Recognition of species belonging to *Meloidogyne ethiopica* group and development of a diagnostic method for its detection

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Abstract

Root-knot nematodes (RKN) of the tropical group reproducing as a rule with mitotic parthenogenesis are highly polyphagous and damaging pests causing great loses in crop production. Morphometrical and molecular characters of three closely related tropical RKN species have been analysed in order to identify species-specific diagnostic parameters and examine the relationship between species. Morphometrical characters of *M. ethiopica, M. luci* and *M. inornata* isolates were similar and overlapped when compared between species and isolates of the same species indicating a very close relationship between the three species. Additionally, structure of *map*-1 genes was analysed, which provided the evidence of identical structure of *map*-1 family genes in *M. ethiopica*, *M. inornata* and *M. luci.* The unique structure of *map*-1 genes when compared to the other tropical RKN species further supports close relationship of the three species and therefore we have classified them together as *M. ethiopica* group. In addition, our results showed that the emergence of a virulent *M. luci* population does not correlate with the loss of *map*-1 genes as it was previously indicated for *M. incognita* (Castagnone-Sereno et al. 2009). Further, we have developed novel molecular methods to aid in identification of these nematodes: a PCR based method with primers specific for the *M. ethiopica* group comprising *M. ethiopica* *M. luci* and *M. inornata;* and a PCR based method with primers specific for the tropical RKN group species. Our results show that species of *Meloidogyne ethiopica* group share high similarity at morphological and genetic level.

Introduction

Root-knot nematodes (RKN) *Meloidogyne* spp. are considered to be the most damaging plant parasitic nematodes for agricultural production. Several species that reproduce by mitotic parthenogenesis, with the exception of *M. floridensis*, belong to the phylogenetic clade I of *Meloidogyne* genus, also referred as the tropical RKN group. Species of this group cause substantial economic losses particularly in vegetable production as they have a wide host range. Tropical group species *M. ethiopica* has been reported to have been found in Africa by Whitehead (1968). The species is widely distributed in vineyards in Chile (Aballay et al. 2009) and was found to be the major root-knot nematode pest of grapevine and other crops in Chile (Carneiro et al. 2007). In Brazil, *M. ethiopica* affected the root growth, yield and quality of kiwi fruit causing serious economic losses (Carneiro et al. 2003) and generated multiple galls on potato roots and protuberances on tubers (Medina et al. 2014). Furthermore, *M. ethiopica* was the most frequently found species in Chilean vineyards and kiwi orchards

(Carneiro et al. 2007). In Europe *M. ethiopica* has been first reported from Slovenia (Širca et al. 2004), followed by Greece (Conceição et al. 2012), Italy (Maleita et al. 2012), and Turkey (Aydinli et al. 2013). Morphologically similar species *M. luci* has been described by Carneiro et al. (2014), which was reported from Brazil, Chile, Iran (Carneiro et al. 2014), Guatemala (Janssen et al. 2016) and Portugal (Maleita et al. 2017). After that, all *M. ethiopica* populations reported from Europe were subsequently re-analysed and re-identified as *M. luci* (Janssen et al. 2016; Gerič Stare et al. 2017). *Meloidogyne luci* was detected on several crops but there are no reports of economic damage except from Europe. In Slovenia, *M. luci* was found at two distinct locations. In the greenhouse in 2003 *M. luci* caused severe damage on tomatoes, however, the pest was eradicated due to omission of the agricultural production. A second infestation was detected in 2015 in a greenhouse near Ljubljana where *M. luci* caused severe damage and decline of more than 80 % of tomato crop (Gerič Stare et al. 2018). The finding of virulent populations of *M. luci* in Turkey that can reproduce on tomato plants bearing *Mi*-1 resistance gene adds to the concern on damaging potential of this species (Aydinli and Mennan 2016a). Although *Mi*-1 resistance gene has been introgressed into many commercial tomato cultivars to control RKN *M. incognita, M. javanica* and *M. arenaria*, it has been shown that *Mi*-1 also prevents the reproduction of *M. luci* (Strajnar and Širca 2011; Conceição et al. 2012). In Middle Black Sea Region of Turkey *Mi*-1 tomato cultivars are widely used to control RKN including *M. luci* (Aydınlı and Mennan 2016b). Another close species *M. inornata* was described from soybean in Brazil by Lordello (1956) and designated also as a sister species to *M. ethiopica* (Whitehead 1968). It was also detected on tobacco, yacon and common bean (Figueiredo 1958; Carneiro et al. 2008; Machado et al. 2013) but there are no reports on the economic damage. As *M. ethiopica* and *M. luci* represent serious phytosanitary risk both species were included on the EPPO Alert List of harmful organisms in 2011 and 2017, respectively (EPPO).

A combination of diagnostic methods is used for the identification of closely related *M. ethiopica, M. luci* and *M. inornata*: morphometry, analysis of the isozyme patterns and different molecular (DNA) markers. The most reliable method to distinguish between the three species is the esterase isozyme phenotype analysis (Janssen et al. 2016; Gerič Stare et al. 2017) however, L3 and E3 phenotypes differ slightly in migration rate of one band only, which is hard to spot unless isolates with both phenotypes are analysed on the same gel (Gerič Stare et al. 2017). The use of morphometrics demands a well-trained specialist as the sister species *M. luci* and *M. ethiopica* are very difficult to distinguish. Identification based on DNA markers is challenging due to closely related lineages indicating a recent speciation (Janssen et al. 2016), hybrid origins of the species (Lunt et al. 2014) and related taxonomic ambiguity (Janssen et al. 2016). ITS, SSU and LSU of the rDNA regions are not

appropriate diagnostic markers while mtDNA regions proved to be useful in phylogenetic studies (Janssen et al. 2016; Gerič Stare et al. 2017). Besides mtDNA, the analysis of taxonomically restricted genes of *map*-1 gene family provided additional information of evolutionary relationship and speciation in the tropical RKN group. The analysis of the *map*-1 genes presence in the RKNs showed that these genes are specifically present in the species that reproduce by mitotic parthenogenesis, with the exception of *M. floridensis*, and could not be detected in RKNs reproducing by either meiotic parthenogenesis or amphimixis (Tomalova et al. 2012). In addition, identical structure of genes of *map*-1 family in *M. ethiopica* and *M. inornata* was observed which was unique in comparison to the rest of the analysed species (Tomalova et al. 2012) suggesting the common evolutionary history of these parasites.

