# Detection and molecular characterization of the rice root-knot nematode *Meloidogyne graminicola* in Italy

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**Abstract** The rice root-knot nematode *Meloidogyne graminicola* was detected in North Italy attacking roots of rice plants in lowland and upland fields. This nematode was previously reported only in South and Southeast Asia, China, South Africa, United States, Columbia and Brazil attacking rice and wheat. The present study provides an integrative diagnosis of the nematode on the basis of morphological and molecular data by sequencing the SCAR species specific marker, the ITS region and the partial mitochondrial *co*II-16srRNA genes. Morphological and morphometric traits of the new intercepted population of *M. graminicola* agree with the original description. The diagnostic SCAR marker was amplified showing the expected size fragment and unequivocally identified the Italian population as *M. graminicola*. Sequence analyses of ITS and *co*II-16SrRNA confirmed this identification. Sequence comparison of both molecular markers among Italian *M. graminicola* populations from lowland and upland rice fields revealed 100% identity. Phylogenetic analysis based on the ITS and *co*II-16SrRNA sequences revealed that Italian *M. graminicola* formed a well supported cluster with *M. graminicola* populations from GenBank confirming the high intraspecific variability among all populations. No correlation between genetic variability and geographic origins occurred. Furthermore, the phylogenetic trees suggest a common ancestor for most of the facultative meiotic parthenogenetic *Meloidogyne* species.

**Keywords** cytochrome c oxidase subunit II (*co*II), *Meloidogyne graminicola*, maximum likelihood, rDNA, rice, SCAR.

**Introduction**

Rice (*Oryza sativa* L.) is the most important cereal crop worldwide since it provides food security for more than half of the world's human population. Severe yield losses can occur in this economically important crop as a result of infestation by nematodes. Among nematode species reported in association with rice, the most important rice pathogen is considered the root-knot nematode *Meloidogyne graminicola* Golden & Birchfield, 1965 which is also listed as a quarantine pest in many countries (Bridge et al. 2005; Pokharel et al. 2010). This species was first described in 1965 (Golden and Birchfield 1965) from grasses and oats in Louisiana, since then it has been found attacking primarily irrigated and rainfed rice, lowland and upland rice, and deepwater rice in South and Southeast Asia, China, South Africa, United States of America, Columbia and Brazil. This nematode, which has a wide host range of more than 98 hosts (Pokharel et al., 2010), has been also found associated to other cereals and grasses, including weeds commonly present in rice fields that may represent the principal hosts for *M. graminicola* (Bridge et al. 2005; Bridge and Starr 2007; Rich et al. 2009).

*Meloidogyne graminicola* is an obligate sedentary endoparasite that once has infected rice is well adapted to flooded conditions. Infestations of *M. graminicola* in rice induces the formation of galls, localized at the root tips, with females laying eggs in masses within the root cortex. Second-stage juveniles (J2) hatch from the eggs remaining in the maternal gall or migrating in the same root in order to multiply even under flooded conditions. In flooded conditions, J2s cannot infect rice plants but when fields are drained they resume their infectivity by attacking the root tips.

To date, *M. graminicola* has never been detected in Europe and in Italy (EPPO; 2015). Italy is the main rice-growing country in Europe with an area devoted to rice production of about 220,000 ha, 90% of which are located in the Po valley, in Vercelli, Pavia, Novara and Milan provinces. The irrigation management in rice fields is of greatest importance to ensure the cultivation, in its various stages, the best conditions of temperature, nutrient availability, weed or pest control. The water level is constantly regulated depending on the crop stage and precipitation, with intercalated dry periods in order to

practice weeding and provide fertilization. Species determination of *M. graminicola* is complex and usually based on the symptoms (hook-like galls produced on the rice roots). The application of molecular methods have been used with success to identify this species, in particular sequences of nuclear ribosomal (rDNA) and mitochondrial DNA (mtDNA) have been largely used (Pokharel et al. 2007, 2010; McClure et al. 2012; Htat et al. 2016). Recently, two molecular methods were developed: one used a diagnostic SCAR marker (Bellafiore et al. 2015) for rapid and reproducible identification of *M. graminicola* and the second one used real-time PCR primers for the quantification of this nematode in soil (Katsuta et al. 2016).

