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1 **Integrative taxonomy of *Meloidogyne ottersoni* (Thorne,1969) Franklin, 1971 (Nematoda:**
2 ***Meloidogynidae*) parasitizing flooded rice in Brazil**

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14

15 *Abstract:* A root-knot nematode (RKN) parasitizing rice (*Oryza sativa* L.) and causing
16 damage in Santa Catarina (SC), Rio Grande do Sul (RS) and Paraná (PR) states (Brazil) was
17 identified as *Meloidogyne ottersoni* (Thorne, 1969) Franklin,1971. The species is redescribed
18 from the Brazilian population from Meleiro (SC) and compared with the type description of
19 *M. ottersoni* from Wind Lake (Wisconsin, USA) with additional morphological, biochemical
20 and molecular characterization. The female and male bear smaller stylets when compared
21 with *M. graminicola* and *M. oryzae* (10-12 µm and 14-16 µm, respectively, vs 12-14 µm and
22 16-18 µm, and 14-16 µm and 28-20 µm, respectively). *Meloidogyne ottersoni* presents
23 perineal patterns located on the contour of a slight protuberance. Striae are mostly continuous,
24 never raised by transverse irregular striae, as frequently observed in *M. graminicola* and *M.*
25 *oryzae*. *Meloidogyne ottersoni* belongs to the RKN group 11 described by Jepson (1987), the
26 reproduction is by meiotic parthenogenesis and the somatic chromosome number is 18. The
27 tail of second-stage juveniles is very long and thin, and tapers to a long, narrow, irregular
28 hyaline terminus (*M. ottersoni*, 20.5 µm vs *M. graminicola*, 17.9 µm and *M. oryzae*, 22.0 µm,
29 respectively). The ability of the Brazilian *M. ottersoni* population to parasitize reed canary
30 grass, *Phalaris arundinacea* L., was confirmed. Biochemically, the esterase profile of *M.*
31 *ottersoni* lacks any band (Est Ot0, Rm=0), which differentiates it from *M. graminicola* and *M.*
32 *oryzae* (Est VS1, Rm=0.70 and Est O1, Rm=1.02, respectively). In Maximum Likelihood
33 analysis of ITS, D2D3 and COXII-16S rRNA sequences, populations of *M. ottersoni* from

34 different states of Brazil clustered together and were separated from other *Meloidogyne* spp.,
35 thus confirming that all four populations are very similar and conspecific.

36

37 Keywords: COXII, D2D3, esterase phenotyping, ITS, morphology, *Oryza*, root-knot
38 nematode.

39

40 **Introduction**

41

42 Rice (*Oryza sativa* L.) is the third most produced cereal worldwide, just after corn and
43 wheat, and plays a key role in the current and future food security across the globe. Outside
44 Asia, Brazil is the largest rice-producing country (Chauhan *et al.*, 2017), the Southern
45 Brazilian states being responsible for more than 80% of all national production, with the
46 predominance of flooded rice (Conab, 2018).

47 Several factors can interfere in rice crop yield, including nematodes. Nematodes from
48 more than 35 genera have been reported parasitizing rice plants, but few species are described
49 causing significant damage (Khan, 2015). In particular, the flooded rice system determines the
50 community of plant parasitic nematodes by selecting the most adapted ones, and several
51 authors reported the predominance of *M. graminicola* Golden and Birchfield, 1965 in this
52 cropping system (De Waele & Elsen, 2007; Khan, 2015).

53 All the populations in the *Meloidogyne* species complex detected on rice in Brazil
54 (Mattos *et al.*, 2017) belong to the 'graminis group' that includes some species with similar
55 characteristics: females deeply embedded in the host and body elongate with different forms,
56 vulva situated on posterior protuberance, second-stage juveniles with hyaline part of the tail
57 very thin and long, meiotic parthenogenesis as the mode of reproduction, males common
58 (Jepson, 1987). A recent survey in Southern Brazil detected *M. graminicola* and other atypical
59 esterase phenotype populations of *Meloidogyne* spp. in rice fields of Rio Grande do Sul (RS),
60 Santa Catarina (SC) and Paraná (PR) states, causing damage under field conditions (Negretti
61 *et al.*, 2017; Mattos *et al.*, 2018). In these studies, enzymatic and molecular analysis revealed
62 the existence of a 'species complex' parasitizing rice in the Southern region of Brazil. In the
63 survey conducted by Negretti *et al.* (2017), a particular population provisionally named
64 *Meloidogyne* sp.0, lacking any band in its esterase profile, was not detected, probably due to
65 this biochemical character. Subsequently, the same population was detected in four different
66 municipalities in three Brazilian southern states: Capão do Leão (RS), Meleiro (SC), Nova
67 Veneza (SC) and Santa Cruz de Monte Castelo (PR) (Mattos *et al.*, 2017). At the same time, a

68 new root-knot nematode parasitizing rice in SC state, Brazil was identified as *Meloidogyne*
69 *oryzae* Maas, Sanders and Dede, 1978. This species was redescribed from this Brazilian
70 population and compared with the type description of *M. oryzae* from Suriname, with
71 additional morphological, biochemical and molecular characterization (Mattos *et al.*, 2018).