The objective of this study was to clarify the relations between three morphologically similar RKN species. According to the analysis of the region of mtDNA stretching from the 3' end portion of the cytochrome oxidase II (COII) gene through a 5' portion of the 16S rRNA sequences of these three species form a monophyletic group in which *M. luci* forms a subclade while *M. ethiopica* and *M. inornata* couldn't be distinguished (Gerič Stare et al. 2017). A close relationship of these three species was also shown based on several mtDNA genes by Janssen et al. 2016. A second objective was to identify species-specific diagnostic parameters which could be of diagnostic value. A third objective was to test if the emergence of virulent *M. luci* populations correlates with the loss of *map*-1 genes as it was previously indicated for *M. incognita* (Castagnone-Sereno et al. 2009). We reviewed information on the host plants in order to find any biological differences, reanalysed morphological characters and sequenced the structural genes of *map*-1 family in the selected species *M. ethiopica, M. luci* and *M. inornata*. We proposed *Meloidogyne ethiopica* group and developed a diagnostic method for its detection.

Materials and Methods

Nematode isolates. RKN isolates were collected from different geographical origin (Table 1 and Table 2). Selected isolates were maintained on tomato cv. Val, type Cuore di bue or cv. Falcon cultures, kept in a greenhouse at 20 - 25°C. Nematodes used for analyses were isolated from the fresh cultures.

Morphometric analysis. Using a dissecting microscope SMZ 800 (Nikon), females and egg masses were isolated from infested roots with a scalpel and a nematological needle. Males and second stage juveniles (J2)

were extracted from the sand by the decanting method (Hržič 1973) followed by Baerman's funnel extraction. Isolated males and J2s were fixed in 2% formalin solution before analyses, while female parameters were analysed on fixed females in 45% lactic acid. Nematode images and morphometrical analyses were performed using Leica, M125 or Nikon TiE light microscope with a system of image analyses. Common nematode body features were measured in a set of *Meloidogyne* spp. isolates (Table 2) on 10 fixed nematode specimens of each nematode life stage.

DNA extraction. DNA was extracted from a single egg mass of each selected isolate (Table 1). A Promega Genomic DNA Wizard purification kit (Madison, WI, USA) was used according to manufacturer's instructions. Extracted DNA was diluted in 10 µl of DNA rehydration solution (10mM Tris-HCl, 1mM EDTA).

Structure of *map*-1 genes. Sequences of repetitive domains in *map*-1 genes in a set of *Meloidogyne* spp. isolates, including *Mi*-1 virulent *M. luci* isolate from Turkey, Ordu (Table 2) were determined. Repetitive domains in *map*-1 genes were amplified with primers m1 and m2 as described by Semblat et al. (2001). Electrophoresis of all amplified DNA products was performed on a 0.8% TBE agarose gel. Individual bands were excised from the gel and DNA was extracted with GeneJetGel Extraction Kit (Thermo Scientific) following manufacturer's instructions. Individual bands were cloned with the pGEM-T Easy Vector System II (Promega) according to the manufacturer's instructions. Plasmid DNA was isolated by JetQuick Plasmid Miniprep (Genomed) following the manufacturer's instructions. Macrogen Inc. (Korea) provided sequencing of plasmids with universal primers SP6 and T7. DNA sequences were edited and aligned using the BioEdit v. 7.0.5.2 software (Hall 1999).

Molecular identification tests. Primers were designed manually in parallel with alignment of *Meloidogyne* mitochondrial DNA available in the public domain. The region of mtDNA stretching from the 3' end portion of the cytochrome oxidase II (COII) gene through a 5' portion of the 16S rRNA (lRNA) gene was selected as a target sequence as sequences of this marker region are available for many nematode species. Specific primers Me309F (CTAATTTGGGTGAATTT) and Me549R (AATCAAAATCTTCTCCT) were selected for *M. ethiopica* group. For the tropical RKN group, the specific reverse primer Mt575R (AGAACTTAAACTCTAAATAAC) was selected to be used in combination with the forward primer C2F3 (GGTCAATGTTCAGAAATTTGTGG) described previously (Powers and Harris 1993). PCR reactions

contained 2 µl of isolated DNA, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 2.5 mM of each of the dNTPs, 1 µM of each corresponding primer (Sigma), 1U Go*Taq* DNA Polymerase (Promega) and distilled water up to 50 µl. The amplification was carried out in a thermal cycler Veriti (Applied Biosystems) using the following program: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, 30 s for annealing at 50°C for *M. ethiopica* group and at 54°C for the tropical group of RKN, elongation at 72°C for 30 s for *M. ethiopica* group and for 1 min for the tropical group of RKN; followed by a final extension at 72°C for 5 min. Amplified DNA products were visualised on a 1% TBE agarose gel.

Results

Morphometric analysis

Morphometric characters of females, males and J2 of thirteen *M. luci* isolates from Turkey, an isolate of *M. luci* from Slovenia and an isolate of *M. inornata* from Chile were measured and compared with previous reports of *M. ethiopica* (Carneiro et al. 2004), *M. inornata* (Carneiro et al. 2008) and *M. luci* (Carneiro et al. 2014) (Table 1S). The values of all life stage morphological characteristics studied here overlapped in all species and isolates. In females, stylets were robust with developed knobs and ranged in length from 11.3 to 18.7 µm in *M. luci*, from 12 to 15 µm in *M. ethiopica* and from 14.7 to 17 µm in *M. inornata*. Mean value of stylet length in *M. ethiopica* females was shorter compared to other two species while stylet knob height in *M. inornata* was higher than in other two analysed species. The mean values of distance from anterior end to metacorpus in females of two *M. luci* isolates were 71.4 and 73.6 µm which is shorter than in *M. inornata* isolates (95.3 and 104.0 µm). Female excretory pore position was similar in *M. luci* and *M. inornata* while more posteriorly positioned excretory pore was observed in *M. ethiopica*. In males, stylet length and spicule length in *M. ethiopica* were longer than in other two species. The rest of the measured and published morphometrical characteristics of males were similar among isolates and species. In juveniles, *M. ethiopica* showed the highest average values for body length (468 µm) and tail length (62 µm), whereas *M. luci* and *M. inornata* had lower average values for these characters. Reported measurements for two characters i.e. the anterior end to metacorpus and the anterior end to excretory pore in *M. inornata* from Brazil seems rather high compared to measurements of these characters in all other species and populations included in Supplementary Table 1, including *M. inornata* from Chile. The measurements of anterior end to metacorpus in juveniles ranged from 47.5 to 67.6 µm and from 46.2 to 66.0 µm in *M. luci* from Turkey and Slovenia, respectively. In *M. inornata* isolate from Chile this character ranged from 55.7 to 61.5 µm

(this study data) which is similar to all studied isolates except for *M. inornata* from Brazil where the character ranged from 102 to 134 µm (Carneiro et al. 2008). Similar deviation was observed in measurements for anterior end to excretory pore.