In June 2016 four rice fields in Vercelli and Biella provinces, North Italy, were showing above-ground symptoms of the nematode attacks, such as stunting, leaf chlorosis and loss of vigour that resulted in poor growth of the crop. In addition, roots of rice plants presented swelling and apical galls typical of root-knot nematode infection. Weeds collected in the vicinity of rice fields were also showing root galls. Soil and root samples from upland and lowland rice fields were analysed for the presence of RKN.

The objectives of the present research were: i) to provide additional morpho-biological data, with illustration of perineal patterns and male features of *M. graminicola* from North Italy; ii) to extend the molecular characterization for this nematode species using ITS, the partial mitochondrial *co*II-16S rDNA and the species-specific SCAR marker; iii) to investigate intraspecific variation between populations of *M. graminicola* from North Italy and those from database; and iv) to explore the phylogenetic relationships among the different populations of *M. graminicola* and other *Meloidogyne* species.

## Material and methods

### *Nematode morphological identification*

Specimens of *M. graminicola* used in this study were isolated from soil and infected root samples of upland and lowland rice fields in Vercelli province, and in three rice fields in Biella province, North Italy. Despite nematodes were also found in the roots of weeds, only specimens from rice roots and soil were used in this study.

For diagnosis and identification, root-knot nematode females and males were collected from rice galled roots, while second-stage juveniles (J2) were extracted from the soil rhizosphere by centrifugal-flotation (Coolen 1979) and from feeder roots by blending in a 0.5 % NaOCl solution for 4 min (Hussey and Barker 1973). Specimens for light microscopic (LM) observation were killed by gentle heat, fixed in a 4 % solution of formaldehyde + propionic acid and processed to glycerin by Seinhorst's rapid method (Hooper, 1986). Specimens were examined using a Leitz Diaplan optical microscope at powers up to 1,000 × magnification equipped with differential interference contrast optics and Leica® DFC425 camera. Observations and measurements were made on glycerine infiltrate specimens, using LAS (Leica Application Suite), Version 3.6.0 software. Photographs were taken on specimens mounted in water agar temporary slides (Troccoli 2002). Perineal patterns of mature females were prepared according to standard procedures (Hartman and Sasser 1985). Therefore, adult females were gently removed from root tissues and teased apart with tweezers and needle. The lip and neck regions of the nematode were excised, and the posterior end was cleared in a solution of 45 % lactic acid. Then, the perineal pattern was trimmed and transferred to a drop of glycerin for microscopic observations and photogragraphs.

*Nematode molecular identification*

Genomic DNA was extracted from twenty-one and thirty-five individual nematodes, from upland and lowland rice fields, respectively, as described by De Luca et al. (2004). The crude DNA isolated from each specimen was directly amplified by using three sets of universal primers. The partial 18S rRNA gene was amplified using the forward primer 18SNF (5'-TGGATAACTGTGGTAATTCTAGAGC-3') and the reverse primer 18SNR (5'-TTACGACTTTTGCCCGGTTC-3') (Kanzaki and Futai 2002), the ITS containing region was