72 The identification of *Meloidogyne* species is not an easy task, considering the large
73 number of species belonging to the genus, their worldwide distribution and their wide host
74 range. The morphological similarity between species is also a factor that makes identification
75 difficult, since inter and intra-specific differences are very subtle (Jepson, 1987; Karssen &
76 Moens, 2006; Moens *et al.*, 2009). Alternatively, molecular methods have been used
77 successfully to identify *Meloidogyne* species. In particular, for *M. graminicola*, sequences of
78 nuclear ribosomal (rDNA) and mitochondrial DNA (mtDNA) have been largely applied
79 (Pokharel *et al.*, 2007, 2010; Bellafiore *et al.*, 2015; McClure *et al.*, 2012; Htay *et al.*, 2016).
80 Recently, two diagnostic SCAR markers were proposed for this species (Bellafiore *et al.*,
81 2015; Mattos *et al.*, 2018) and also for *M. oryzae* and *M. salasi* Lopez, 1984 (Mattos *et al.*,
82 2018).

83 In a recent survey, RKN samples exhibiting an esterase phenotype without any band
84 (i.e., corresponding to the unknown species *Meloidogyne* sp.0) were detected as pure
85 populations on rice in field conditions, predominantly in the SC state and less frequently in
86 the PR and RS states (Mattos *et al.*, 2017). Therefore, the aim of this study was to identify and
87 characterize this unknown *Meloidogyne* sp.0 using an integrative taxonomy approach. Based
88 on morphological, morphometrical and molecular differences with other *Meloidogyne* species
89 from the 'graminis group', we assign *Meloidogyne* sp.0 to the species *Meloidogyne ottersoni*
90 (Thorne, 1969) Franklin, 1971, and we report its first occurrence on rice in the Southern
91 region of Brazil.

92

93 **Materials and Methods**

94

95 Populations of nematodes

96

97 Four pure RKN populations exhibiting an esterase phenotype with no band (i.e., *Meloidogyne*
98 sp.0, Rm=0) were sampled in fields from RS and SC states and multiplied on rice plants *O.*
99 *sativa* (IRGA-410 and IRGA-424), in greenhouse conditions, with an average temperature of
100 15-28 °C. All biochemical and molecular studies were done using these four pure cultures
101 (Table 1). Morphological and cytogenetic studies were performed with one population from

102 Meleiro, SC. Egg masses and females were obtained directly from the infected rice roots.
103 Second-stage juveniles (J2) were hatched in humid chambers. Males were obtained placing
104 the infected rice roots in aerated water using an aquarium pump. The specimens were
105 collected periodically from the water (every 24 hours for J2 and 48 hours for males,
106 respectively) (Carneiro *et al.*, 1998).

107

108 Morphological and morphometric studies

109

110 Morphological and morphometric studies were performed with at least 30 specimens from
111 each developmental stage. Males and J2 were killed in cold 2% formaldehyde (5 °C),
112 measured and photographed immediately under a light microscope. Morphometric studies
113 were performed using the measurements suggested by Eisenback & Hunt (2009). The
114 biometric studies were performed with adult females, cut in 45% lactic acid and their anterior
115 region mounted with 2% formaldehyde at room temperature. The perineal cuts were mounted
116 in glycerin (Taylor & Netscher, 1974). The micrographs and measurements were performed
117 in a Zeiss - Axiophot Microscope under white light. Females, males, J2 and stylets (from
118 females and males) were prepared for scanning electron microscopy (SEM) according to the
119 methods proposed by Eisenback (1985). Specimens were viewed and photographed with a
120 scanning electron microscope JEOL-JSM-7001F.

121

122 Biochemical and cytogenetic studies

123

124 The biochemical characterization of the four RKN populations was performed on 7%
125 polyacrylamide gel slabs isozymes in a horizontal electrophoresis system, according to
126 Carneiro & Almeida (2001). *Meloidogyne javanica* (Treub,1885) Chitwood, 1949 (EST J3,
127 MDH N1) was used as reference. Both esterase (EST) and malate dehydrogenase (MDH)
128 enzymes were investigated. Cytogenetic studies were carried out using a propionic orcein
129 staining method (Triantaphyllou, 1985).

