ARTICLE (Discoveries)

Population genomics supports clonal reproduction and multiple gains and losses of parasitic abilities in the most devastating nematode plant pest

Georgios D. Koutsovoulos[1*], Eder Marques[2], Marie-Jeanne Arguel[3], Laurent Duret[4], Andressa C.Z. Machado[5], Regina M.D.G. Carneiro[2], Djampa K. Kozlowski[1], Marc Bailly-Bechet[1], Philippe Castagnone-Sereno[1], Etienne G.J. Danchin[1*], Erika V.S. Albuquerque[2*]

[1]INRA, CNRS, Université Côte d'Azur, ISA, France

[2]Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brasil

[3]CNRS, Université Côte d'Azur, IPMC, France

[4]Université de Lyon, Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive UMR 5558, F-69100, Villeurbanne, France

[5]Área de Proteção de Plantas, Instituto Agronômico do Paraná, Londrina, Paraná, Brasil

Corresponding author: Etienne GJ Danchin, etienne.danchin@inra.fr

* equal contributions

Abstract

The most devastating nematodes to worldwide agriculture are the root-knot nematodes with *Meloidogyne incognita* being the most widely distributed and damaging species. This parasitic and ecological success seem surprising given its supposed obligatory clonal reproduction. Clonal reproduction has been suspected based on cytological observations but, so far, never confirmed by population genomics data. At the species level, *M. incognita* is highly polyphagous with thousands of host plants. However, the host range varies among different *M. incognita* isolates that may present distinct and more restricted host compatibilities. Historically, four 'host races' had been defined as a function of ranges of compatible and incompatible plants. We sequenced the genomes of 11 isolates across Brazil, covering these four distinct races to assess (i) how clonal reproduction is and (ii) how the level of genome variability associates with biological traits such as the host races, affected agronomic culture, and geographical distribution. By aligning the genomic reads of the isolates to the *M. incognita* reference genome assembly, we identified SNPs and small-scale insertions/deletions. Analysis of linkage disequilibrium and 4-gametes test, showed no sign of recombination, confirming the clonal mode of reproduction of *M. incognita.* We showed that there are relatively few point variations between the different isolates, and these variations show no significant association with either the host races, the geographical origin of the samples or the host plant on which they have been collected. Due to the lack of phylogenetic signal underlying their existence, we recommend the discontinuation of the terminology "race". Overall, these results suggest that multiple gains and losses of parasitic abilities and adaptations to different environmental conditions account for the broad host spectrum and wide geographic distribution of *M. incognita*. Hence, this metazoan constitutes a model species to study adaptability without sexual recombination and overall low genomic variations in animals.

Introduction

Nematodes cause severe damages to the world agricultural production every year with the root-knot nematodes (RKN, genus *Meloidogyne*) being the most economically harmful in all temperate and tropical producing areas (Moens et al. 2009; Jones et al. 2013). Curiously, the most polyphagous RKN species, able to parasitize the vast majority of flowering plants on Earth (Trudgill and Blok 2001), are described as mitotic parthenogenetic, based on cytogenetics comparisons with outcrossing relatives (Triantaphyllou 1981; Triantaphyllou 1985). This suggests absence of meiosis and obligatory asexual reproduction. Among these mitotic parthenogenetic RKN, *M. incognita* is the most widespread and is present, at least, in all the countries where the lowest temperature exceeds 3°C. Greenhouses over the world also extend its geographic distribution (Sasser et al. 1983). *M. incognita* is so widely distributed that it is not even included on the list of regulated pests (Singh et al. 2013). Due to its worldwide distribution and extremely large range of hosts, *M. incognita* has been deemed the most damaging species of crop pest worldwide (Trudgill and Blok 2001).