Structure of *map*-1 genes

The presence of two *map*-1 genes was determined in all of our tested RKN isolates (Table 2). In accordance with gene designations by Tomolova et al (2012) the determined genes belong to *map*-1 alleles 1 and 6 each with certain length and unique structure of repetitive domains. Combination of *map*-1 genes denominated as allele 1 and allele 6 was found in all three tested species: *M. luci, M. ethiopica* and *M. inornata* (Table 2, Fig. 1), confirming their close relationship. Two genes/alleles with the same structure of repetitive domains were present in the avirulent as well as in the virulent isolate of *M. luci*. The emergence of virulence does not correlate with the loss of *map*-1 gene in *M. luci*.

The sequences generated during the current study were deposited at ENA database (acc. nos. LT835071-LT835099, LT996917 - LT996918).

Molecular identification tests

We have developed two PCR identification methods based on the mtDNA sequences of RKNs from the database. The group of three species, *M. ethiopica, M. luci* and *M. inornata* also referred to as *M. ethiopica* group can be identified based on amplification of a group-specific, 241 bp long amplicon with group-specific PCR primers designed in this study (Fig. 2). With an additional PCR reaction presence of RKNs belonging to the tropical group can be checked by amplifying a group-specific, 621 bp long amplicon (Fig. 2). Tropical RKN test was performed with one group-specific primer designed in this study and the primer C2F3 (Powers and Harris 1993), which is a widely used primer for molecular identification of *Meloidogyne* species. Specificity of both identification methods was tested on a range of RKN species and isolates of diverse geographical origin: eight *M. arenaria*, three *M. chitwoodi*, five *M. ethiopica*, one *M. fallax*, three *M. hapla*, two *M. hispanica*, twelve *M. incognita*, two *M. inornata*, seven *M. javanica* and twenty-six *M. luci* isolates (Table 1).

Discussion

The biological species concept defines a species as members of populations capable of interbreeding in nature and producing fertile offspring, not according to similarity of appearance (Mayr, 1942, cited in Adams,

1998). However, biological species concept cannot be applied to organisms that do not reproduce sexually. One such example is the tropical group of RKNs, where majority of species reproduces by obligate mitotic parthenogenesis (Castagnone-Sereno & Danchin, 2014). In such cases we can apply the Linnean species concept or typological morphospecies concept, which delimits species as groups of organisms that have the most overall similarity (Mayr, 1963, cited in Adams, 1998). However, this approach is most prone to errors including overestimating and underestimating the number of species and misrepresenting their phylogenetic relationships. To overcome the species problem De Queiroz (2007) proposed a unified species concept, not as a new species concept but rather a view that encompasses all the above species concepts (and others not mentioned in this paper) in the light of continuum of speciation process. A unified species concept treats existence as a separately evolving metapopulation lineage as the only necessary property of species while evidence (operational criteria) relevant to assessing lineage separation can be presence of any of the different important biological properties (e.g., intrinsic reproductive isolation, diagnosability, monophyly, occupation of a distinct niche, phenetic distinguishability). But he points out that these different secondary properties arise at different times during the process of speciation. So, in species that are in process of speciation, delimitation of species can be based on different properties (operational criterion) emphasized in different species concepts. This argument seems in favour of *M. ethiopica*, *M. luci* and *M. inornata* being recognised as separate species as any evidence of delimitation under unified concept is a proof of a new species. As *M. ethiopica*, *M. luci* and *M. inornata* exhibit many similarities on biological (common hosts), morphological (overlapping morphological characteristics) and molecular levels (demonstrated on mtDNA), and share the evolutionary path distinct from the other tropical RKN species (demonstrated by the unique structure of *map*-1 genes) we have classified the three studied species as a *M. ethiopica* group.

Meloidogyne ethiopica can affect numerous plant species, including many important crops such as grapevine and potato (Whitehead 1968; O'Bannon 1975; Carneiro et al. 2003, 2004; Lima et al. 2009). As there is no reliable differential host between *M. ethiopica* and *M. luci* found so far, it seems that these two species are so closely related that there is no biological difference between them in terms of parasitizing different plant species (Gerič Stare et al. 2017). Therefore both species represent a serious threat to agriculture (Carneiro et al. 2003, 2007; Aballay et al. 2009; Medina et al. 2014; Maleita et al. 2017) and were therefore included in the EPPO Alert list of harmful organisms. Less host plant species have been reported for *M. inornata* (Lordello 1956; Figueiredo 1958; Carneiro et al. 2008; Machado et al. 2013). *Meloidogyne inornata* had the same response as *M. javanica* in differential host dependent tests where tomato cv. Rutgers, tobacco cv. NC95 and watermelon

cv. Charleston Gray were defined as good hosts, whereas cotton cv. Deltapine 61, pepper cv. California Wonder and peanut cv. Florunner were non-hosts (Carneiro et al. 2008). Although not many plant host species are reported for *M. inornata* and there has not been an extensive study to determine its host range, all reported hosts of *M. inornata* can also be parasitized by *M. ethiopica* and/or *M. luci.*

In addition to the common hosts the three studied species exhibit also morphological similarities. In the species description of *M. inornata* Lordello (1956) a close relation to *M. incognita* was noticed but some morphological characteristics including broader eggs, second-stage juvenile heading with only one post-labial annule and female excretory pore located further posterior differ from *M. incognita*. Moreover, the author considered that the single wide annule immediately posterior to the head cap of the male was the most outstanding character for distinguishing these species. Whitehead (1968) and Hewlett and Tarjan (1983) considered *M. inornata* to be closely related to *M. incognita*. Therefore, Jepson (1987) and Eisenback and Triantaphyllou (1991) synonymised these two species based on morphological characteristics. In 2008 Carneiro et al. re-described this species and indicated some distinctive morphological characteristics of females, males and J2s including the perineal pattern with *incognita*-type and excretory pore location of female, a single annule posterior to the head cap in males and J2s, tail length and c ratio of the J2, and stylet morphology of males. According to Carneiro et al. (2008), the isolate of *M. inornata* from Brazil had the metacorpus positioned much more posteriorly (ranges from 102 to 134 µm) compared to the J2s of *M. inornata* from Chile (55.7 to 61.5 µm) or *M. luci* from Turkey and Slovenia. However, our measurements of *M. inornata* from Chile were consistent with *M. inornata* species original description where the character ranged between 46.5 and 64.8 µm (Lordello, 1956). Morphological characteristics of *M. luci*, *M. inornata* and *M. ethiopica* recorded in this study and in previous reports (Carneiro et al. 2004, 2008, 2014) were similar and the ranges for certain characters overlapped (Table 1S). In previous reports, morphology of these species was examined in detail and morphometric values from different nematode stages were reported. Carneiro et al (2014) stated that distinctly different morphology of knobs shape in male stylet was observed between three species on electron scans but other morphological characteristics of females, males and J2s were similar for at least two species. For example, the perineal patterns of *M. luci* and *M. ethiopica* were similar whereas stylet conus of female in *M. luci* and *M. inornata* was similar and at the same time different from *M. ethiopica* (Carneiro et al. 2014). These differences are difficult to observe using the most widely used light microscopy and even then highly trained and experienced diagnostic specialists are needed. Therefore, we evaluated the morphological characters that can be easily measured with light microscopy and do not require highly experienced analyst. Jepson (1983) noted tail length as the least variable