130

131 Host Test

132

133 The response of *Meloidogyne* sp.0 to the host type plant of *M. ottersoni*, reed canary grass,
134 *Phalaris arundinacea* L., was evaluated on seeds of this plant obtained from Cobase
135 Cenargen, Embrapa were germinated in 3-l pots. Seedlings 5-cm long were inoculated with

136 5,000 eggs of *Meloidogyne* sp.0 and kept in a greenhouse at an average temperature between
137 15 and 25°C, with watering and fertilization as needed. Fifty-days after inoculation (DAI),
138 the roots were washed thoroughly with tap water, then stained with acid fuchsin (Byrd *et al.*,
139 1983) and observed under a stereoscopic microscope to document the J2 penetration and the
140 development of females and egg laying within the roots.

141

142 Phylogenetic analyses

143

144 For the phylogenetic analyses, three rDNA sequences from the four populations of
145 *Meloidogyne* sp.0 from rice, originating from Brazil (Table 1) were amplified. Namely, the
146 ITS1-5.8S-ITS2 region of rRNA (primer set: forward 5'-TTGATTACGTCCTGCCCTTT-3'
147 and reverse 5'-TCCTCCGCTAAATGATATG-3'; Schmitz *et al.*, 1998); the D2-D3 fragment
148 of the 28S rRNA gene (primer set: forward 5'-ACAAAGTACCGTGAGGGAAAGTTG-3'
149 and reverse 5'-TCGGAAGGAACCAGCTACTA-3'; De Ley *et al.*, 1999); and the 3' portion
150 of COXII and the 5' portion of 16S rRNA mitochondrial region (primer set: forward 5'-
151 GGTCAATGTTTCAGAAATTTGTGG-3' and reverse 5'-TACCTTTGACCAATCACGCT-3';
152 Powers & Harris, 1993), using the PCR conditions described by Subbotin *et al.* (2000). PCR
153 products were cleaned using the Wizard® SV Gel/PCR Clean Up System (Promega) and
154 cloned into the pGem-T® Easy Vector (Promega), following the manufacturer's instructions.
155 Sequencing of the insert was carried out on two independent clones by Macrogen.

156 Sequences cloned were aligned using ClustalW in Mega 5.0.3 (Tamura *et al.*, 2011) and
157 compared with other retrieved sequences from the NCBI database. The program MrModeltest
158 (Nylander, 2004) implemented in PAUP (Phylogenetic Analysis Using Parsimony; v.4.0 b10)
159 was used to identify the best-fit models of each analysis. The phylogenetic trees were
160 generated based on Maximum Likelihood (ML) analysis in IQtree (Trifinopoulos *et al.*,
161 2016). The phylogram was bootstrapped 1,000 times and only support values above 50%
162 were considered.

163

164 Results

165

166 *Meloidogyne* sp.0 infection on rice forms an arcuate, elongated gall and then proceeds
167 to develop a series of similar galls containing some females completely embedded in the root
168 tissue where the eggs are laid. The egg-masses are internal or external. Males are very
169 frequent and they are often found inside the root with females and second-stage juveniles. The

170 population from Meleiro reproduced also on reed canary grass, *Phalaris. arundinacea*,
171 showing J2 within the roots and females with egg masses, 50 days after inoculation (Fig. 9).

172

173 Morphological and morphometric studies

174

175 *Females.* Measurements of 30 females are shown in Table 2. The body is variable in shape
176 and size (410-960, 668 μ m), general oval to pear shaped or elongate, translucent-white, neck
177 variable (78-118 μ m, 92.2 μ m) projecting at different angles, vulval and anus region usually
178 with slight protuberance (Fig.1 a, b), but sometimes this elevation is not present. Perineal
179 patterns rounded/oval dorsoventrally with mostly continuous striae and without marked lateral
180 lines (Fig. 1 a-f), striae coarse, dorsal arch low and rounded. Perivulval region usually free of
181 striae and no striae above de anus. No punctuations observed. Tail terminus and lateral fields
182 not clearly defined and phasmids distinct. Ventral region rounded. Excretory pore located
183 two stylet lenght or less below head end (Fig. 2 a, f). In SEM (Fig. 2 b, c) labial disc fused
184 with medial lips forming a continuous head cap. Lateral lips distinct, triangular. Amphid
185 apertures slightly oval, located at apices of lateral lips, sometimes continuous with the first
186 annule clearly demarcated (Fig. 2 b, c). Stylet robust (9-13 (10.7) μ m), cone straight or
187 slightly curved dorsally and gradually increasing in diameter posteriorly. Shaft gradually
188 widening posteriorly to near junction with stylet knobs, knobs rounded and set off from the
189 shaft (Fig. 2 c, d). Dorsal gland orifice (DGO) 3-6 μ m (4.4 μ m) from the base of stylet.

