# *Meloidogyne* spp. from Preserved Areas of Atlantic Forest in the State of Rio de Janeiro, Brazil*

INORBERT M. LIMA[1*], RICARDO M. SOUZA[1], CARINA P. SILVA[1] & REGINA M.D.G. CARNEIRO[2]

* Part of the first author's MS Dissertation.
[1] Universidade Estadual do Norte Fluminense, CCTA/LPP. Av. Alberto Lamego, 2000, 28015-620, Campos dos Goytacazes (RJ), Brazil, ricmsouza@censanet.com.br
[2] Embrapa/Cenargen, C.P. 2372, 70849-970, Brasília (DF), Brazil.

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**Summary** – Lima, I.M., R.M. Souza, C.P. Silva & R.M.D.G. Carneiro. 2005. *Meloidogyne* spp. from preserved areas of Atlantic Forest in the State of Rio de Janeiro, Brazil.

A survey for the presence of root-knot nematodes (RKN), *Meloidogyne* spp., was conducted in six areas of Atlantic Forest with the vegetation types montane forest or "restinga". A total of 360 samples, each composed of 2.5 L of soil and roots, were taken from the rizosphere of trees, palms, shrubs and herbaceous species. In order to detect RKN in the samples, bioassays were carried out using tomato as indicators for a period of 75 to 90 days. RKN were isolated from 21 samples collected in the montane forest, from which 23 monospecific isolates were obtained. These isolates were identified on the basis of esterase isozyme phenotypes and morphology of females, males and second-stage juveniles. Twelve isolates were identified as *M. javanica*, six as *M. exigua*, two as *M. incognita*, one as *M. arenaria*, one as *M. mayaguensis*, and one remained unidentified. This work highlights the diversity of RKN in the montane forest, while our failure in recovering isolates from the "restinga" suggests that this vegetation type may not be conducive to *Meloidogyne*, or at least to those species found in the montane forest.

**Keywords**: montane forest, nematofauna, restinga, root-knot nematode, biodiversity

**Resumo** - Lima, I.M., R.M. Souza, C.P. Silva & R.M.D.G. Carneiro. 2005. *Meloidogyne* spp. oriundas de áreas preservadas de Mata Atlântica do Estado do Rio de Janeiro, Brasil.

Um levantamento para detecção do nematóide das galhas (NDG), *Meloidogyne* spp., foi conduzido em seis áreas de Mata Atlântica com os tipos de vegetação floresta de altitude ou restinga. Trezentas e sessenta amostras de 2,5 L de solo e raízes foram coletadas da rizosfera de árvores lenhosas, palmeiras, arbustos e herbáceas. Para detecção do NDG, as amostras foram cultivadas com tomateiros por 75-90 dias. NDG foram isolados de 21 amostras coletadas na floresta de altitude, das quais 23 isolados monoespecíficos foram obtidos. A identificação destes isolados foi baseada no fenótipo da isoenzima esterase e na morfologia de fêmeas, machos e juvenis de segundo estádio. Doze isolados foram identificados como *M. javanica*, seis como *M. exigua*, dois como *M. incognita*, um como *M. arenaria*, um como *M. mayaguensis*, e um isolado não foi identificado. Esse trabalho evidencia a diversidade de NDG na floresta de altitude, ao passo que a não recuperação de isolados da restinga sugere que esse tipo de vegetação não é favorável aos NDG, ou pelo menos às espécies encontradas na floresta de altitude.

**Palavras-chave**: nematofauna, floresta de altitude, restinga, nematóide das galhas, biodiversidade.

## Introduction

The signs of a recent resurgence of interest in systematics are everywhere. The worldwide concern for nature conservancy and biodiversity has been generously covered by the media, and scientists are debating over the perspectives and challenges ahead (Godfray, 2002; Mallet & Willmot, 2003; Wilson, 2003). New funding opportunities exist, and technological support from software, the internet, and molecular biology have attracted scientists to work on the systematics of many groups of organisms, sometimes in the framework of initiatives such as Biota (www.fapesp.br), All-Species (http://www.all-species.org/), and The Tree of Life (http://tolweb.org/tree/phylogeny.html). Specifically on Nematoda, even high category classification is under debate (De Ley & Blaxter, 2001), and many surveys have been conducted focusing on several groups of nematodes.

