stage juvenile morphological and morphometrical observations.

Females

The important morphological characters, like female stylet knob and perineal pattern shape do not differ between the species, as can already be observed by comparing the original illustrations between *M. mayaguensis* and *M. enterolobii* (see original descriptions respectively Fig. 2 A–D & Fig. 3 A–D). This perineal pattern type is not species specific within the genus *Meloidogyne* and can best be marked as typical for many species within the *M. incognita*-group, including the observed variation within the dorsal part. Additionally we observed a relatively large tail remnant area, free of any striae, just above the covered anus (Fig. 1 A–D). Also the observed stylet knob position variation,
slightly sloping backward to set off from the shaft, is a common *Meloidogyne* feature. Strangely this variation is also clearly visible in the SEM photographs of excised female stylets of *M. mayaguensis* (see original description, Fig. 3 A-C), but not described. With the light microscope one can observe a weak longitudinal indentation, for both species, in the female stylet knobs at the anterior side. The reported differences “not divided so conspicuously as those of *M. enterolobii*” as mentioned in the *M. mayaguensis* descrip-

**Figure 1.** LM photographs of perineal patterns of *M. mayaguensis* (A, B) and *M. enterolobii* (C, D). Bar = 25 µm.
tion (see diagnosis original description), was not confirmed by our observations. Also the described position of one of the *M. mayaguensis* stylet knobs “the dorsal knob is slightly sloping posteriad in lateral view” was not observed by us.

Males

The male head shape for *M. mayaguensis* is described as “not set off”, while a slightly set off head region was observed as described for *M. enterolobii*. Comparing the original SEM pictures of the head for *M. mayaguensis* and *M. enterolobii* (see original descript-
tions respectively Fig. 6 A–D & 5 A,B) shows clearly not any differences in head morphology. Also the male stylet knobs have been SEM studied for the original descriptions (Fig. 3 E, F & Fig. 6 B) of both species. Large oval to rounded shaped knobs, slightly sloping backwards are clearly visible. This was also observed by LM for both species, however described as “rounded and set off” for *M. enterolobii* and “set off from the shaft, rounded, sloping backward” for *M. mayaguensis*. The later description of the knobs is rather odd, i.e. set off and sloping backward at the same time! The same results were described and observed for the second-stage juvenile knobs for both species.

**Second-stage juveniles**

The second-stage juvenile stylet knob size is described as small for *M. mayaguensis* and large for *M. enterolobii*. We indeed observed a larger size variation for *M. enterolobii*
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Table 3. Morphometrical (in µm) observations (mean, SD & range) of female Meloidogyne mayaguensis and M. enterolobii holo- (single female) and paratypes (perineal patterns) compared to described data.

| Character               | M. mayaguensis | M. enterolobii |
|-------------------------|----------------|----------------|
|                         | holotype (N)   | paratypes (N)  |
| Body length             | 720            | 667            |
| Body width              | 570            | 415            |
| Neck length             | 190            | 265            |
| Neck width              | 160            | 169            |
| DGO                     | 6.2            | 3.7            |
| Excretory pore to head end | 46.4          | 44.8          |
| Stylet length           | 15.1           | 13.4           |
| Stylet knob height      | 2.2            | 2.7            |
| Stylet knob width       | 4.4            | 4.3            |
| Interphasmidial dist.  | 23.2 ± 2.5     | 28.8 ± 3.7     |
| Vulval slit length      | 26.1 ± 1.9     | 27.0 ± 1.4     |
| Vulva-anus distance     | 18.4 ± 1.5     | 21.4 ± 3.1     |
| DGO                     | 4.8 ± 0.8      | 4.9 ± 0.8      |
| Excretory pore to head end | 48.2 ± 13.6   | 62.9 ± 10.5   |
| Stylet length           | 15.8 ± 0.8     | 15.1 ± 1.4     |

However when observing live second-stage juveniles, the same large stylet knob width variation was observed for both species.