amplified using the forward primer 18S (5-TGATTACGTCCCTGCCTTT-3) and the reverse primer 26S (5'-TTTCACTCGCCGTTACTAAGG-3') (Vrain et al. 1992), while the region of the mitochondrial genome between the cytochrome oxidase subunit II (*co*II) and the 16S rRNA genes was amplified using the forward primer C2F3 (5'-GGTCAATGTTCAGAAATTTGTGG-3') and the reverse primer 1108 (5'-TACCTTTGACCAATCACGCT-3') (Powers and Harris 1993). PCR cycling conditions used for amplification of the partial 18S rRNA gene and ITS region were: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 50 s, annealing at 55°C for 50 s and extension at 72°C for 1 min and a final step at 72°C for 7 min. For the *co*II these conditions were: an initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 30 s and extension at 60°C for 30s and a final step at 72°C for 7 min. In order to quickly identify the species, the crude DNA extracted from individual juveniles was amplified by using the following SCAR primer set: SCAR-MgFW/SCAR-MgRev (Bellafiore et al. 2015) for *M. graminicola*, Finc/Rinc (Zijlstra et al. 2000) and INCK14F/INCK14 (Randing et al. 2002) for *M. incognita*, Fjav/Rjav (Zijlstra et al. 2000) and SCAR MJF/SCARMJR (Meng et al. 2004) for *M. javanica*, Mn28SFs/RK28SUR (Ye et al. 2015) and MnSCARF/MnSCARev (Zijlstra et al. 2004) for *M. naasi*. Amplifications were performed using the following conditions: an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min and a final step at 72°C for 7 min. The size of amplification products was determined by comparison with the molecular weight marker ladder 100 (Fermentas, St Leon-Rot, Germany) following electrophoresis of 10 $\mu$l on a 1% agarose gel.

*Sequencing*

PCR products of the partial 18S rRNA gene (2 individual specimens, one for each sample), the ITS containing region (5 specimens, 2 from lowland and 3 from upland rice fields), the mitochondrial *co*II (5 specimens, 2 from lowland and 3 from upland rice fields) and the specific SCAR marker (2 individual

nematodes for each sample) were purified for sequencing using the protocol listed by manufacturer (NucleoSpin® Gel and PCR Clean-up, Macherey-Nagel). Purified DNA fragments were cloned and sequenced in both directions. A BLAST (Basic Local Alignment Search Tool) search at NCBI (National Center for Biotechnology Information) was performed using the 18S rRNA gene, ITS, *co*II and SCAR sequences as queries to confirm their nematode origins (Altschul et al. 1997) and the identification as *M. graminicola*. The consensus sequences of newly obtained sequences were submitted to the GenBank database under accession numbers: LT669809-LT669810 for the ITS, LT669811-LT669812 for COII, LT669813-LT669814 for the 18S rRNA gene and LT718706-LT718709 for the specific SCAR marker.

*RFLP analysis*

The RFLP analyses were conducted on six individual specimens (2 and 4 from lowland and upland rice fields, respectively). Ten $\mu$l of each ITS product from upland and lowland rice populations of *M. graminicola* was digested with five units of the following restriction enzymes: *Alu* I, *Hae* III, *Hind* III and *Hin*f I (Promega). Digested products were separated onto a 2.5% agarose gel by electrophoresis, stained with gel red, visualized on a UV transilluminator and recorded by photography with a digital system.

*Phylogenetic analyses*

ITS rDNA and *co*II-16SrRNA sequences of different *Meloidogyne* species including *M. graminicola* populations from GenBank, along with those determined in this study, were used for phylogenetic reconstruction. The newly obtained and published sequences for each gene were aligned using Clustal W (Thompson et al. 1994) with default parameters. Sequence alignments were manually edited using BioEdit (Hall et al. 1999). Based on previous studies, *Pratylenchus penetrans* and *Meloidogyne mali* were chosen as outgroups. Phylogenetic trees, obtained for ITS dataset, and the COII were performed with Neighbour-Joining (NJ), Minimum Evolution (ME), Maximum Likelihood (ML) and Maximum Parsimony

(MP) methods using MEGA version 6 software (Tamura et al. 2013). No significant conflict in branching order and support level among methods was observed and, therefore, only ML tree is shown for each marker. The phylograms were bootstrapped 1,000 times to assess the degree of support for the phylogenetic branching indicated by the optimal tree for each method.