In Brazil, recent surveys of plant-parasitic nematodes have focused on specific crops, such as banana, soybean and coffee, and on the root-knot nematodes (RKN), *Meloidogyne* Goeldi, 1892, in agricultural or natural areas. Of the Brazilian biomes, some areas of the Amazon Forest were surveyed by Cares (1984), while the "cerrado" (Brazilian savannah) has been the subject of several taxonomic and ecological studies, such as those of Cares & Huang (1991), Souza *et al.* (1994), and Mattos (1999).

Until the 16th century, the Atlantic Forest was one of the most extensive and biodiverse biomes of what is now Brazil. Its vegetation types include lowland and montane forests, high-altitude grassland, mangrove swamp, and "restingas" www.rma.org.br/mataatlantica/mata_centro.htm). "Restinga" denotes fauna and flora thriving in marine sand deposits along the Brazilian shore, extending up to 30 kilometers inland in the State of Rio de Janeiro (Lacerda *et al.*, 1993).

Although reduced to around 10% of its original area, the Atlantic Forest still holds astonishing biodiversity and a high level of endemism in several groups of organisms, which led Myers *et al.* (2000) to include it among the biomes that demand urgent conservancy efforts. As pointed out by Rocha *et al.* (2003), the Atlantic Forest's fauna and flora have been the focus of an increasing number of studies, but apparently nothing is known about its nematofauna. This article reports the results of a RKN-focused survey carried out in preserved areas of Atlantic Forest of the State of Rio de Janeiro.

## Materials and Methods

Areas of montane forest and "restinga" in the North and "Serrana" regions of the State (Figure 1) were sampled from April 2001 through September 2002. The four montane forest areas sampled are well preserved are generally difficult to access because of the accentuated topography and/or high altitude, and have been unaffected by agricultural activities in the past; nor are there such activities near them today. Two of these areas suffered logging for hardwoods decades ago. In the municipality of Campos dos Goytacazes, 70 samples were collected in the locality of Fazenda Babilônia, at an altitude of 200-300 m.a.s. In Santa Maria Madalena, 40 samples were collected in the locality of Terras Frias, at 600-750 m.a.s. These two areas are part of the Desengano State Park. In Macaé, 70 samples were collected in the locality of Bicuda Pequena, at 200-300 m.a.s. In Nova Friburgo, 100 samples were collected in the locality of Serra da Sibéria, at 1,200-1,400 m.a.s., for a total of 280 samples collected from the montane forest.

The "restinga" areas sampled are part of the Restinga de Jurubatiba National Park, which spreads over the municipalities of Carapebus and Quissamã, at an altitude of 10-20 m.a.s. Forty samples were collected in a locality with sparse shruby vegetation, and 40 samples were collected in a locality with forest, for a total of 80 samples collected from the "restinga".

In all the localities, samples were taken in at least two different locales (e.g., two distinct hills, with the collection sites spread from 5 to 30 m apart), along arbitrarily chosen paths open through the forest or the "restinga". Each collection site was numbered, and it received a position marker - colorful spray paints on the tree trunks, or colorful plastic tags or stripes attached to the plants. Samples consisted of 2.5 L of soil and roots collected at one point, to a depth of 25 cm, from the rhizosphere of woody trees, palms, shrubs, and herbaceous plant species. Because the plants sampled were never growing far from others, the samples might include roots from more than one plant species. The soil collected was taken to a greenhouse, where tomato (*Lycopersicum esculentum* L.) plants cv Santa Cruz were grown in a bioassay to detect RKN. These plants were irrigated with filtered water only. After 75 to 90 days, the root systems of all plants were inspected for RKN egg masses. Whenever positive, the RKN isolates were established by chopping the root systems and using them to reinoculate tomato plants grown in autoclaved soil.