As for the males, the published SEM second-stage juvenile head shape is absolute identical for M. mayaguensis and M. enterolobii (see original descriptions respectively Fig. 7 A–D & Fig. 8 A, B). The tail is distinctly tapering and in the posterior tail (roughly the hyaline tail part) nearly straight and running parallel for both second-stage juvenile paratypes. Also, for both species the hyaline tail part is described as “distinctly set off” or “clearly defined”. We observed for both species however not a clearly anterior delimited hyaline tail part, in fact the body content runs deep into the hyaline tail part (Fig 2 A, B), as comparable to M. hapla (Karssen, 2002). The second-stage juvenile drawings for both species descriptions (Fig. 4 E, F & Fig. 7 E–F) show a clearly delimited anterior hyaline tail part, while the original photographs (Fig. 5 F, G & Fig. 9 B) do not show this at all. The fact that both descriptions did not include the hyaline tail measurements (a standard procedure), suggest strongly that the hyaline tail part is not clearly defined. Also in live second-stage juveniles we did not observe a clearly defined hyaline tail part (Table 2).
### Table 4. Morphometrical (in µm) observations (mean, SD & range) of male *Meloidogyne mayaguensis* and *M. enterolobii* paratypes compared to described data.

| Species | *M. mayaguensis* | *M. enterolobii* |
|---------|------------------|------------------|
| Character | description | observed | description | observed |
| N | 30 | 7 | 20 | 11 |
| Body length | 1503 ± 142 (1175–1742) | 1431 ± 63 (1337–1496) | 1600 ± 160 (1349–1913) | 1230 ± 316 (865–1667) |
| Greatest body width | 37.8 ± 3.1 (32.2–44.4) | 34.5 ± 1.9 (32.0–37.4) | 42.3 ± 3.6 (37.0–48.3) | 32.0 ± 6.0 (23.7–39.2) |
| Stylet length | 22.9 ± 0.8 (20.7–24.6) | 22.1 ± 0.7 (20.8–23.0) | 23.4 ± 1.0 (21.2–25.5) | 21.5 ± 1.7 (19.2–23.4) |
| Stylet knob height | 3.0 ± 0.3 (2.4–3.7) | 3.2 ± 0.3 (2.6–3.4) | 3.3 ± 0.3 (2.6–3.9) | 2.5 ± 0.3 (2.1–3.2) |
| Stylet knob width | 5.0 ± 0.3 (4.3–5.6) | 5.3 ± 0.5 (4.5–5.8) | 5.4 ± 0.3 (4.5–5.8) | 4.5 ± 0.6 (3.5–5.0) |
| DGO | 4.1 ± 0.4 (3.3–5.0) | 4.1 ± 0.7 (3.2–5.1) | 4.7 ± 0.4 (3.7–5.3) | 4.7 ± 0.6 (3.7–5.8) |
| Excretory pore to head end | 166.4 ± 8.8 (147.2–180.8) | 158.6 ± 14.9 (132.5–177.9) | 178.2 ± 11.2 (159.7–206.2) | 155.8 ± 22.3 (129.9–199.7) |
| Spicule length | 28.3 ± 1.5 (24.4–31.3) | 29.0 ± 2.4 (25.6–32.3) | 30.4 ± 1.2 (27.3–32.1) | 28.0 ± 1.1 (26.2–29.4) |
| Gubernaculum length | 7.1 ± 0.6 (6.1–9.3) | 7.5 ± 1.0 (6.4–9.0) | 6.2 ± 1.0 (4.8–8.0) | 6.5 ± 0.8 (6.1–8.0) |
| Tail length | 14.3 ± 1.1 (11.3–16.3) | 13.0 ± 1.1 (10.9–14.7) | 12.5 ± 2.2 (8.6–20.2) | 11.9 ± 1.2 (10.2–13.4) |
| A | 39.9 ± 3.9 (31.1–49.6) | 41.6 ± 2.9 (37.2–44.7) | 37.9 ± 3.2 (34.1–45.5) | 38.1 ± 4.0 (30.0–43.4) |
| C | 105.7 ± 10.0 (85.8–124.3) | 110.5 ± 10.8 (98.5–133.7) | 131.6 ± 24.2 (72.0–173.4) | 105.2 ± 23.7 (71.4–135.9) |

### Morphometrics

The morphometrical characters between the types of *M. mayaguensis* and *M. enterolobii* (Table 3–5), are comparable for the described and observed data, i.e. all mean data are the same or at least within the calculated range. Body length and body width data are generally slightly smaller when comparing observed to described data, this is a well known effect due to a slight shrinking of the nematode body within permanent slides. For *M. enterolobii* males we noticed however an unusual difference in greatest body width between the described 42.3 µm (37–48 µm) and observed 32.0 µm (24–39) µm data. The differences can not only be explained due to a shrinking effect, particularly as the observed greatest body width data agrees with the observed data for *M. mayaguensis*. Also for the *M. enterolobii* female holotype unexplainable differences were noticed between described and observed data for the DGO (3.7 µm versus 4.8 µm) and stylet length (13.4 µm versus 14.7 µm).