190

191 *Males.* Measurements of 30 males are shown in Table 2. Body vermiform, variable in size
192 (760-1,420 μ m, 1,181.5 μ m) tapering anteriorly, tail variable in form, sometimes with an
193 expansion (Fig. 3 f), usually twisting 90°. Cuticular annulations coarse (Fig. 3 a-c). Head cap
194 high, rounded, dome shaped; head region distinct from the first body annule, without (Fig 3 a,
195 d) or with some weak annulations (Fig. 3 b, c) under SEM. Labial disc and medial lips fused
196 forming a smooth, continuous head cap (Fig. 3 a-d). Labial disc slightly elevated above
197 medial lips. Lateral lips demarked or not on the head region (Fig. 3 a-d). Amphid apertures
198 forming elongate slits (Fig. 3 a-d). Stylet robust (13-18 μ m, 16.3 μ m), cone pointed at the tip,
199 widening at junction with shaft, shaft cylindrical. Knobs round to oval-shaped sloping
200 backward (Fig. 3 h, i). Excretory canal prominent twisting irregularly visible to the end of
201 gland lobe. DGO 4-6 μ m from stylet base. Lateral fields variables composed of different
202 forms and numbers of incisures (4-7). Spicules and gubernaculum arcuate, phasmids almost
203 terminal.

204

205 *Second-stage juveniles (J2)*. Measurements of 30 J2 are given in Table 2. Body cylindrical
206 vermiform tapering to an elongated tail. Head with slight cephalic frameworks not offset
207 from the body without postlabial annules (Fig. 4 a, b). Stylet delicate (9-13 μm , 10.9 μm),
208 knobs rounded tapering onto shaft. Ampula obscure. Dorsal gland orifice to stylet base (2-5
209 μm , 3.7 μm). Metacarpus broadly oval, valve large and heavily sclerotized (Fig. 4 c).
210 Excretory pore below nerve ring. Anal opening obscure. In SEM, labial disc and medial lips
211 fused forming a smooth, continuous head cap, bow-tie shaped. Six depressed sensilla
212 surrounded pre-stoma (Fig. 4 a, b). Amphid apertures large slit-like. Lateral lips elongated
213 (Fig. 4a, b). Excretory pore immediately posterior to hemizonid. Variable lateral fields usually
214 with 4 incisures. Rectum not inflated. Tail elongated conoid (62-76 μm , 69.2 μm). Hyaline
215 region of tail long, usually without regular annulation (15-25 μm , 20.5 μm), constricting at
216 one, two or three places, tapering to a slender, terminal digitiform tip (Fig. 4 e-i).

217

218 Biochemical and biological studies

219

220 The enzymatic analyzes revealed the lack of any esterase band ($R_m=0$) for the four
221 populations of *Meloidogyne* sp.0. This observation was strengthened by using more than 20
222 macerated females as substrate in each individual analysis (Fig. 5). For the enzyme Mdh, the
223 obtained phenotype is N1a ($R_m: 1.4$), the same as in the species *M. graminicola*, *M. oryzae*
224 and *M. naasi* (Esbenshade & Trianthaphyllou, 1985). The reproduction of *Meloidogyne* sp.0
225 is by meiotic parthenogenesis and the somatic chromosome number is 18.

226

227 Molecular analysis

228

229 Phylogenetic analyses were further conducted to validate the results obtained with
230 morphological approaches and isozymes, comparing sequences of the ITS1-5.8S-ITS2 rRNA
231 segment, the D2-D3 fragment of 28S rRNA, and the partial mitochondrial region between the
232 coxII and the 16S rRNA genes of *Meloidogyne* sp. populations to homologous sequences
233 from other *Meloidogyne* spp. obtained from GenBank. The individual ML trees obtained for
234 all sequences were similar, and showed that the four isolates of *Meloidogyne* sp.0 from Brazil
235 were distributed into a single cluster (99-100% bootstrap values), as shown in Figures 6, 7
236 and 8, thus strongly suggesting that they belong to the same species.