After 90 days, each RKN isolate was purified by extraction of 27 egg-laying females from the tomato plants, with their respective egg masses being kept in water. The females were submitted to eletrophoresis to reveal their esterase isozyme phenotypes, according to Carneiro & Almeida (2001). In each electrophoretic run, a lane was reserved for a *M. javanica* (Treub, 1985) Chitwood, 1949 female used as a phenotyping reference. The egg masses of females with the same phenotypes

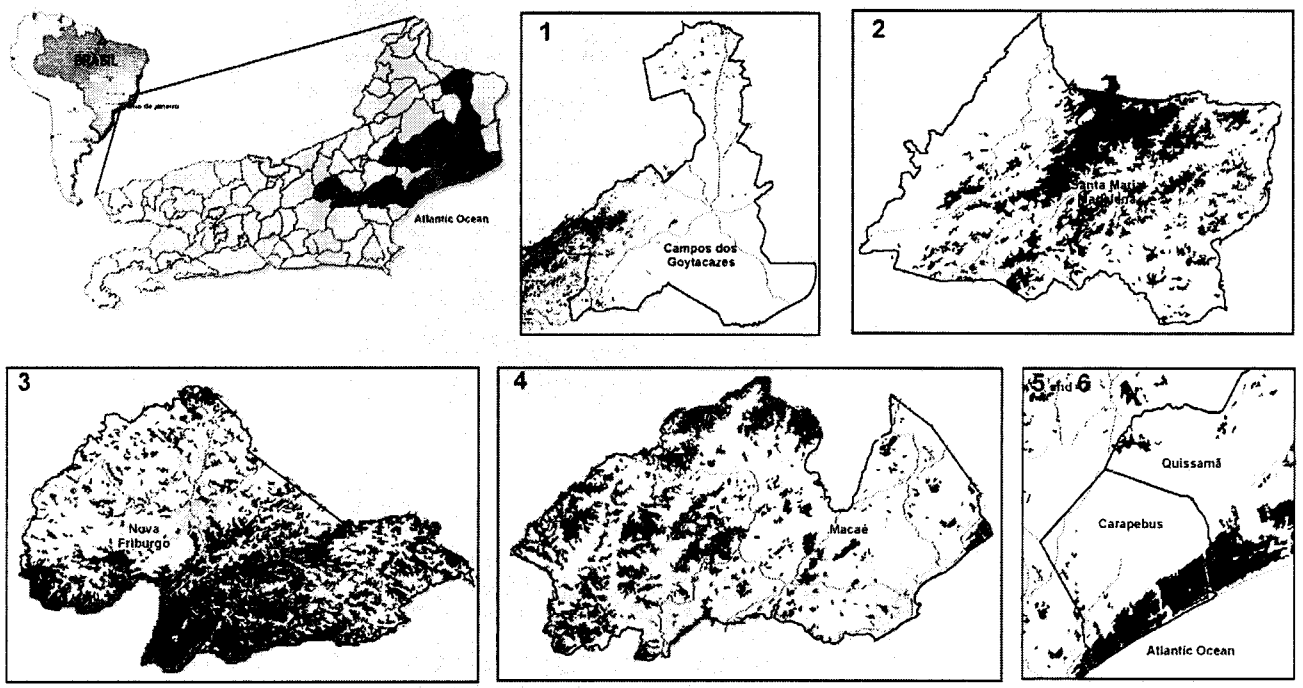


Figure 1 – Municipalities sampled for detection of Meloidogyne spp. in the State of Rio de Janeiro, Brazil. 1: Campos dos Goytacazes, 2: Santa Maria Madalena, 3: Nova Friburgo, 4: Macaé, 5: Carepebus, and 6: Quissamã. Shaded areas represent preserved areas of Atlantic Forest