The described and discussed *M. mayaguensis* differences (see diagnosis original description) within the female perineal pattern for the interphasmidial distance, vulval...
slit length and vulva-anus distance is not confirmed by our observations. All these measurements are within the observed range. Perineal pattern measurements are generally highly variable and a logical reason for Jepson (1987) not to list this type of data when discussing differential characters for the genus *Meloidogyne*.

**Reproduction and cytogenetics**

The two species descriptions report also on the mode of reproduction and number of chromosomes, both reproduce by mitotic parthenogenesis (= apomixes) and have a somatic chromosome number of $2n = 44–45$ for *M. mayaguensis* and $2n = 44–46$ for *M. enterolobii*. In conclusion, both species have the same mode of reproduction and somatic chromosome number.

**Table 5.** Morphometrical (in µm) observations (mean, SD & range) of second-stage juvenile *Meloidogyne mayaguensis* and *M. enterolobii* paratypes compared to described data.

| Species | *M. mayaguensis* | *M. enterolobii* |
|---------|------------------|------------------|
| Character | description | observed | description | observed |
| N | 35 | 25 | 30 | 25 |
| Body length | $454 \pm 28$ (390–528) | $420 \pm 21$ (386–456) | $437 \pm 17$ (405–473) | $408 \pm 18$ (380–442) |
| Greatest body width | $14.7 \pm 0.5$ (13.8–15.8) | $13.9 \pm 0.7$ (13.1–15.4) | $15.3 \pm 0.9$ (13.9–17.8) | $14.8 \pm 2.1$ (11.0–18.0) |
| Body width at anus | $10.9 \pm 0.5$ (10.2–12.2) | $9.8 \pm 0.6$ (9.0–11.2) | – | $9.8 \pm 0.9$ (8.0–11.0) |
| Stylet length | $11.6 \pm 0.3$ (11.1–12.2) | $11.5 \pm 0.4$ (10.9–12.1) | $11.7 \pm 0.5$ (10.8–13.0) | $11.3 \pm 0.7$ (10.5–13.0) |
| Stylet base to head end | $15.2 \pm 0.3$ (14.8–15.8) | $15.4 \pm 0.3$ (14.7–16.0) | – | $15.0 \pm 0.7$ (14.0–16.0) |
| Stylet knob height | – | $1.5 \pm 0.1$ (1.2–1.7) | $1.6 \pm 0.1$ (1.3–1.8) | $1.8 \pm 0.3$ (1.5–2.0) |
| Stylet knob width | – | $2.5 \pm 0.2$ (2.2–2.9) | $2.9 \pm 0.3$ (2.4–3.4) | $3.0 \pm 0.4$ (2.5–4.0) |
| DGO | $3.9 \pm 0.2$ (3.3–4.3) | $3.7 \pm 0.4$ (3.2–4.2) | $3.4 \pm 0.3$ (2.8–4.3) | $3.8 \pm 0.3$ (3.0–4.5) |
| Excretory pore to head end | $87.6 \pm 3.3$ (79.9–97.9) | $88.3 \pm 3.0$ (83.5–95.3) | $91.7 \pm 3.3$ (84.0–98.6) | $80.8 \pm 4.4$ (70.0–88.0) |
| Tail length | $54.4 \pm 3.6$ (49.2–62.9) | $54.2 \pm 2.7$ (48.7–58.5) | $56.4 \pm 4.5$ (41.5–63.4) | $52.1 \pm 3.4$ (45.0–57.0) |
| a | $30.9 \pm 1.9$ (26.4–34.7) | $30.1 \pm 1.6$ (26.9–32.8) | $28.6 \pm 1.9$ (24.0–32.5) | $28.0 \pm 3.7$ (23.3–34.6) |
| c | $8.3 \pm 0.4$ (7.0–9.2) | $7.8 \pm 0.3$ (7.1–8.4) | $7.8 \pm 0.7$ (6.8–10.1) | $7.9 \pm 0.6$ (7.0–9.0) |
| Excretory pore (%) | $19.4 \pm 1.0$ (17.8–22.3) | $21.1 \pm 0.9$ (19.2–22.7) | – | $19.8 \pm 1.1$ (17.6–21.9) |
Host plants

Additionally, both species descriptions report in their introduction part some hosts, i.e. they both previously applied the North Carolina differential host test (Hartman and Sasser, 1985). Both species showed the same positive host response for tobacco, pepper, watermelon and tomato and no host response on peanut. Beside this, *M. mayaguensis* did not infest cotton, while *M. enterolobii* moderately infested cotton. As the details of the previously applied host tests have not been described in the material and method part of the species descriptions, we can not explain the reported host response differences on cotton for *M. mayaguensis* and *M. enterolobii*. Interesting is the *M. mayaguensis* study by Brito et al. (2004) with four isolates from Florida (USA). All four isolates, maintained on tomato, reproduced also on cotton, tobacco, pepper and watermelon but not on peanut, i.e. identical to the published results for *M. enterolobii*.