However, it has become more and more evident that the full broad host range of *M. incognita* as well as the other major RKN species is not present in all the individuals within the species but that different 'populations' or 'isolates' have different and overlapping ranges of compatible hosts (Moens et al. 2009). Variations regarding host range within one given species gave rise to the concept of 'host race' as soon as 1952 (Sasser 1952). Although RKN species can be differentiated based on morphological descriptions (Eisenback and Hunt 2009), isozyme phenotypes (Esbenshade and Triantaphyllou 1985; Carneiro et al. 2000), and molecular analysis (Blok and Powers 2009), this is not the case of host races within a species (Triantaphyllou 1985). Consequently, the pattern of compatibility/incompatibility of the nematode interaction with a set of different host plants was standardised into the North Carolina Differential Host Test (NCDHT, (Hartman and Sasser 1985)) to differentiate races within Meloidogyne spp. In *M. incognita*, all the populations originally tested reproduced on tomato, watermelon, and pepper and none infected peanut, but they differed in their response to tobacco and cotton defining four distinct host races (Hartman and Sasser 1985) (Table S1). Whether some genetic features are associated with the *M. incognita* races remains unknown. Indeed, diversity studies using RAPD and ISSR markers across eight isolates and the four races of *M. incognita* found no correlation between phylogeny and host races (Cenis 1993; Baum 1994; Santos et al. 2012). Although in one of these studies (Santos et al. 2012), two out of three esterase phenotypes were monophyletic in the phylogenetic tree of the *M. incognita* isolates, they did not segregate according to the host races. A different molecular approach to try to differentiate host races was also proposed based on repeated sequence sets in the mitochondrial sequence (Okimoto et al. 1991). Although the pattern of repeats allowed differentiating one isolate of race 1, one of race 2 and one of race 4; the study encompassed only

one isolate per race, and thus the segregation could be due to differences between isolates unrelated to the host race status itself.

Hence, no clear genetic determinant underlying the phenotypic diversity of *M. incognita* isolates in terms of host compatibility patterns have been identified so far (Castagnone-Sereno 2006). This lack of phylogenetic signal underlying the host races is surprising because it would suggest multiple independent gains and losses of host compatibly patterns despite clonal reproduction. Theoretically, animal clones have poorer adaptability because the efficiency of selection is impaired, advantageous alleles from different individuals cannot be combined and deleterious mutations are predicted to progressively accumulate in an irreversible ratchet-like mode (Muller 1964; Hill and Robertson 1966; Kondrashov 1988; Glémin and Galtier 2012).

For these reasons, the parasitic success of *M. incognita* has long been described as a surprising evolutionary paradox (Castagnone-Sereno and Danchin 2014). However, this apparent paradox holds true only if this species does actually reproduce without sex and meiosis while presenting substantial adaptability. So far, no conclusive study conducted at the whole genome level, support these tenets.

A first version of the genome of *M. incognita* was initially published in 2008 (Abad et al. 2008) and re-sequenced at higher resolution in 2017, providing the most complete *M. incognita* reference genome available to date (Blanc-Mathieu et al. 2017). This study showed that the genome is triploid with high average divergence between the genome copies most likely because of hybridization. Due to the high divergence between the homoeologous genome copies, and the supposed lack of meiosis, it was assumed that the genome was effectively haploid. The genome structure itself showed synteny breakpoints between the homoeologous regions and some of them formed tandem repeats and palindromes. These same structures were also described in the genome of the bdelloid rotifer *Adineta vaga* and considered as incompatible with meiosis (Flot et al. 2013; Blanc-Mathieu et al. 2017). However, whether these structures represent a biological reality or artefacts of genome assembly remains to be clarified. Indeed, both genomes have been assembled using the same techniques and no independent biological validation for these structures has been performed. Hence, so far no strong evidence supporting the absence of meiosis was available at the genome level.

Furthermore, because the reference genome was obtained from the offspring of one single female (originally from Morelos, Mexico), no information about the genomic variability between different populations or isolates was available. More recently, a comparative genomics analysis, including different strains of *M. incognita*, showed little variation at the protein-coding genome level between strains collected across different geographical locations (Szitenberg et al. 2017), confirming previous observations with RAPD and ISSR markers (Cenis 1993; Baum 1994; Santos et al. 2012). However, no attempt was made to associate these few variations with biological traits

113 such as the host-race or geographic origin. Moreover, the variability between isolates at the non-
114 coding level, which represents the vast majority of the genome, was not described in this initial
115 analysis.