## Results and discussion

### *Field symptoms and morphological identification*

Upland and lowland rice fields in North Italy infected by *M. graminicola* showed patches in which plants were showing poor growth and stunting, chlorosis and heavily affected root systems. Diseased roots showed swelling and galls of different shape and size. Infected roots contained a large number of eggs and J2s. Eggs were laid within the root cortex in an egg sac that was not easy to observe. Female and male specimens were present in the same gall. Severe infection and large galls were also observed on weeds (*Panicum dichotomiflorum*, *Heteranthera reniformis*, *Cyperus difformis*, *Echinochloa crus-galli*, *Oryza sativa* var. *selvatica*, *Murdannia keisak* and *Alisma plantago*) on which the symptoms of the above ground part of the plants were similar to those described on rice.

Additional morphological and morphometric data of males and second-stage juveniles of populations of the present study, from upland rice fields, are presented in Table 1. A few measurements, including body, stylet, pharynx and tail length were also taken in live specimens from lowland fields, although they are not presented here.

The average measurements and ranges of *M. graminicola* Italian population were within the range of measurements reported for *M. graminicola* (Golden and Birchfield 1965) (Fig. 1). Minor differences were found in the male total pharyngeal length (from anterior end to base of gland lobe) which was slightly shorter (mean value 193 vs 222 µm) than that reported for *M. graminicola*, and in the male spicules, slightly shorter in the Italian population (23.5-26.9 vs 27.4-29.1 µm of type population).

Males were present in large number within galls, but scarcely present in the soil. However, no morphological difference between upland and lowland rice populations of *M. graminicola* was observed.

Perineal patterns of females of the Italian populations appeared oval in shape, dorso-ventrally oriented, with moderately high dorsal arc and no lateral incisures, except in rare specimens. Phasmids were usually not observed, unless in very few perineal patterns (Fig. 1B). Tail tip sometimes visible in form of a coarser, semicircular ridge, above the anus (Fig. 1K,L). In most of perineal mounts, a rather large, roughly rhomboidal footprint was visible beneath the vulva. Perineal pattern of these Italian populations appear similar to the pattern described for *M. graminicola*.

*Molecular characterization and phylogenetic relationships of* Meloidogyne graminicola

In this study, PCR amplification was conducted on individual J2, males and females. The amplification of the partial 18S rDNA, ITS, the partial mitochondrial region between the *cox* II and the 16S rRNA genes and the species-specific SCAR fragment yielded a single fragment of approximately 1618 bp, 800 bp, 531 bp, and 640 bp, respectively, based on sequencing. The partial 18S rRNA sequences from upland and lowland Italian populations of *M. graminicola* were identical and showed a similarity of 99% with *M. graminicola* from the database (1-5 nucleotides different) and with *M. naasi* (10-18 nucleotide different). ITS region of the Italian *M. graminicola* specimens (LT669809-LT669810) matched well (98% similarity) with ITS of *M. graminicola* deposited in GenBank (KF751065; HM581973), differing by 18 nucleotides (787/805 identities), and presented 13 gaps (1%, 13/7805) and 97% similarity with all *M. graminicola* present in the database. Low variability of the ITS sequences was observed within individual juveniles of the Italian populations and between the upland and lowland Italian populations of *M. graminicola*. The Italian *M. graminicola* populations showed high intraspecific variability compared with the other *Meloidogyne* species that could infect rice including *M. incognita*, *M. javanica*, and *M. arenaria*. Several microsatellites were present in both ITS regions of *M. graminicola* as reported for

other nematodes. Stretches of (A)n and (T)n were also observed that contributes to length variation in the number of repeats among populations and also within individual nematodes without altering the identification of this species or the relationships between species.

Digestion of the ITS products of the upland and lowland rice populations with four restriction enzymes (*Alu* I, *Hae* III, *Hind* III and *Hin*f I) produced identical patterns (Fig. 2) suggesting that ITS-RFLP is a reliable and rapid method of diagnosis for *M. graminicola*.