and most differential quantitative character in second-stage juveniles. Overlapping values were obtained in three species for tail length of J2s. However, *M. ethiopica* isolate from Brazil (Carneiro et al. 2004) exhibits longer tail in J2s (52-72 µm) compared the original described *M. ethiopica* (Whitehead,1968) tail length in J2s (41-52 µm) which indicates greater intra-specific variability for this character. Tail length in *M. luci* and *M. inornata* J2s exhibited similar ranges. For the distance from anterior end to excretory pore of J2s only *M. inornata* isolate from Brazil (Carneiro et al. 2008) exhibited much higher values ranging from 152 to 164 µm, but the parameter was in the same range in all isolates including *M. inornata* from Chile (this study data) which ranged from 60.2 to 93.5 µm. Similar deviation was observed in measurements for anterior end to excretory pore in *M. inornata* isolate from Brazil which indicates possible incorrect measurements taken by Carneiro et al. (2008) for these two characters. Similarly, morphological measurements from *M. luci* and *M. inornata* in this study contradicted the finding of Carneiro et al (2014) who reported that the distance of DGO to stylet base in males were different between these species. The values for this character in males of *M. inornata* isolate from Chile were in the same range as in *M. luci* isolates. Few morphometric characters in all there analysed species exhibited species distinguishing potential but when comparing morphometry of different isolates and original species description data the overlapping between the species was noticed. Based on these findings, the sister species are very difficult to distinguish on morphological characteristics alone.

The biochemical properties as the isozyme esterase phenotypes were reported as the most distinguishing character for the studied species (Carneiro et al., 2008, 2014; Gerič Stare et al., 2017). In general, analysing phenotypes of malate dehydrogenase (MDH) and esterase (EST) isozymes has proved to be a useful approach in RKN species identification (Esbenshade and Triantaphyllou 1985). Species of *M. ethiopica* group can be differentiated by their EST profiles E3 or E2, L3 and I3 found in *M. ethiopica*, *M. luci* and *M. inornata*, respectively. However, L3 and E3 phenotypes differ in migration rate of one band only which is hard to spot unless isolates with both phenotypes are analysed on the same gel (Gerič Stare et al., 2017).

The accurate RKN species identification is the first step toward appropriate management strategies. A combination of several diagnostic methods is used for the RKN identification: morphology, determination of the host plants, analysis of the isozyme patterns and different molecular (DNA) markers. Advantages of molecular approaches in identification of RKNs are that live material and different live stages are not needed. Further, it should be stressed that rDNA repeating units have been shown to be of valuable diagnostic benefit in the case of species for which a risk of confusion exists, e.g., distinguishing *M. enterolobii* from the other tropical RKN species or *M. chitwoodi* from its sibling species *M. fallax* and *M. hapla*. However, these rDNA repeating units

299 cannot be used with confidence to identify the tropical RKN species, where intraspecific and inter-individual
300 variations were observed (Castagnone-Sereno et al. 2013). Many species-specific primer pairs have been
301 developed for tropical RKNs and some of them seem to be more reliable and robust than others (Groover 2017).
302 While species-specific primer pairs are developed for the more common *Meloidoyne* species (Zijlstra et al. 2000;
303 Adam et al. 2007; Tigano et al. 2010), they are not available or are not working reliably for many other 'minor'
304 species including *M. ethiopica M. luci* and *M. inornata* (Correa et al. 2014). Similar is true for PCR-RFLP
305 approach; while it seems to work for identification of some species, the approach does not enable identification
306 of all or at least the majority of clade I species in the genus *Meloidoyne* (Powers & Harris, 1993; Zijlstra et al.
307 1995; Stanton et al. 1997; Maleita et al. 2012; Pagan et al. 2015; Baidoo et al. 2016). While we could not find
308 any species-specific diagnostic parameters that could be used for an easy identification on morphological level,
309 we were able to identify group-specific parameters (conserved nucleotide sites) on molecular level. The methods
310 for *M. ethiopica* group and tropical RKN identification developed in this study could be a valuable tool for
311 quick, simple and unambiguous identification to this taxonomical level or one step in a scheme towards selection
312 of further species-specific tests. Specificity of both developed tests was tested with a wide selection of RKN
313 species and isolates from different geographical origin. Further, the specificity of the method for *M. ethiopica*
314 group identification was tested with alignment of the primers to the mtDNA sequences of *M. luci* reported from
315 Portugal. As primers Me309F and Me549R showed 100% identity to the sequences with accession numbers
316 KM042847 and KM042848, this *in silico* annealing test is indicating that this isolate would probably give a
317 positive result in a PCR test, although *in vitro* test would be needed for confirmation. A substantial number of *M.*
318 *luci* isolates from Turkey was included in this study as this nematode species is widely distributed in the Black
319 See region of Turkey in greenhouses as well as in open field production (Aydinli and Mennan 2016b; Aydinli
320 2018). Six *M. luci* isolates from Turkish Samsun province included in our testing originate from open fields
321 (Aydinli 2018). Although this nematode was first recorded in Turkey only in 2009 (Aydinli et al. 2013) and at
322 that time reported as *M. ethiopica*, but later recognised as *M. luci* (Gerič Stare et al. 2017), there is an indication
323 that *M. luci* may have been present in Turkey much earlier. Esbenshade and Triantaphyllou (1985) reported an
324 esterase profile designated as M3 in one *Meloidogyne* spp. isolate from Turkey which could not be identified to
325 species. This esterase profile M3 was later renamed as L3 and reported as a species specific esterase profile
326 characteristic for *M. luci* (Carneiro et al. 2014).

327 While real-time PCR based diagnostic methods have been on the rise for over a decade as they among
328 other advantages ensure a higher sensitivity of detection than a conventional PCR, molecular identification

methods developed in this work were deliberately developed for the conventional PCR, because many diagnostic laboratories do not have the real-time PCR thermocyclers. Further, developed methods based on conventional PCR are sensitive enough to give a positive result with DNA extracted from a single egg mass. Additionally, the lengths of PCR amplicons (241 and 621 bp) are not optimal for real-time PCR where shorter amplicons are preferred to ensure a good efficiency (E value) of the real-time PCR reaction. The lengths of PCR amplicons are dependent on positions of variable and conserved sites in target DNA, which were crucial in primers design. We were able to upgrade *M. ethiopica* group identification method to a real-time PCR, but the E value was not within the proposed range for an optimal real-time PCR reaction (data not shown.)