116 In the present study, we used population genomics analyses to investigate, (i) whether the
117 supposed absence of meiosis is supported by the properties of genome-wide SNP markers
118 between different isolates, (ii) the level of variation between different isolates at the whole
119 genome level, and (iii) whether these variations follow a phylogenetic signal underlying several
120 life history traits such as the host compatibility patterns, the geographic distribution or the current
121 host crop plant.

122 To address these questions, we have sequenced the genomes of 11 isolates representing
123 the four *M. incognita* host-races from populations parasitizing six crops from geographically
124 different sites across Brazil (Figure 1). We used isozymes profiles, SCAR markers, and the
125 NCDHT to characterize the biological materials before the DNA extraction and high coverage
126 genome sequencing. We identified SNPs at the whole genome level by comparing the *M.*
127 *incognita* isolates to the reference genome (Morelos strain from Mexico). We conducted several
128 SNP-based genetic tests to investigate the evidences (or lack thereof) of recombination. Using
129 two different approaches, we classified the *M. incognita* isolates according to their SNP patterns
130 and investigated whether the classification was associated to the following biological traits: host
131 compatibility, geographical localisation and current host plant.

132 Our population genomics analysis allowed addressing key evolutionary questions such
133 as how asexual is reproduction in this animal species. We were also able to clarify the adaptive
134 potential of this devastating plant pest in relation to its mode of reproduction. In particular, we
135 determined whether there is a phylogenetic signal underlying variations in biological traits of
136 agro-economic importance such as the patterns of host compatibility (host races) in *M. incognita*.
137 While association between phylogenetic signal and patterns of host compatibilities would tend to
138 show stable inheritance from ancestral states, the non-association would support multiple gains
139 and losses of parasitic abilities and substantial adaptability.

140 This resolution has important agricultural and economic implications since crop rotation
141 and other control strategies should take into account the adaptive potential of this nematode pest.
142

143

Results

The *M. incognita* genome is mostly haploid and shows few short-scale variations

We collected 11 *M. incognita* populations from six different states across Brazil and from six different crops (soybean, cotton, coffee, cucumber, tobacco, watermelon) (Figure 1). Each isolate was reared by multiplication of the egg mass of one single female on tomato plants (methods). After having confirmed that the 11 isolates we collected showed the characteristic isozyme profiles and molecular signatures of *M. incognita*, we characterized their host race status using the NCDHT (methods, Table 1). We characterised three isolates as race 1, two as race 2, three as race 3, and three as race 4.

We generated high-quality paired-end genome reads (~76 million per isolate) for each *M. incognita* isolate. These reads covered the ~184Mb *M. incognita* genome assembly (Blanc-Mathieu et al. 2017) at a depth > 100X (Table S2) for each isolate. Variant calling, performed in regions with at least 10x coverage per sample, identified 338,960 polymorphic positions (~0.19% of the total number of non-ambiguous nucleotides). Around 20% of these positions corresponded to 1/1 SNPs, homozygous within isolates. We examined the distribution of base coverage of homozygous (1/1) and heterozygous (0/1) SNPS. We observed that the heterozygous SNPs showed a peak of distribution at ~twice the coverage of the peak for homozygous SNPs in the 11 isolates (Fig. S1). This parallels the distribution of base coverage in the *M. incognita* reference genome scaffolds which shows a major peak at ~65X and a second minor peak at ~130X (twice the coverage; Fig. S2). These genome regions at double coverage were considered as representing highly similar homoeologous genome copies that were collapsed during the assembly (Blanc-Mathieu et al. 2017). Although these regions are minority in the genome assembly, they seem to be responsible for many heterozygous (0/1) SNPs. The SNPs in these minority regions of double coverage probably result from genome reads of two homoeologous regions mapped to a single collapsed region in the reference assembly. Hence, most of these heterozygous SNPs might not represent variations between isolates or between individuals but between the few collapsed homoeologous regions. Since most of the reference genome is assembled in haploid status (Fig. S2), and the status of heterozygous SNPs is unsure, we will utilise only homozygous SNPs for all downstream analyses.