The partial *coII*-16S rRNA sequences, obtained in this study, matched well (97-98% similarity, from 9 to 15 different nucleotides) with the corresponding sequences of *M. graminicola* from the database. Intraspecific variability for *co*II-16S rRNA region of upland and lowland Italian populations was of 99.82% (1-2 nucleotides in 531). Mitochondrial sequences of *M. graminicola* showed high A-T rich content as all other *Meloidogyne* species. The *coII*-16S rRNA region contains a variable intergenic sequence that is useful for species identification and for phylogenetic study.

The SCAR species-specific marker for *M. graminicola* produced the expected fragment (644 bp; Fig. 3) and the sequence was identical to that present in Genbank allowing a quick identification of this species. No amplification was observed on *M. graminicola* DNA as template when the SCAR primer set for *M. javanica*, *M. incognita*, and *M. naasi* were tested. The SCAR primer set Mn28SFs/RK28SUR, designed on the conserved 28S rRNA gene, for *M. naasi* (Ye et al. 2015), in our study amplified a light band of the expected size (data not shown) on *M. graminicola* suggesting that this region is very conserved between *M. graminicola* and *M. naasi*. In contrast, the second SCAR primer set MnSCARF/MnSCARev for *M. naasi* (Zijlstra et al. 2004) did not amplify any band on *M. graminicola*. This finding clearly suggests that the Mn28SFs/RK28SUR primer set is not species-specific because this primer set was validated on few populations of *M. naasi* as also observed by the mentioned authors. This report shows that the SCAR specific data set for *M. graminicola* easily amplified the expected fragment from individual juveniles, females and males and can be used for routine diagnostic purposes. Consequently, species identification based on sequencing of rDNA regions (18S, ITS), mtDNA (partial

*coII*-16SrRNA) and SCAR marker was congruent with morphological and morphometrical studies, thus the nematode species from Italian rice fields was clearly identified as *M. graminicola*.

Phylogenetic relationships of *M. graminicola* based on ITS1-5.8S-ITS2 of a multiple edited alignment including 80 sequences and 635 total characters using maximum likelihood originated the ML tree as given in Figure 4. This ML tree showed that all *M. graminicola* populations formed a well supported group (97% support). The different sequences obtained from different isolates of *M. graminicola* did not cluster according to geographical origin, suggesting a very high level of intra-population variability. The Nepalese isolates (NP 18, Np 29 and NP 50) together with the isolates from USA (FL1) and Bangladesh (BP 3) showed relatively long branch in the tree reported in Fig. 4 due to higher sequence variation of these isolates compared with the other *M. graminicola* isolates. Furthermore, our data confirm that the high variability observed in *M. graminicola* is not due to the presence of cryptic species but to sequence variation among isolates as reported in other *Meloidogyne* species (Hugall et al. 1999; Power et al. 2005). In the present study, the two sequences of *M. trifoliophila* clustered with all *M. graminicola* isolates suggesting that these populations could represent either cryptic species or races of *M. graminicola* as *M. trifoliophila* is presently known to infect white clover only. *Meloidogyne naasi*, *M. minor*, *M. chitwoodi* and *M. hapla* formed three different and well supported groups (100% support) (Fig. 4), while the three mitotic parthenogenetic species *M. arenaria*, *M. incognita* and *M. javanica* grouped together with high support value (98%).

Phylogenetic relationships of *M. graminicola* based only on ITS1 of a multiple edited alignment including 96 sequences, 62 of which *M. graminicola* sequences, and 303 total characters was also determined (data not shown). The groupings were the same as those observed in Fig. 4; *M. graminicola* sequences formed a well supported group that clustered all isolates coming from different countries. These results, together with those obtained when the entire ITS was used, confirmed the high intraspecific variability among *M. graminicola* isolates that do not correspond to geographical location and agree with previous results (Pokarel et al. 2007; Bellafiore et al. 2015; Htay et al. 2016). The low level of genetic distance among the 62 *M. graminicola* isolates suggests a recent expansion of this