Although mtDNA regions have a certain discriminatory power to distinguish the sister species in phylogenetic analyses (Janssen et al. 2016; Gerič Stare et al. 2017), we have opted for the analysis of structural genes linked to pests' ability for parasitism in order to test phylogenetic relationships of tropical RKN species. MAP-1 protein and corresponding *map*-1 gene were first described from *M. incognita* by Semblat et al. (2001) and thought to be secreted from the amphids, chemosensory organs in the nematode head region. The internal part of the protein is characterised by 58AA and 13AA long tandemly arranged repetitive motives. Although MAP-1 was initially hypothesised to be an expansin-like protein (Tomalova et al. 2012; Semblat et al. 2001), further studies suggested it contained a 14 AA mofits with sequence similarity to CLE-like peptides, a plant peptide hormone mimic and that the gene may be expressed in the subventral gland cells (Rutter et al. 2014). CLE effectors secreted by cyst nematodes (*Globodera* and *Heterodera* spp.) influence plant signalling pathways resulting in the formation and maintenance of syncytia (Guo et al. 2011), while the function of MAP-1 proteins in *Meloidogyne* spp. parasitism is not well understood. However, it has been shown that *map*-1 gene family is a set of taxonomically restricted genes found only in tropical RKNs (Tomolova et al. 2012). In addition to the morphometric similarity between species and close relationship revealed on mtDNA level (Jansen et al. 2016, Gerič Stare et al. 2017), the identical structure of genes in *map*-1 family in *M. ethiopica*, *M. luci* and *M. inornata* supports a close relationship of these three species and therefore we have classified them together as *M. ethiopica* group. Further, the loss of one copy of *map*-1 gene in *M. incognita* near-isogenic lines has been reported to correlate with the break of *Mi*-1 mediated resistance in tomatoes (Castagnone-Sereno et al. 2009). In order to test if the same mechanism linked with loss of *map*-1 gene is responsible for virulence observed in *M. luci*, we have determined the sequence of repetitive domains in *map*-1 genes in a set of tropical RKN isolates, including avirulent and virulent *M. luci* isolates from Turkey. While the loss of one copy of *map*-1 gene in *M. incognita* correlated with the break of *Mi*-1 mediated resistance (Castagnone-Sereno et al. 2009), we have shown

that the emergence of virulence does not correlate with the loss of *map*-1 genes in *M. luci*. Similar to our observation, *M. javanica* isolate virulent to the *Mi*-resistant gene retains two homologues of *map*-1 genes (Adam et al. 2009). Molecular mechanics for resistance break in *M. luci* virulent populations remains to be elucidated. Analysis of whole genomes could reveal the basis for this resistance break as well as resolve phylogenetic relationships of closely related populations of *M. ethiopica*, *M. luci* and *M. inornata*. As tropical RKN species have evolved with hybridisation (Lunt et al. 2014), the whole genome datasets will be more useful for establishing evolutionary trails and phylogenetic relationships of these species than any partial DNA sequence / molecular marker.

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Fig. 1 Representation of different combination of alleles from *map*-1 gene family present in certain tropical RKN species representing sequences determined in this study and by Tomolova et al. (2012) and Humphreys-Pereira et al (2014). Modular architecture of the internal part schematically represented with light grey rectangles for 58-aa domains, dark grey squares for 13-aa domains and dark grey triangles for 5-aa domains (adopted from Tomalova et al. 2012 and Castagnone-Sereno et al. 2009)

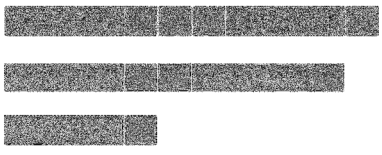
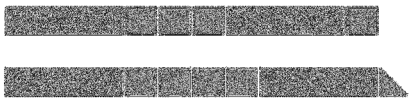
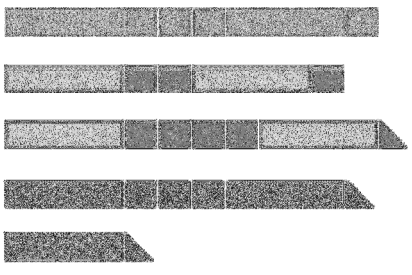
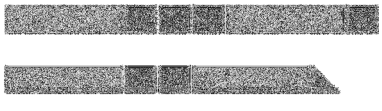
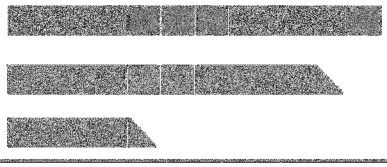
Species	Allele	Structure of *map*-1 repetative domains
M. arabidicida *M. enterolobii* *M. floridensis*	Allele 1	
M. hispanica *M. koanensis* *M. lopezi*	Allele 1 Allele 2	
M. paranaensis	Allele 1 Allele 2 Allele 3	
M. arenaria *M. cruciani* *M. javanica*	Allele 1 Allele 4	
M. izalcoensis	Allele 1 Allele 2 Allele 4 Allele 5 Allele 7	
M. ethiopica *M. luci* *M. inornata*	Allele 1 Allele 6	
M. incognita	Allele 1 Allele 6 Allele 7	

Fig. 2 PCR Amplification with *M. ethiopica* group specific primers (above) and with tropical RKN specific primers (below). From left to right: M – DNA ladder 100 bp Plus (Thermo Scientific), 1 – *M. luci* Slovenia, 2 - *M. luci* Turkey, 3 - *M. luci* Iran, 4 – *M. luci* Guatemala, 5 - *M. ethiopica* Brazil, 6 - *M. ethiopica* South Africa, 7 - *M. inornata* Chile, 8 – *M. arenaria*, 9 - *M. incognita*, 10 - *M. javanica*, 11 - *M. hispanica,* 12 - *M. hapla*, 13 - *M. chitwoodi*, 14 - *M. fallax*, 15 – negative control, M – DNA ladder 100 bp Plus (Thermo Scientific)

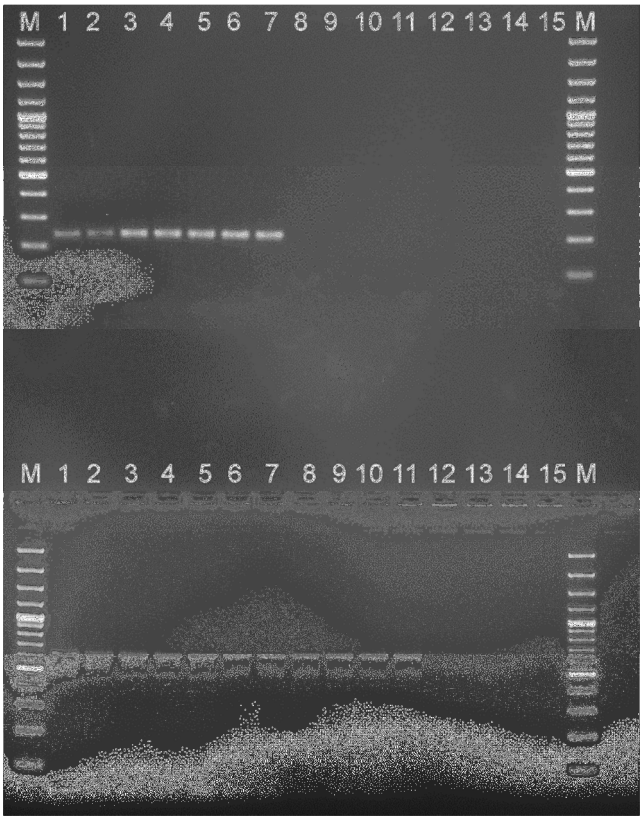


Table 1 *Meloidogyne* spp. isolates used for specificity testing of the molecular identification methods.