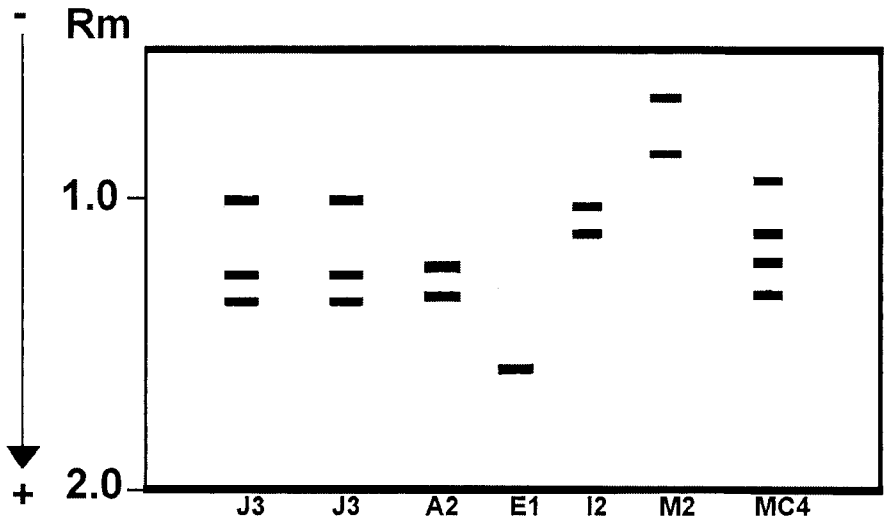


Figure 2 - Schematic representation of esterase isozyme phenotypes (Est) resolved from *Meloidogyne* spp. isolates from preserved montane forest in the State of Rio de Janeiro, Brazil. First lane from left to right, *M. javanica* (Est J3) used as reference, and isolates MC83 (*M. javanica,* Est J3), CG58b (*M. arenaria,* Est A2), MM04 (*M. exigua,* Est E1), CG74 (*M. incognita,* Est I2), CG52 (*M. mayaguensis,* Est M2) and isolate MC 100b (*Meloidogyne* sp., Est MC4)

were collectively reinoculated into tomato plants grown in autoclaved soil for another 90 days. These purified RKN isolates received codes after the municipalities which they came from, followed by the number of the collection site.

Before proceeding to the taxonomic identification of the purified RKN isolates, another round of esterase phenotyping was performed with 27 females per isolate, to confirm their purity.

The taxonomic identification of all isolates was performed by esterase isozyme phenotypes, and light microscopy examination. For each isolate, at least 15 perineal patterns and other morphological characters were examined in females, males and second-stage juveniles (J2), as suggested by Hirschmann (1985), Jepson (1987), and Eisenback & Triantaphyllou (1991).

For each case where RKN were recovered, the authors returned to the collection site to obtain plant material for the taxonomic identification of the associated (putative) host, from which rizosphere the sample was collected.

## Results

RKN were recovered from 21 (7.5%) of the 280 samples collected in the montane forest. From these positive samples, 23 monospecific RKN isolates were established. In the "restinga" areas, RKN were not recovered from any of the samples.

Six esterase phenotypes were found amongst the 23 RKN isolates (Figure 2). The phenotypes J3 (*M. javanica*), A2 (*M. arenaria* (Neal, 1889), Chitwood, 1949), E1 (*M. exigua,*