species as reported by Mantelin et al. (2016). Furthermore, the high bootstrap value in the ML tree confirmed that *M. graminicola* formed a well separated cluster from the other *Meloidogyne* species resulting phylogenetically related to *M. naasi*. Both species, in fact, possess similar morphological traits and infect rice; however they can be morphologically easily distinguished by the pattern of female perineal region (with a distinct fold over the anus and large phasmids in *M. naasi*, both absent in *M. graminicola*), by the position of excretory pore in female (ca. at level of stylet knobs vs more than one stylet length posterior to stylet knobs in *M. graminicola*) and by the tail terminus shape of J2, tapered and sharply pointed in *M. naasi* vs rounded and often clavate in *M. graminicola*. Phylogenetic relationships of *M. graminicola* based on *co*II-16SrRNA of a multiple edited alignment including 42 sequences, of which 14 from *M. graminicola*, showed seven well supported groups (Fig. 5). *Meloidogyne graminicola* clade contained all sequences *of M. graminicola* including those from Italy with a high bootstrap support value of 97%. Italian *M. graminicola* populations formed a well supported subgrouping with *M. graminicola* from the United States populations that were designed by McClure et al. (2012) as type A for the presence of one *Dra*I and four *Ssp*I restriction sites. *In silico* analysis of *mt*DNA sequences of Italian *M. graminicola* revealed the presence of one *Dra*I and four *Ssp*I restriction sites. Both observations clearly confirmed that the Italian *M. graminicola mt*DNA also belongs to the *mt*DNA type A. Furthermore, *M. graminicola* clade resulted phylogenetically close to two well supported clades: one containing *M. chitwoodi* and the second containing *M. naasi*, resulting well separated from the other *Meloidogyne* species (98% support). Although the topologies of the two trees (nuclear and mitochondrial) were a little different (Figs. 4 and 5) Italian and database *M. graminicola* populations clustered all together with a high bootstrap value. In addition to previous studies, our results clearly show that mitotic parthenogenetic *Meloidogyne* (*M. hapla*, *M. arenaria*, *M. javanica*, and *M. incognita*) species are evolutionary distant from facultative meiotic partenogenetic species (*M. graminicola*, *M. naasi*, *M. chitwoodi* and *M. minor*). These results suggest a monophyletic origin of facultative meiotic parthenogenetic *Meloidogyne* spp. and could explain the high heterogeneity of these species compared to the mitotic parthenogenetic species.

In conclusion, our phylogenetic analyses demonstrates that *M. graminicola* was present in all rice fields surveyed, regardless of climatic and environmental conditions and rice cultivars that might contribute to the high genetic heterogeneity of the nematode. The presence of *M. graminicola* was also observed on weeds associated to rice fields in Italy as in other countries suggesting that they could have an important role in reproduction and diffusion of the rice root-knot nematode.

In summary, the present study enlarges the molecular characterization of *M. graminicola* and provides additional sequences for precise and unequivocal diagnosis of this species. Furthermore, this research add more information to the wide dissemination and phylogenetic relationships of *M. graminicola*, highlighting the paramount importance for appropriate adoption of control measures leading to the exclusion of the nematode from rice fields.

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**Figure legends**

**Fig. 1**. Light micrographs of female, second-stage juveniles and male of *Meloidogyne graminicola* from Italy. A-C: Female perineal pattern of population from upland rice field; D: Mature female entire body; E: Second-stage juvenile (left) and male (right) entire body; F: Male pharyngeal region; G: Male anterior end; H: Male lateral field at midbody; I,J: Male tail showing spicula; K,L: Female perineal pattern of population from lowland rice. (Scale bars: A-C, K, L = 20 µm; D, E = 100 µm; F-J = 10 µm).

**Fig. 2**. Restriction profiles of the ITS region of upland and lowland rice populations of *Meloidogyne graminicola* from Italy separated on 2.5% agarose gel. M: 100 bp DNA ladder; 1) and 5) *Alu* I; 2) and 6) *Hinf* I; 3) and 7) *Hind* III; 4) and 8) *Hae* III.