237 Intraspecific variability among the four populations of *Meloidogyne* sp.0, between 1 to
238 4 nucleotides (in a total of 755 nucleotides) for ITS sequences and about 11-16 nucleotides (in
239 743 nucleotides) for D2D3. Population from RS (Ot1) seems to be genetically different from
240 the others from SC (Ot2, Ot3 and Ot4). The coxII-16S rRNA region of *Meloidogyne* sp.0
241 populations also showed a low intraspecific variability; i.e., 1–2 nucleotides in 522. However,
242 this region contains a variable intergenic sequence which clearly separates *Meloidogyne* sp.0
243 populations from other RKN rice related species.

244 *Meloidogyne* sp.0 is closely related to meiotic species like *M. graminicola*, *M. salasi*,
245 *M. naasi* Franklin, 1965, *M. trifoliophila* Bernard and Eisenback, 1997 and other species
246 belonging to Jepson's group 11. Contrarily, *M. incognita*, *M. javanica*, *M. minor* Karszen,
247 Bolk, van Aelst, van den Beld, Kox, Korthals, Molendijk, Zijlstra, van Hoof and Cook, 2004
248 are genetically distant from this species as demonstrated in our ML trees. *Meloidogyne oryzae*
249 was closely related to *Meloidogyne* sp.0 in the D2D3 and CoxII ML trees. However, in the
250 ITS analysis, this species appeared close to mitotic obligatory parthenogenetic species such as
251 *M. incognita* and *M. javanica* (Fig. 6).

252

253 Diagnosis and Relationships

254

255 Based on the results obtained here, we concluded that the four *Meloidogyne* sp.0
256 populations from Brazil characterized in this study indeed belong to the RKN species *M.*
257 *ottersoni*. The correct identification of *M. ottersoni* requires a detailed study of distinctive
258 morphological features of the females, males and J2. Unfortunately, the type material was not
259 available (Jepson, 1987), nor live populations in US collections (Jonathan D. Eisenback and
260 Zafar Handoo, personal information). Comparing the populations from Brazil with the
261 description made by Thorne (1969) and Jepson (1987), it was possible to see that they present
262 similar morphological and morphometrical features, as well as the following common
263 characters described for the population of *M. ottersoni* from Wisconsin, USA. Females
264 elongate, lemon shaped to nearly rounded. Perineal patterns located on the contour of the
265 protuberance, rounded to oval; dorsal arch hemispherical with slightly spaced striae. Striae
266 mostly continuous (Fig. 1), never raised by transverse irregular striae frequently observed in
267 *M. graminicola* and *M. oryzae*. Quantitative differences between species belonging to the
268 Jepson group 11 are reduced, but *M. ottersoni* shows a small female stylet (9-12 μ m),
269 different from others species: *M. graminicola*, 12-14 μ m and *M. oryzae*, 14-16 μ m (Jepson,
270 1987, Mattos et al., 2018). The stylet knobs also can differentiate these three species also:

271 large transverse elongate knobs in *M. graminicola* (Eisenback & Triantaphyllou, 1991), set
272 off from the shaft in *M. ottersoni* and sloping backward in *M. oryzae* (Mattos *et al.*, 2018).
273 Now, considering the size of cone in relation to shaft, it is bigger in *M. graminicola*, smaller
274 in *M. oryzae* and the same size or bigger in *M. ottersoni* (Jepson, 1987; Eisenback &
275 Triantaphyllou, 1991; Mattos *et al.*, 2018). The length range of male stylets are different also
276 among the three species: 13-16 μm for *M. ottersoni*, 16-18 μm for *M. graminicola* and 18-20
277 μm for *M. oryzae* (Jepson, 1987). The tail of J2 of the three species are very long and thin and
278 tapers to a long, narrow irregular hyaline terminus (*M. ottersoni*, 20.5 μm , *M. graminicola*,
279 17.9 μm and *M. oryzae*, 22.0 μm), ending in a slightly clavate tip (Fig. 4 e-i). Populations of
280 *M. ottersoni* shows esterase phenotype without bands or enzymatic activity (Rm=0, Est Ot0),
281 completely different from *M. graminicola* (Est VS1) and *M. oryzae* (Est O1) with a very large
282 drawn-out area of enzymatic activity (Mattos *et al.*, 2018). The esterase phenotype is
283 therefore a great tool for identifying *M. ottersoni* (Est Ot0) from other species parasites of rice
284 (Fig. 5). Considering now the SCAR markers developed by Belafiore *et al.* (2015), Htay *et al.*
285 (2016) and Mattos *et al.* (2018), unfortunately, they were not species-specific for *M.*
286 *graminicola*, since they also amplify some populations of *M. ottersoni*. Nonetheless, *M.*
287 *ottersoni* has specific ITS, D2-D3 and partial coxII-16S rRNA sequences (Figs. 6, 7, 8).
288 *Meloidogyne ottersoni* from rice reproduced also on the type host plant, reed canary grass, *P.*
289 *arundinacea*, showing J2 within the roots and females with egg masses, 50 days after
290 inoculation (Fig. 9), confirming the Koch's postulate.