Species	Origin	Isolate designation	Kept at	*M. ethiopica* group test	Tropical RKN test
M. arenaria	Slovenia	*M. arenaria* SI	1	-	+
M. arenaria	Bulgaria	P66	4	-	+
M. arenaria	Turkey, Antalya province	K18	3	-	+
M. arenaria	Turkey, Muğla province	O1	3	-	+
M. arenaria	Turkey, Samsun province	Çr-1	2	-	+

M. arenaria	Turkey, Samsun province	108	5	-	+
M. arenaria	Turkey, Samsun province	114	5	-	+
M. arenaria	Turkey, Tokat province	Er-1	2	-	+
M. chitwoodi	Netherlands		4	-	-
M. chitwoodi	Turkey, Niğde province	NIG11	2 [h]	-	-
M. chitwoodi	Turkey, Nevşehir province	NEV10	2 [h]	-	-
M. ethiopica	Brazil	*M. ethiopica* BR	1 [e]	+	+
M. ethiopica	Brazil		4 [a]	+	+
M. ethiopica	South Africa	M. ethiopica JA	1,2 [b]	+	+
M. ethiopica	Chile	*M. ethiopica* ČI1	1 [c]	+	+
M. ethiopica	Chile	*M. ethiopica* ČI2	1 [c]	+	+
M. fallax	Unknown		4	-	-
M. hapla	Slovenia	*M. hapla* SI	1	-	-
M. hapla	Slovenia	10166017	4	-	-
M. hapla	Turkey, Ordu province		5	-	-
M. hispanica	Spain		4 [d]	-	+
M. hispanica	Portugal		2 [g]	-	+
M. incognita	Slovenia	*M. incognita* SI	1	-	+
M. incognita	Slovenia	V463	4	-	+
M. incognita	Slovenia	47613	4	-	+
M. incognita	Monte Negro	ČG4	4	-	+
M. incognita	Turkey, Antalya province	G3	3	-	+
M. incognita	Turkey, Antalya province	A2	3	-	+
M. incognita	Turkey, Antalya province	S6	3	-	+
M. incognita	Turkey, Antalya province	M1	3	-	+
M. incognita	Turkey, Antalya province	K5	3	-	+
M. incognita	Turkey, Antalya province	G2	3	-	+
M. incognita	Turkey, Samsun province	109	5	-	+
M. incognita	Turkey, Samsun province	Çr-20	2	-	+
M. inornata	Chile	639	4 [e]	+	+

M. inornata	Chile	695	4 [e]	+	+
M. javanica	Turkey, Antalya province	A1	3	-	+
M. javanica	Turkey, Antalya province	A4	3	-	+
M. javanica	Turkey, Antalya province	AKS2	3	-	+
M. javanica	Turkey, Antalya province	K16	3	-	+
M. javanica	Turkey, Antalya province	D3	3	-	+
M. javanica	Turkey, Antalya province	KA6	3	-	+
M. javanica	Turkey, Samsun province	B-26	2	-	+
M. luci	Slovenia, Dornberk	*M. luci* Dornberk	1,2	+	+
M. luci	Slovenia, Šmartno	*M. luci* Šmartno	1	+	+
M. luci	Greece	*M. luci* GR	1 [f]	+	+
M. luci	Iran	*M. luci* IR	1 [e]	+	+
M. luci	Guatemala	*M. luci* GVA	1 [e]	+	+
M. luci	Turkey, Samsun	*M. luci* TR1	1,2	+	+
M. luci	Turkey, Sinop province	Sn-3	2	+	+
M. luci	Turkey, Sinop province	Sn-12	2	+	+
M. luci	Turkey, Samsun province	Al-4	2	+	+
M. luci	Turkey, Samsun province	Tk-4	2	+	+
M. luci	Turkey, Samsun province	Tr-19	2	+	+
M. luci	Turkey, Samsun province	Çr-5	2	+	+
M. luci	Turkey, Samsun province	Çr-10	2	+	+
M. luci	Turkey, Samsun province	Çr-25	2	+	+
M. luci	Turkey, Samsun province	Çr-34	2	+	+
M. luci	Turkey, Samsun province	Çr-36	2	+	+
M. luci	Turkey, Samsun province	Çr-39	2	+	+
M. luci	Turkey, Ordu province	Or-1	2	+	+
M. luci	Turkey, Ordu province	Or-2	1,2	+	+
M. luci	Turkey, Samsun province	Çr-40	5	+	+
M. luci	Turkey, Samsun province	103	5	+	+
M. luci	Turkey, Samsun province	110	5	+	+

M. luci	Turkey, Samsun province	111	5	+	+
M. luci	Turkey, Samsun province	112	5	+	+
M. luci	Turkey, Samsun province	113	5	+	+
M. luci	Turkey, Samsun province	125	5	+	+

Isolates maintained in the following culture collections:

1: Plant Protection Department Agricultural Institute of Slovenia, Ljubljana, Slovenia;

2: Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Turkey;

3: Department of Plant Protection, Faculty of Agriculture, Akdeniz University, Antalya, Turkey.

Material included in the nematode DNA collections:

4: Plant Protection Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia;

5: Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Turkey.

Isolates or DNA obtained from these collections:

[a] Vanessa Silva Mattos, University of Brasília, Department of Plant Pathology, Brasília, Brazil;

[b] Dr. Mariette Marais, Agricultural research council, Pretoria, South Africa;

[c] Dr. Pablo Meza D., Nematology Laboratory of INIA-La Platina, Santiago, Chile;

[d] Dr. Philippe Castagnone-Sereno, INRA UMR1301, UNSA, CNRS UMR6243, Sophia Antipolis, France;

[e] Dr. Gerrit Karssen, Plant Protection Service, Wageningen, The Netherlands;

[f] Dr. Isabel Luci Conceição, IMAR-CMA, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra, Portugal;

[g] Dr. Carla Maleita, Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Portugal;

[h] Dr. Emre Evlice, Plant Protection Central Research Institute, Ankara, Turkey.