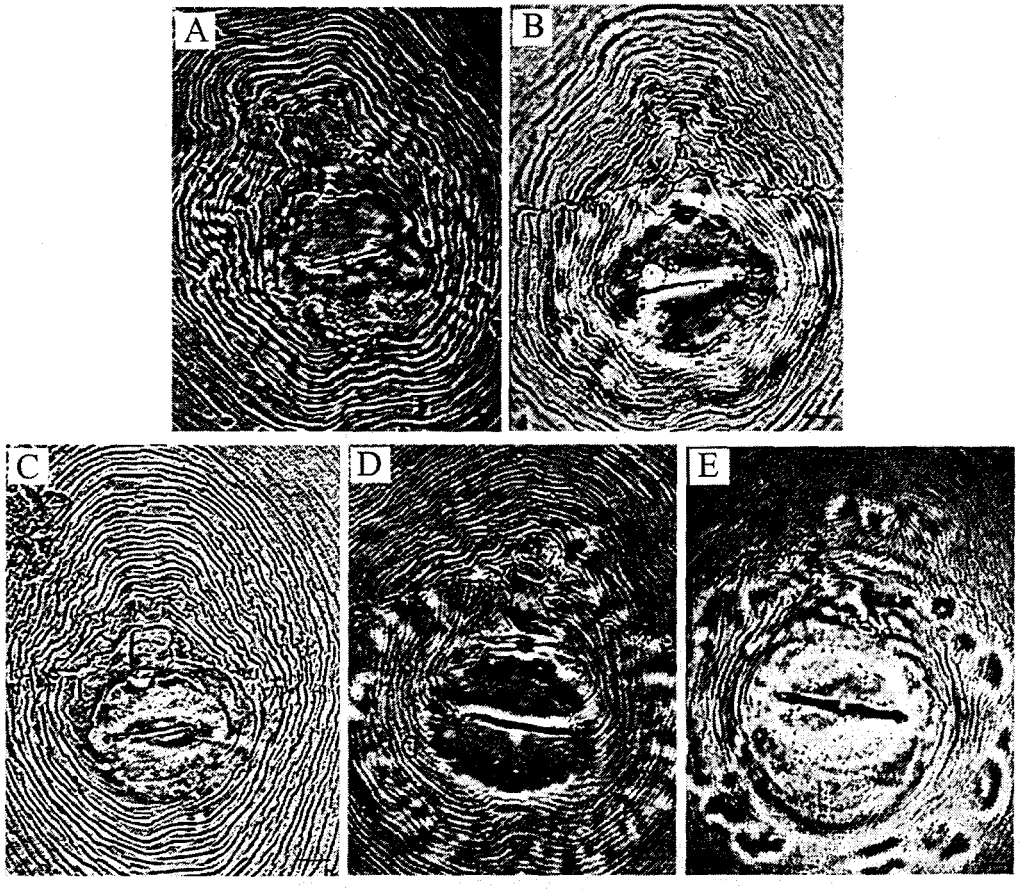


Figure 3 - Perineal patterns of *Meloidogyne* isolates from preserved montane forest in the State of Rio de Janeiro, Brazil. A: isolate CG72 (*M. incognita*), B: CG117 (*M. javanica*), C: MM16 (*M. exigua*), D: CG58b (*M. arenaria*), and E: CG52 (*M. mayaguensis*). Scale = 10 μm

Goeldi, 1892), I2 (*M. incognita* (Kofoid &White, 1919) Chitwood, 1949), and M2 (*M. mayaguensis* Rammah &Hirschmann, 1988) had been reported by Esbenshade & Triantaphyllou (1985), Pais & Abrantes (1989), and Carneiro *et al.* (2000). An unreported phenotype MC4 (*Meloidogyne* sp.) was resolved for one RKN isolate. The relative mobility of the esterase bands were 1.0, 1.25, and 1.33 for the phenotype J3, 1.2, and 1.3 for A2, 1.55 for E1, 1.1, and 1.2 for I2, 0.7,

Table 1. Identification of *Meloidogyne* isolates found in preserved areas of montane forest in the State of Rio de Janeiro, Brazil.

| Species | Isolate Codes |
|---|---|
| *M. mayaguensis* | CG52[1] |
| *M. arenaria* | CG58b |
| *M. incognita* | CG 72, and 74 |
| *M. exigua* | MM 04, 16, 17, 19, 21, and 27 |
| *M. javanica* | CG 58a, 75, 76, 111, 112, 114, 116, and 117; MC83, and 100a; NF84, and 93 |
| *Meloidogyne* sp. | MC 100b |