291

292 Discussion

293

294 The traditional identification methods of RKN are based on morphology (Jepson,
295 1987; Eisenback & Triantaphyllou, 1991; Hunt & Handoo, 2009), esterase phenotypes
296 (Esbenshade & Triantaphyllou 1985; Carneiro *et al.*, 2000) and molecular markers (Blok &
297 Powers, 2009). The identification of *Meloidogyne* spp. from rice (group 11) has been a
298 challenge with conservative morphology, unclear species boundaries and complex taxonomy
299 (Jepson, 1987), and a lack of SCAR markers for several species (Belafiore *et al.*, 2015;
300 Mattos *et al.*, 2018) complicates the diagnosis.

301 The RKN species found on rice have been generally classified as *M. graminicola*,
302 based largely on morphometric characters, the symptoms and the ability of this species to
303 cause infection in lowland rice (Pokharel *et al.*, 2007). However, detailed information on
304 identity and variability of observed RKN is still lacking. Identification and diagnosis of *M.*

305 *graminicola* has always been a challenge. Recently, Salalia *et al.* (2017), studying 14
306 populations belonging to the '*M. graminicola* group' from India, suggested that cryptic
307 species may be occurring mixed with the rice RKN. In a recent survey in southern Brazil, 21
308 populations of *Meloidogyne* spp. were detected in SC State. *Meloidogyne* sp.0, now identified
309 as *M. ottersoni*, occurred in 19% of the samples, always by itself without mixtures with other
310 species (Mattos *et al.*, 2017). *Meloidogyne graminicola* was the most widespread nematode
311 detected in the southern region of Brazil (Negretti *et al.*, 2017).

312 *Meloidogyne ottersoni* from the Wind Lake, Wisconsin, USA is a species well adapted
313 to flood conditions and reproduces well on reed canary grass, *P. arundinaceae*. This plant is a
314 perennial bunchgrass that commonly forms extensive single-species stands along the margins
315 of lakes and streams and in wet open areas, with a wide distribution in Europe, Asia, northern
316 Africa and North America

317 In Brazil, *M. ottersoni* (= *Meloidogyne* sp.0) was detected in rice regions in flooded
318 soil, with low drainage capacity. The average temperature in this region is 19.5°C, ranging
319 from 10 °C to 25 °C in summer and from 10 °C to 18 °C in winter. Rice flooding occurs via
320 irrigation channels and the water comes from the local river Aranguará and streams. This
321 region covers 9.500 ha of rice grown by at least 320 families and it is characterized by
322 prolonged flooding in the growing areas. During the inter-crop season, the soils with poor
323 natural drainage remain flooded during the winter, where some weeds as swamp rice grass,
324 *Leersia hexandra* Sw. contribute to maintain or to proliferate nematode populations during
325 this season. In Argentina (Tucumã), *M. ottersoni* was detected on barnyard grass, *Echinochloa*
326 *crus-galli* (L.) P. Beauv. by Doucet & Pinochet (1992) using the typical perineal-pattern
327 described by Thorne (1969) and Jepson (1987).

328 Tail morphology of the second-stage juvenile of *M. ottersoni* places it in Jepson's
329 Group 11 (Jepson, 1987). This is the most well defined group within the genus *Meloidogyne*,
330 which includes now ten species that parasitize rice and present differential hyaline tail length:
331 *M. graminicola* (17.9 µm), *M. graminis* (Sledge and Golden, 1964) Whitehead, 1968 (18.5
332 µm), *M. lini* Yang, Hu and Xu, 1988 (~ 14.0 µm), *M. hainanensis* Liao and Feng, 1995 (~15.3
333 µm), *M. salasi* (19.7 µm), *M. triticoryzae* Gaur, Saha and Khan, 1993 (17.6 µm), *M.*
334 *marylandi* Jepson and Golden, 1987 (11.8 µm), *M. sasseri* Handoo, Huettel and Golden, 1995
335 (19.5 µm), *M. oryzae* (22.0 µm) (Mattos *et al.*, 2018), and finally *M. ottersoni* (20.5 µm).