Isozymes

The observed esterase (VS1-S1 type) and malate dehydrogenase (N1a type) isozyme patters are identical for both species and agrees with previous results (Carneiro et al. 2000; Xu et al. 2004).

Conclusion

In conclusion, the holo- and paratype material of *Meloidogyne mayaguensis* and *M. enterolobii* is morphological and morphometrical identical and it confirms the taxonomical status of *M. mayaguensis* as a junior synonym for *M. enterolobii*.

Acknowledgement

This work was supported by the special fund for agro-scientific research in the public interest of China (grant no. 201103018).

References

Anonymous (2008) An emerging root-knot nematode, *Meloidogyne enterolobii*: addition to the EPPO Alert List. EPPO Reporting Service 5: 9–10.
Blok VC, Wishart J, Fargette M, Berthier K, Phillips MS (2002) Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. Nematology 4: 773–781. doi: 10.1163/156854102760402559
Brito J, Powers TO, Mullin PG, Inserra RN, Dickson DW (2004) Morphological and molecular characterization of Meloidogyne mayaguensis isolates from Florida. Journal of Nematology 36: 232–240.

Carneiro RMDG, Almeida MRA, Quencherve P (2000) Enzyme phenotypes of Meloidogyne spp. populations. Nematology 2: 645–654. doi: 10.1163/156854100509510

Fargette M, Braaksma R (1990) Use of the esterase phenotype in the taxonomy of the genus Meloidogyne. 3. A study of some “B” race lines and their taxonomic position. Revue de Nématologie 13: 375–386.

Fargette M, Phillips MS, Blok VC, Waugh R, Trudgill DL (1996) An RFLP study of relationships between species, populations and resistance-breaking lines of tropical species of Meloidogyne. Fundamental and Applied Nematology 19: 193–200.

Hartman KM, Sasser JN (1985) Identification of Meloidogyne species on the basis of differential host test and perineal pattern morphology. In: Barker KR, Carter CC, Sasser JN (Eds.) An advanced treatise on Meloidogyne, vol. 2, Methodology, pp. 69–77, North Carolina State University, Raleigh, USA.

Jepson SB (1987) Identification of root-knot nematodes (Meloidogyne species). CAB International, Wallingford, UK, 265 pp.

Karssen G (1996) Description of Meloidogyne fallax n. sp. (Nematoda: Heteroderidae), a root-knot nematode from The Netherlands. Fundamental and Applied Nematology 19: 593–599.

Karssen G (2002) The plant-parasitic nematode genus Meloidogyne Göldi, 1892 (Tylenchida) in Europe. Brill, Leiden, The Netherlands, 157 pp.

Karssen G, van Hoenselaar T, Verkerk-Bakker B, Janssen R (1995) Species identification of root-knot nematodes from potato by electrophoresis of individual females. Electrophoresis 16: 105–109. doi: 10.1002/elps.1150160119

Kiewnick S, Karssen G, Brito JA, Oggenfuss M, Frey B, Frey J-E (2008) First report of root-knot nematode Meloidogyne enterolobii in Switzerland. Plant Disease 92: 1370. doi: 10.1094/PDIS-92-9-1370A

Kiewnick S, Dessimoz M, Franck L (2009) Effects of the Mi-1 and the N root-knot nematode-resistance gene on the infection and reproduction of Meloidogyne enterolobii on tomato and pepper cultivars. Journal of Nematology 41: 134–139.

Meng Q, Long H, Xu J (2004) PCR assays for rapid and sensitive identification of three major root-knot nematodes, Meloidogyne incognita, M. javanica and M. arenaria. Acta Phytopathologica Sinica 34: 204–210.

Rammah A, Hirschmann H (1988) Meloidogyne mayaguensis n. sp. (Meloidogynidae), a root-knot nematode from Puerto Rico. Journal of Nematology 20: 58–69.

Xu J, Liu P, Meng Q, Long H (2004) Characterisation of Meloidogyne species from China using isozyme phenotypes and amplified mitochondrial DNA restriction fragment length polymorphism. European Journal of Plant Pathology 110: 309–315. doi: 10.1023/B:JEPP.0000019800.47389.31

Yang B, Eisenback JD (1983) Meloidogyne enterolobii n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpod tree in China. Journal of Nematology 15: 381–391.