[1] The letters in the isolate codes indicate the municipalities from which the isolates were recovered. CG: Campos dos Goytacazes, MM: Santa Maria Madalena. NF: Nova Friburgo, and MC: Macaé

Table 2. Morphometrics of *Meloidogyne* spp. found in preserved areas of montane forest in municipalities of the State of Rio de Janeiro, Brazil (measurements in μm)

| Species | Municipality | Characters | | | | | |
|---|---|---|---|---|---|---|---|
| | | Female | | Male | | J2 | |
| | | **Stylet length** | **DEGO[3]** | **Stylet length** | **DEGO** | **Stylet length** | **DEGO** |
| *M. javanica* | CG[1] | 15.9[2] | 3.6 | 19.7 | 2.8 | 54.5 | 14.7 |
| | | (13.5-18.1) | (2.3-5.6) | (18.3-23.1) | (1.9-4.1) | (49.5-56.2) | (49.5-56.2) |
| | | 119 | 119 | 91 | 91 | 103 | 103 |
| | MC | 15.1 | 4.9 | 18.1 | 2.5 | 59.1 | 15.3 |
| | | (12.9-17.6) | (2.9-7.0) | (16.7-23) | (2.1-3.3) | (51.3-62.6) | (9-18.1) |
| | | 28 | 28 | 22 | 22 | 27 | 27 |
| | NF | 17.4 | 5.1 | 21.1 | 3.1 | 61.2 | 17 |
| | | (13.8-19.3) | (4.2-8.6) | (18.2-25) | (2.8-4.7) | (53.6-65.8) | (10.3-11.7) |
| | | 29 | 29 | 23 | 23 | 29 | 29 |
| *M. exigua* | MM | 13.8 | 4.2 | 18.8 | 3.2 | 45.5 | 12.8 |
| | | (11.5-14.7) | (3.6-7.6) | (17.1-20.1) | (2.3-4.7) | (43.1-46.5) | (11.6-15.1) |
| | | 87 | 87 | 83 | 83 | 90 | 90 |
| *M. arenaria* | CG | 14.3 | 5.3 | 24.7 | 5.8 | 55.3 | 14.2 |
| | | (12.7-18.3) | (2.9-7.6) | (20-27.6) | (4.1-9) | (45.8-71.2) | (8.5-15.7) |
| | | 14 | 14 | 13 | 13 | 15 | 15 |
| *M. incognita* | CG | 15.8 | 2.7 | 24.1 | 2.7 | 48.2 | 7.4 |
| | | (13.9-17.2) | (2.1-4.8) | (23.3-26.8) | (2-4.6) | (42.2-58.1) | (6-12.2) |
| | | 28 | 28 | 26 | 26 | 30 | 30 |
| *M. mayaguensis* | CG | 14.9 | 4.1 | 22.7 | 3.9 | 51.5 | 11.1 |
| | | (13.5-17.3) | (3.7-6.1) | (21.1-25.6) | (3.2-4.6) | (48.6-55.5) | (10.3-13.1) |
| | | 15 | 15 | 15 | 15 | 15 | 15 |

[1] CG: Campos dos Goytacazes, MC: Macaé, NF: Nova Friburgo, and MM: Santa Maria Madalena

[2] Mean, minimum, and maximum values (between parenthesis), and number of specimens measured

[3] DEGO: Distance from the dorsal esophageal gland orifice to the base of the stylet