336 Most of *Meloidogyne* species from rice (Group 11) were poorly described, as well as
337 poorly cytogenetically, enzymatically and molecularly characterized. Some morphological
338 and biological characters are common in these species: female body elongated, vulva

339 sometimes situated on a posterior protuberance, association with an amphimictic or
340 parthenogenetic mode of reproduction, semi-endoparasitism, with males abundant and
341 females deeply embedded in the host (Jepson, 1987). Exception is made for *M. oryzae* that
342 reproduces by mitotic parthenogenesis and presents few males (Mattos *et al.*, 2018).

343 In Thorne's (1969) description of *M. ottersoni*, a very small number of specimens were
344 obtained and measured: females (10), males (7) and J2 (10), directly picked up from roots of
345 reed canary grass collected in the field. In our situation, the populations of *M. ottersoni* were
346 multiplied on rice under greenhouse conditions for three months. In general, the morphology
347 and morphometric data of *M. ottersoni* populations from Brazil were similar to the original
348 description (Thorne, 1969; Jepson, 1987), with expected variations frequent to the genus
349 *Meloidogyne*. The populations from Brazil had bigger female, male and J2 compared to the
350 type population from US. This variation in body length can occur in populations of the same
351 species (Handoo *et al.*, 1993). The stylet of females and males were similar in length, but the
352 population from US presented J2 with longer stylet. Some authors consider morphological
353 features much more relevant in species characterization than morphometric characters
354 (Jepson, 1987; Eisenback & Triantaphyllou, 1991). Considering these aspects, *M. ottersoni*
355 populations collected in Brazil were very close to the described population from USA and
356 parasitize the same type host plant *P. arundinaceae*, corroborating Koch's postulates.

357 Biochemically, *M. ottersoni*, *M. graminicola* and *M. oryzae* showed distinct esterase
358 profiles; the first one without bands ($R_m=0$), and the others with drawn-out bands in different
359 positions (Esbenshade & Triantaphyllou, 1985; Mattos *et al.*, 2018). This phenotype was
360 never clearly characterized before and seems to be new and a species-specific character for *M.*
361 *ottersoni*. A similar phenomenon occurs with *Meloidogyne exigua* Göldi, 1987: more than
362 10 females are required to reveal esterases satisfactorily (Carneiro *et al.*, 2000). This is due to
363 the reduced size of females and low concentration of the esterase enzyme. For *M. ottersoni*,
364 even if more than 20 females per individual analysis were used in this study, no esterase band
365 was observed. However, to use this criterium as a diagnostic character, it is necessary to
366 reveal an other enzymatic activity with the same extract, to ensure that the lack of esterase
367 activity is not due to protein degradation within the sample.

368 Oogenesis and spermatogenesis of the population of *M. ottersoni* from Wisconsin, USA
369 were of the meiotic type ($n=18$). When males were abundant, reproduction was by
370 amphimixis. Considering the cytogenetic studies, *M. ottersoni* is closely related to *M.*
371 *graminicola*, *M. graminis* and *M. naasi* (Triantaphyllou, 1973). The population of *M.*
372 *ottersoni* from Meleiro studied in this work reproduced by meiotic parthenogenesis and

373 presented 18 chromosomes, being similar to *M. ottersoni* type population and *M.*
374 *graminicola* and completely different from *M. oryzae* (3n=50-56 chromosomes) (Esbenshade
375 & Triantaphyllou, 1985).

376 *Meloidogyne oryzae* is the only triploid rice-related species and as previously studied
377 (Mattos *et al.*, 2018) clusters together with other apomictic species in ITS-based phylogenetic
378 trees. Our molecular results for this region confirmed that *M. oryzae* was closer to other
379 mitotic parthenogenetic species, while *M. ottersoni* clustered with meiotic parthenogenetic
380 species. Species with about 51–56 chromosomes were most likely derived following
381 hybridization of meiotic parthenogenetic forms like *M. graminicola*, involving the
382 fertilization of an unreduced egg with 36 (18 + 18) chromosomes, with a normal
383 spermatozoon having 18 chromosomes (Triantaphyllou, 1985). A probable speciation
384 process may be occurring with these RKN rice-related species as suggested by Negretti *et al.*
385 (2017) and Mattos *et al.* (2018).