The levels of variations between isolates are low and not specific to races

Each isolate showed a different level of divergence from the reference genome with R1-2 having the highest number of homozygous variable positions (41,518) and R1-6 having the least (17,194) variants (Figure 2). The R3-4 isolate originated from a pool of four populations. However, the low number of SNPs compared to the reference indicates either that the genomes

179 of these four populations were very close or that a specific population displaced the other three
180 (Figure 2). Thus, the R3-4 isolate was analysed exactly as the other isolates. Overall, the
181 percentages of homozygous variable positions on the nuclear genomes of the eleven isolates,
182 compared to the Morelos reference strain, ranged between 0.01 % and 0.02 %. In comparison, the
183 percentages of variable positions in the mitochondrial genome ranged between 0.04% and 0.18%.
184 Interestingly, race-specific variants exist only for race 2, which exhibited 30 race-specific
185 variations. This is possibly due to the fact that race 2 is represented by only two isolates (vs. 3 for
186 the rest of the isolates). The vast majority (~78%) of SNPs were outside of coding regions; only
187 14,704 variable positions fell in coding regions and covered 7,259 out of 43,718 predicted protein-
188 coding genes. In these coding regions, 8,179 were synonymous substitutions, 3,854 SNPs yielded
189 non-synonymous substitution, 93 nonsense mutations and the rest other disruptive mutations.
190 From the SNPs falling in coding regions, we also measured nucleotide diversity at
191 synonymous (π_s) and non-synonymous (π_n) sites for the 11 isolates as well as the π_n/π_s ratio as a
192 measure of the efficiency of selection. Consistent with the overall low number of SNPs, the π_s
193 values were quite low for the 11 isolates (ranging from 2.82 10^{-04} (R2-1) to 5.60 10^{-04} (R1-2) and
194 median value = 3.53 10^{-04}, Table S3). This is two orders of magnitude lower than the values
195 measured for two outcrosser nematodes from the *Caenorhabditis* genus (Romiguier et al. 2014),
196 *C. doughertyi* (formerly sp. 10: 4.93 10^{-02}) and *C. brenneri* (3.22 10^{-02}). A similar difference of
197 two orders of magnitude was also observed for the diversity at non-synonymous sites with π_n
198 values ranging from 4.63 to 8.56 10^{-05} for *M. incognita* and values reaching 2.53 10^{-03} and 1.28
199 10^{-03} for *C. doughetryi* and *C. brenneri*, respectively. However the π_n/π_s ratio was substantially
200 higher for *M. incognita* (median ratio = 0.156 and range 0.149-0.164) than for the two outcrossing
201 Caenorhabditis (0.051 and 0.040 for *C. doughetryi* and *C. brenneri*, respectively). This would
202 suggest a lower efficacy of selection in the obligate parthenogenetic *M. incognita* than in these
203 two outcrossing *Caenorhabditis* nematodes.
204

205 There is no significant association between the short-scale variants and biological 206 traits

207 Using principal component analysis on the whole set of homozygous SNPs (PCA,
208 methods), we showed that the eleven *M. incognita* isolates formed three distinct clusters, which
209 we named A, B and C (Figure 3). Cluster A is represented by isolate R1-2 alone, which has the
210 highest number of variants. Cluster B is constituted by R3-2 and R4-4. The rest of the isolates fall
211 in a single dense cluster C of overall low variation. There was no significant association between
212 the clusters and the host race status (Fisher's exact test p-value=1, Sup. Text, Table S4). This
213 implies that isolates of the same host race are not more similar to each other than isolates of
214 different host races. There was also no significant association between the SNP-based clusters

215 and the original host plant from which the nematodes have been collected (Fisher's exact test p-
216 value=0.69, Sup. Text, Table S4). Interestingly, the four different host races are all represented in
217 one single cluster (C). Within this cluster, the total number of variable positions was 29,597.
218 Meaning that the whole range of host-race diversity is present in a cluster that represents only
219 44% of the total existing genomic variation. We also conducted an isolation by distance (IBD)
220 analysis, which showed no correlation between the genetic distance and the geographical distance
221 (Fig. S3).