Table 2 Determined *map*-1 genes with specific structure of repetitive domains designated as different alleles with accession numbers for sequences deposited at ENA database. Isolate *M. luci* from Turkey, Ordu (Or-2) represents a virulent population.

Species	Origin	map-1, allele 1	map-1, allele 6
M. ethiopica	Brazil [b]	LT835071, LT835072	LT835084, LT835085
M. ethiopica	South Africa [c]	LT835073	LT835086, LT835087
M. inornata	Chile [b]	LT996917	LT996918
M. luci	Turkey, Samsun [a]	LT835078	LT835092, LT835093
M. luci	Turkey, Ordu [a]	LT835079	LT835094, LT835095
M. luci	Slovenia, Dornberk [d]	LT835074, LT835075	LT835088, LT835089
M. luci	Slovenia, Šmartno [d]	LT835076, LT835077	LT835090, LT835091
M. luci	Greece [e]	LT835080, LT835081	LT835096, LT835097
M. luci	Iran [b]	LT835082, LT835083	LT835098, LT835099

Isolates obtained from these collections:

[a] Dr. Gökhan Aydınlı, Bafra Vocational High School, Ondokuz Mayıs, Samsun, Turkey;

[b] Prof. Dr. Gerrit Karssen, Netherlands Food and Consumer Product Safety Authority, Wageningen, The Netherlands;

[c] Dr. Mariette Marais, Agricultural research council, Pretoria, South Africa;

[d] Dr. Saša Širca, Agricultural Institute of Slovenia, Ljubljana, Slovenia;

[e] Dr. Isabel Luci Conceição, IMAR-CMA, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, Coimbra, Portugal.

Supplementary Table 1 Morphometric comparison of females, males and second stage juveniles of *Meloidogyne luci* from Turkey, Slovenia and Brazil (Carneiro et al. 2014), *M. ethiopica* from Brazil (Carneiro et al. 2004), *M. inornata* from Brazil (Carneiro et al. 2008) and *M. inornata* from Chile. Data are presented as means ± standard deviation and ranges. All measurements are in µm. Data of *M. luci* from Turkey represent means of 13 isolates (locations) from the Middle Black Sea region (isolates designated Sn-3, Sn-12, Al-4, Tk-4, Tr-19, Çr-5, Çr-10, Çr-25, Çr-34, Çr-39, Çr-40, Or-1, Or-2). Further, isolates *M. luci* from Slovenia (Šmartno) and *M. inornata* from Chile (F2642/1) were used to measure morphometric characters in this study. * denotes possible incorrect measurements by Carneiro et al. (2008).

Character\Species, Isolate	*M. luci*, Turkey, this study n=13x10=130	*M. luci*, Slovenia, this study n=20	*M. luci*, Brazil Carneiro et al. 2014 n=30	*M. ethiopica,* Brazil, Carneiro et al. 2004 n=30	*M. inornata*, Brazil, Carneiro et al. 2008 n=30	*M. inornata*, Chile, this study n=20
Females						
Body length (L)	702±94 (555-920)	865.1±84.4 (785.0-982.9)	704±115 (570-800)	700±13 (594-798)	606±15 (594-781)	789.2±135.0 (676.2-1012.5)
Maximum body width	508±78 (314-682)	520±69.9 (433.4-602.6)	503±55 (440-550)	522±12 (120-282)	517±12 (437-675)	509.5±39.0 (448.8-555.3)
Body length without neck	536±82 (400-774)	594.9±49.0 (533.1-657.3)	-	530±17 (420-750)	460±17.8 (300-656)	460±19.6 (552-726)
Stylet length	14.1±1.9 (11.3-18.7)	15.2±1.2 (13.5-16.8)	15.5±0.5 (15-16)	13.5±0.1 (12-15)	15.3±0.2 (15-17)	15.2±0.6 (14.7-16.1)
Stylet knob height	2.0±0.3 (1.0-2.7)	1.8±0.5 (1.3-2.8)	2.0±0.1 (1.8-2.3)	2.0±0.1 (1.5-2.5)	2.8±0.2 (2.5-3.5)	2.5±0.3 (2.0-2.9)
Stylet knob width	3.7±0.6 (2.4-5.0)	3.6±0.8 (2.4-4.8)	3.6±0.5 (3.2-4.0)	3.4±0.1 (3.0-5.0)	4.0±0.5 (4.0-4.5)	3.8±0.2 (3.5-4.1)
Stylet knob width/height	1.9±0.3 (1.2-2.7)	2.1±0.4 (1.5-2.8)	-	1.7±0.3 (1.5-2.5)	-	1.6±0.2 (1.4-1.9)
Dorsal esophageal gland orifice (DGO)	3.7±0.7 (2.6-5.2)	3.3±0.6 (2.3-4.1)	3.2±0.8 (3.0-4.0)	3.8±0.1 (3.0-5.0)	3.9±0.1 (3.5-4.5)	4.1±0.6 (3.2-4.6)
Anterior end to metacorpus	73.6±9.9 (52.6-101.8)	71.4±13.4 (50.9-89.8)	-	-	104±11 (90-120)	95.3±18.4 (77.6-122.0)
Metacorpus length	46.4±8.3 (34.1-70.9)	45.9±7.3 (36.3-55.6)	-	-	-	41.2±6.5 (32.2-52.6)
Metacorpus diameter	42.8±8.0 (29.4-68.0)	44.4±9.8 (33.6-59.1)	-	-	-	39.4±8.6 (28.9-40.6)