386 The multi-copy basis of rDNA provides sample target for PCR amplification and it
387 allows detecting intraspecific variation in *Meloidogyne* spp. populations (Zijlstra *et al.*, 1995;
388 Adam *et al.*, 2007). Similar to *M. ottersoni*, the presence of intraspecific variability in rDNA
389 sequences was reported for *M. graminicola* (Pokharel *et al.*, 2007) and *M. oryzae* (Mattos *et*
390 *al.*, 2018; Besnard *et al.*, 2019), two other RKN rice-related species.

391 From the perspective of identification, the mitochondrial genome provides a rich
392 source of genetic markers for identification (Rubinoff & Holland, 2005; Hu & Gasser, 2006).
393 This region has a faster rate of evolution than the corresponding nuclear genes (Hugall *et al.*,
394 1994; Blok & Powers, 2009). In fact, mitochondrial coding region is already considered a
395 better potential barcode region for some *Meloidogyne* spp. groups, since intergenic regions
396 have been shown to contain AT repeats that appear not to correlate with speciation events
397 (Hugall *et al.*, 1997; Pagan *et al.*, 2015). *Meloidogyne ottersoni* presents a very unique COII
398 sequence with low intraspecific variability, which can be useful for species identification and
399 for phylogenetic studies.

400 Our results clarified the taxonomic position of *M. ottersoni*, and illustrated the interest
401 of using integrative taxonomy for accurate species redescription (Padial *et al.* 2010; Pante *et*
402 *al.*, 2015, Mattos *et al.*, 2018). Indeed, detailed morphological, morphometrical, cytological
403 and molecular studies were performed to correctly characterize this nematode from rice fields.
404 Precise identification of nematode species is important to elucidate future biological or
405 agronomic studies in order to clarify the distribution and importance of this species in the

406 field, and thus to plan efficient control measures for the management of this rice disease in the
407 Southern region of Brazil.

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419

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568 **Figure legends**

569 **Fig. 1.** *Meloidogyne ottersoni* perineal patterns. a, b: Scanning electron microscopy
570 micrographs (SEM); c-f: light microscopy micrographs. Arrows showing slight protuberance
571 of vulval region.

572 **Fig. 2.** *Meloidogyne ottersoni* females. a-e: Scanning electron microscopy (SEM)
573 micrographs of anterior region (a-c) and stylets (d, e). Light microscopy (LM) micrograph of
574 anterior region (f). Arrow showing excretory pore of female.

575 **Fig. 3.** *Meloidogyne ottersoni* males. a-d: Scanning electron microscopy (SEM) micrographs of
576 anterior region. e, f: Light microscopy (LM) micrographs of anterior region (e) and tail (f).
577 g: SEM of lateral fields; h, i: SEM of excised stylets.

578 **Fig. 4.** *Meloidogyne ottersoni*, second-stage juveniles. a, b: Scanning electron microscopy
579 (SEM) micrographs of anterior region. c: Light microscopy (LM) micrographs of anterior
580 region; d: SEM of lateral fields; e, f: SEM of tails in lateral and ventral views, g-i: LM of tails
581 in ventral and lateral views.

582 **Fig. 5** . Esterase (a) and malate dehydrogenase (b) phenotypes of *Meloidogyne ottersoni*
583 (*Ot0*, *N1A*). *Meloidogyne javanica* (*J3*, *N1*) was used as reference.

584 **Fig. 6**. Maximum Likelihood analysis of the phylogenetic relationships of *Meloidogyne*
585 *ottersoni* (*M. sp.0*) and other *Meloidogyne* spp. populations based on the ITS1-5.8S-ITS2
586 rRNA sequences. Numbers to the left of the branches are bootstrap values for 1,000
587 replications. Accession numbers from GenBank are listed before the species names.

588

589 **Fig. 7**. Maximum Likelihood analysis of the phylogenetic relationships of *Meloidogyne*
590 *ottersoni* (*M. sp.0*) and other *Meloidogyne* spp. populations based on the D2-D3 fragment of
591 28S rRNA sequences. Numbers to the left of the branches are bootstrap values for 1,000
592 replications. Accession numbers from GenBank are listed before the species names.

593

594 **Fig. 8**. Maximum Likelihood analysis of the phylogenetic relationships of *Meloidogyne*
595 *ottersoni* (*M. sp.0*) and other *Meloidogyne* spp. populations based on the *coxII*-16S rRNA
596 sequences. Numbers to the left of the branches are bootstrap values for 1,000 replications.
597 Accession numbers from GenBank are listed before the species names.

598

599 **Fig. 9**. Photomicrographs of *Meloidogyne ottersoni* parasitizing reed canary grass, *Phalaris*
600 *arundinacea*. a: second-stage juveniles inside the root; b: female and eggs inside the roots.