Table 3. *Meloidogyne* spp. found in preserved areas of montane forest in the State of Rio de Janeiro, Brazil, and their putative hosts, from whose rizosphere the nematodes were recovered

| Nematode species | Putative host | |
|---|---|---|
| | Common name | Scientific name |
| *M. mayaguensis* | Sucanga | *Senefeldera multiflora* Mart |
| *M. arenaria* | Açá | *Ecclinusa ramiflora* Mart |
| *M. incognita* | Jacatirão | *Miconia cinnamonifolia* Naud |
| | Sucupira | *Sclerolobium* sp |
| *M. exigua* | Açoita-cavalo | *Luhea* sp. |
| | Pindaíba | *Guatteria nigrensis* Mart |
| | Pau-magro | *Cupania oblongifolia* Mart |
| | Pitomba | *Talisia esculenta* Mart |
| | Guapeba | *Bathysa stipulata* Prest |
| | Sobrasil | *Colubrina glandulosa* Perk |
| *M. javanica* | Aça | *Ecclinusa ramiflora* Mart. |
| | Pindaíba | *Casearia* sp. |
| | Iricurana | *Hyeronima alchomeoides* Fr. All. |
| | Goiabão | *Eugenia leitonni* Legr. |
| | Embaúba | *Pourouma guianensis* Aubl. |
| | Sucanga | *Senefeldera multiflora* Mart. |
| | Macuqueira | *Bathysa meridionalis* Smith |
| | Irí | *Attalea dubia* Mart. |
| | Carrapeta | *Guarea* sp. |
| | Cambará | *Gochnatia polymorpha* Cabr. |
| | Jacatirão | *Miconia cinnamonifolia* Naud. |
| *Meloidogyne* sp., isolate MC100 | Carrapeta | *Guarea* sp. |

and 0.9 for M2, and 0.9, 1.1, 1.2, and 1.28 for MC4. The band 1.1 of MC4 was sometimes not visible, and the band 1.2 of I2 could only be seen with five macerated females.

The identification of 22 of the 23 RKN isolates (Table 1) was achieved by the esterase phenotypes, and the morphology of perineal patterns, males, females and J2 (Figure 3, Table 2). The associated hosts are listed in Table 3.

## Discussion

The isolate CG52 was identified as *M. mayaguensis* Rammah & Hirschmann, 1988, an economically important species first reported in Brazil by Carneiro *et al.* (2001). The collection site is located around 50 km from guava (*Psidium guajava* L.) fields that were known to be infested by this species, in the municipality of São João da Barra. The indigenous ocurrence of *M. mayaguensis* in Brazil still needs to be confirmed.

No morphological data or perineal patterns are presented for the isolate MC100b of *Meloidogyne* sp., which grew poorly under greenhouse conditions. Further studies will be conducted to better characterize this isolate.

This work suggests interesting patterns for RKN distribution in the Atlantic Forest that deserve further investigations. In 19 of the 21 samples that were positive for RKN, only one species was recovered. This contradicts the notion that in natural areas related nematode species could coexist, parasitizing the same plant species.

Also, forests at lower altitudes seemed more biodiverse for RKN, with *M. javanica* being the dominant species. In recent surveys in Brazil, *M. javanica* showed to be the most widespread species on soybean (Cartro *et al.*, 2003) and *Musa* (Cofcewicz *et al.*, 2004).

*Meloidogyne exigua*, a widespread species affecting coffee plantations in the State of Rio de Janeiro (Barbosa *et al.* 2004), also was found in the montane forest. It seems reasonable to think that indigenous populations of *M. exigua* existed in the montane forest and adapted to "arabica" coffee when large areas of forests were cleared for agriculture in the 19th century. This could lead to the widespread yield losses described by Goeldi (1892). The same is thought to have happened to *M.*

*coffeicola* (Jaehn *et al.*, 1980). These reports suggest that forest soils should not be used in nurseries without proper fumigation or other treatment against nematodes.

Finally, none of the 80 samples collected in the "restinga" was positive for RKN. This was an expected result because one would expect to recover around six isolates if one considers the same rate (7.5%) as observed in the montane forest.

This work highlights the RKN biodiversity of the Atlantic Forest, and further studies should be encouraged in order to get more information on the nematofauna diversity in the different Brazilian biomes. Furthermore, this work helped to increase the existing RKN collection, which will be available to researchers who are interested in *Meloidogyne* biogeography and systematics.

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