Anterior end to excretory pore	38.9±11.6 (19.2-77.1)	46.9±16.9 (28.8-73.8)	31.3±18 (18.5-48.5)	65.3±2.1 (41-79)	36.9±2.8 (25.0-53.0)	49.1±4.7 (42.3-55.1)
Vulva length	25.1±2.5 (16.2-31.2)	25.6±2.7 (20.9-29.4)	23.3±0.5 (20.0-26.0)	25.2±0.7 (25.4-26.9)	24.4±0.8 (22.5-26.2)	24.8±1.1 (23.2-25.9)
Vulva-anus distance	18.3±2.7 (10.8-23.6)	20.1±3.0 (16.3-24.9)	17.4±0.6 (15.0-26.0)	19.2±0.5 (17.3-21.0)	19.0±0.5 (17.5-22.5)	20.5±1.7 (18.6-22.8)
Interphasmidial distance	23.1±4.6 (11.5-35.0)	27.8±5.9 (18.2-35.2)	26.3±0.8 (20.0-36.5)	20.4±0.3 (19.0-23.0)	24.5±0.7 (22.5-26.3)	23.5±1.7 (21.6-25.3)
Body length/Maximum body width (a)	1.4±0.2 (1.0-1.8)	1.7±0.3 (1.3-2.0)	1.43±0.39 (1.04-1.82)	-	1.28±0.8 (1.11-1.44)	1.6±0.3 (1.4-2.0)
Males						
Body length (L)	1252±308 (706-2038)	1534.1±171.7 (1340.5-1940)	1602±520 (1090-2130)	1171±48 (890-1500)	1594±58 (1101-2063)	1181.8±216.5 (881.2-1423.0)
Maximum body width	41±7.9 (23-56)	37.3±7.2 (29.0-56.0)	43±5.0 (37-50)	48±0.8 (32-59)	47±0.9 (32-51)	33.7±4.0 (28.3-38.9)
Stylet length	20.7±1.4 (17.3-23.6)	21.2±1.7 (17.5-23.7)	22.1±2.7 (20.8-23.0)	24.8±0.6 (23-27)	21.7±0.6 (20.0-25.0)	22.3±1.5 (20.8-24.9)
Stylet knob height	2.6±0.2 (2.0-3.3)	2.8±0.6 (2.0-3.7)	2.6±0.3 (2.5-3.0)	3.3±0.1 (3.0-4.0)	3.0±0.5 (2.5-3.5)	3.2±0.3 (3.0-3.7)
Stylet knob width	4.0±0.5 (3.1-5.4)	4.2±0.4 (3.3-4.6)	4.2±0.4 (3.8-4.5)	4.3±0.6 (3.5-5.0)	4.8±0.6 (4.0-5.0)	4.0±0.6 (3.2-4.9)
Stylet knob width/height	1.6±0.1 (1.2-2.0)	1.6±0.3 (1.1-2.1)	-	1.3±0.2 (1.1-1.5)	-	1.2±0.2 (1.1-1.6)
Dorsal esophageal gland orifice (DGO)	3.7±0.7 (2.2-5.4)	3.2±0.5 (2.4-3.8)	3.5±1.0 (2.5-4.5)	2.5±0.1 (2.0-3.5)	4.5±0.2 (4.0-5.0)	3.4±0.5 (2.8-4.2)
Anterior end to metacorpus	86.8±12.8 (60.7-114.7)	75.3.0±8.9 (62.2-89.0)	-	-	84.0±3.2 (75.0-120.0)	95.7±13.7 (74.5-116.2)
Anterior end to excretory pore	162.1±24.6 (108.9-233.4)	150.3±30.2 (111.0-181.0)	199±30.2 (150-217)	200±31 (187-215)	167.3±3.1 (135-200)	154.9±14.6 (134.5-172.0)
Tail length	12.4±1.9 (9.3-18.1)	13.0±2.1 (9.7-18.0)	9.5±1.0 (3.0-15.0)	13.4±0.5 (10.2-17.0)	13.5±0.5 (10-15)	12.9±1.2 (11.2-14.5)
Spicule length	26.8±2.6 (22.7-36.2)	31.2±3.0 (27.0-37.6)	31.3±4.0 (24.0-35.0)	39±0.6 (34-42)	33.0±0.6 (26-38)	31.2±4.3 (27.0-37.2)
Body length/Maximum body width (a)	30.6±5.5 (21.6-49.3)	42.1±7.0 (30.9-52.5)	39.7±9.8 (21.8-57.6)	27.7±0.8 (24.8-31.0)	27.7±0.8 (24.4-51.0)	35.0±4.6 (31.1-42.7)
Body length/tail length (c)	102±26.5 (56.1-180.5)	82.4±38.9 (30.9-156.5)	322±72 (73-710)	114±12.2 (69.5-147.2)	152±12.2 (104.5-187.2)	91.9±17.9 (64.3-117.6)

Second-stage juveniles (J2)						
Body length (L)	383±26 (321-439)	351.3±28.7 (321.2-408.0)	383±85 (300-470)	468±3 (326-510)	418±3 (394-487)	421.6±16.4 (401.0-446.2)
Maximum body width	16.7±1.8 (13.6-21.0)	15.3±2.1 (12.4-21.0)	16±1.5 (13-20)	20±0.3 (15-22)	19.3±0.6 (17.0-22.0)	15.6±1.2 (13.9-16.8)
Body width at anus	11.1±1.4 (8.3-13.7)	10.9±1.0 (9.5-13.0)	-	-	-	10.6±0.9 (9.5-11.9)
Stylet length	13.6±0.7 (11.6-15.3)	13.6±0.4 (13.0-14.0)	12.5±0.2 (12.0-13.5)	12.2±0.1 (11-14)	11.5±0.1 (10-13)	12.6±0.9 (11.4-13.9)
Dorsal esophageal gland orifice (DGO)	3.0±0.4 (2.1-3.8)	2.6±0.4 (2.1-3.4)	2.9±0.5 (2.3-3.3)	2.6±0.1 (2.0-3.0)	3.0±0.1 (2.5-3.5)	3.3±0.4 (2.9-4.0)
Anterior end to metacorpus	54.3±3.6 (47.5-67.6)	55.2±4.6 (46.2-66.0)	-	-	* 122±2.8 (102-134)	58.4±2.7 (55.7-61.5)
Anterior end to excretory pore	80.1±8.4 (64.3-103.8)	76.2±10.7 (63.0-92.0)	73±10.0 (62-82)	93±0.9 (75-106)	* 158±0.9 (152-164)	77.5±12.2 (60.2-93.5)
Tail length	47.5±6.1 (30.6-58.6)	45.5±5.0 (37.6-58.0)	44.0±4.5 (40.0-48.5)	62±0.6 (52-72)	49.4±0.6 (35.0-58.0)	50.1±5.0 (42.6-55.8)
Hyaline tail terminus length	11.3±1.3 (8.0-13.5)	11.1±1.4 (8.9-14.0)	11.7±3.0 (9-15)	13.5±0.2 (12.0-15.0)	13.9±0.25 (10.0-15.0)	11.1±1.6 (9.5-13.7)
Body length/Maximum body width (a)	23.2±2.2 (17.8-27.6)	23.2±2.1 (19.4-26.8)	25.6±10.5 (15.0-36.1)	24.0±0.27 (21.3-28.2)	23.0±0.27 (17.9-28.7)	27.2±2.1 (25.3-31.1)
Body length/tail length (c)	8.2±1.2 (5.5-12.1)	7.8±0.7 (5.8-8.7)	8.7±2.6 (6.2-11.5)	4.8±0.1 (3.9-6.4)	10.2±0.4 (6.7-13.9)	8.5±0.6 (7.7-9.4)
Tail length/body width at anus (c')	4.3±0.6 (3.0-5.5)	4.2±0.3 (3.6-4.5)	-	-	-	4.8±0.7 (3.9-5.9)