**Fig. 3.** Specific amplification by using the SCAR primers SCAR-MgFW/SCAR-MgRev for *Meloidogyne grminicola*. M: 100 bp DNA ladder: 1) SCAR-MgFW/SCAR-MgRev; 2) ITS region; 3) MnSCARF/MnSCARev; 4) Finc/Rinc; 5) INCK14F/INCK14R; 6) Fjav/Rjav; 7) SCARMjF/SCARMjR; 8) coII-16SrRNA; 9) SCAR-MgFW/SCAR-MgRev.

**Fig. 4.** Phylogenetic tree of ITS containing region describing the evolutionary relationships among different geographical populations of *Meloidogyne graminicola* using Maximum Likely (ML) method. Branch lengths are proportional to the distances as derived from the distance matrix obtained using the GTR method with the invariant site plus gamma options. Numbers at nodes indicate bootstrap values.

**Fig. 5.** Phylogenetic tree of partial mitochondrial *co*II-16SrRNA sequences describing the evolutionary relationships among different geographical populations of *Meloidogyne graminicola* using Maximum Likely (ML) method. Branch lengths are proportional to the distances as derived from the distance

matrix obtained using the GTR method with the invariant site plus gamma options. Numbers at nodes indicate bootstrap values.

**Table 1.** Morphometrics of males and second-stage juveniles (J2s) of *Meloidogyne graminicola*. All measurements are in µm and in the form: mean ± s.d. (range).

| Characters | Males | J2s |
|---|---|---|
| n* | 10 | 10 |
| L | 1264 ± 105.3 (1052-1420) | 441 ± 22.3 (416-485) |
| a | 42.1 ± 3.2 (37.6-47.3) | 28.0 ± 1.9 (25.8-30.9) |
| b | 11.6 ± 0.8 (10.8-12.6) | 5.6 ± 0.2 (5.3-5.7) (5) |
| b' | 6.6 ± 0.6 (5.3-7.5) | 3.3 ± 0.4 (2.4-4.0) |
| c | 129 ± 31.2 (94.1-180) | 6.3 ± 0.5 (5.8-7.1) |
| c' | 0.3 ± 0.1 (0.2-0.5) | 6.4 ± 0.4 (5.6-7.2) |
| Stylet | 15.9 ± 0.5 (15.5-16.9) | 10.6 ± 0.6 (10.0-11.8) |
| D.G.O. | 4.3 ± 0.8 (3.5-5.8) | 2.9 ± 0.3 (2.6-3.6) |
| Anterior end to centre of | | |
| cardia | 110 ± 10.8 (99-120) (5) | 77 ± 3.9 (73.8-82.5) (5) |
| end of pharyngeal gland lobe | 193 ± 10.9 (180-218) | 137 ± 19.4 (112-176) |
| secretory/excretory pore | 122.5 ± 13.6 (107-153) | 74 ± 4.0 (70.8-80) |
| Maximum body diam | 30.1 ± 1.7 (27.3-31.7) | 15.8 ± 0.5 (15.5-16.5) |
| Testis length | 801 ± 140.8 (533-961) | - |
| T (%) | 63.2 ± 8.9 (43.8-74.2) | - |
| Tail length | 10.2 ± 2.2 (7.2-13.5) | 70.0 ± 5.7 (60.0-78.5) |
| Tail jaline region | - | 21.0 ± 1.1 (19.5-23.0) |
| Anal body diam. | 14.8 ± 1.7 (12.0-17.7) | 11.0 ± 0.9 (9.8-12) |
| Spicule length (*) | 25.9 ± 1.1 (23.5-26.9) (8) | - |
| Gubernaculum (*) | 6.5 ± 0.6 (5.5-7.0) (6) | - |

* A number in parentheses after the range refers to the number of specimens measured when this is less than the headline number.