222 To assess the levels of separation vs. past genetic exchanges between these clusters, we
223 calculated fixation index values (F_{ST}). Weighted F_{ST} values between clusters were all >0.83,
224 suggesting a lack of genetic connections between the clusters (Table S5). Using the mean F_{ST}
225 values, in contrast, while we observed a mean F_{ST} >0.98 between clusters A and B, indicating a
226 lack of genetic connection between R1-2 and cluster B, the F_{ST} values were much lower between
227 A and C (0.35) and between B and C (0.52). This would suggest isolates from clusters A and B
228 both result from a past bottlenecked dispersal and propagation from some isolates in cluster C.
229

230 Phylogenetic networks confirm the lack of association of SNPs with biological 231 traits and support clonal evolution

232 Using a phylogenetic network analysis based on SNP present in coding regions, we
233 could confirm the same three clusters (Figure 4). This further supports the absence of
234 phylogenetic signal underlying the host races (patterns of host compatibilities). Interestingly,
235 this network analysis based on homozygous SNPs yielded a bifurcating tree and not a network.
236 This result suggests a lack of genetic exchanges between the isolates, as expected from a clonal
237 species. To confirm this result, we conducted separate phylogenetic analyses for each of the
238 longest 14 scaffolds with sufficient number of phylogenetically informative variable positions
239 and the mitochondrial genome. All these analyses showed a clear separation between the three
240 clusters (A, B, and C) with some minor polytomies within cluster C (Fig. S5 and Fig; S6).

241 According to the two classification methods (PCA and phylogenetic network), isolate R1-
242 2 seemed to be the most divergent from the rest of isolates, which is consistent with its higher
243 total number of SNPs and number of isolate-specific SNPs. Then, a small cluster was composed
244 of isolates R3-2 and R4-3 (equivalent to cluster B of the PCA). Finally, a cluster (equivalent to
245 PCA cluster C) grouped the rest of the eight isolates and covered all the defined host races as well
246 as 5 of the 6 different host plants

247 Consistent with the PCA and phylogenetic network analysis, we also did not observe
248 significant association between the number of repeats in the two repeat regions in the
249 mitochondrial genome (63R and 102R) and races, geographical origin or host plant of origin
250 (Table 2).

No evidence for meiotic recombination in *M. incognita*

Based on cytogenetics observation, *M. incognita* and other tropical root-knot nematodes have been described as mitotic parthenogenetic species (Triantaphyllou 1981; Triantaphyllou 1985). However, this evolutionary important claim has never been confirmed by genome-wide analyses so far. Using the SNP markers at the whole-genome scale, we conducted linkage disequilibrium (LD) analysis as well as 4-gametes test to search for evidence for recombination (or lack thereof). In an outcrossing species, although physically close markers should be in high LD, this LD should substantially decrease with distance between the markers, because of recombination, and eventually reach absence of LD as for markers present on different chromosomes. In clonal species, however, in the absence of recombination, the LD between markers should remain high and not rapidly decrease with distance between markers. By conducting an analysis of LD, we did not find any trend for a decrease of LD between markers as a function of their physical distance (Figure 5). In contrast, the LD values remained high regardless the distance and oscillated between 0.85 – 0.94. Hence, we did not observe the expected characteristic signatures of meiosis. An inversely contrasted situation between outcrossing and clonal genomes should be observed for the 4-gametes test. Taking SNP markers that exist in two states among the 11 isolates, the proportion of pairs of markers that pass the 4-gametes test (i.e. that represent the 4 products of meiosis) should rapidly increase with distance between the markers, in case of recombination. In contrast, in the absence of recombination, no trend for an increase of the proportion of pairs or markers passing the 4-gametes test with distance between markers should be observed. By conducting an analysis of 2-states markers, we observed no trend for a change in the proportion of markers passing the test with distance. In contrast, the distribution remained flat and close to a value of 0.0. Again, this trend does not correspond to the expected characteristic of meiotic recombination.

To assess the sensitivity of our methods in finding evidence for recombination, we conducted the same analyses (LD and 4-gametes tests) in the outcrossing diploid meiotic plant-parasitic nematode *Globodera rostochiensis* (Eves-van den Akker et al. 2016). Because the *G. rostochiensis* genome assembly mostly consists of merged paternal and maternal haplotypes, we had to phase the SNPs before conducting LD and 4-gametes tests. The results were totally contrasted between *M. incognita* and *G. rostochiensis* (Figure 6). In *G. rostochiensis*, the LD and 4-gametes curves started at relatively lower (<0.7) and higher (>0.15) values, respectively (Figure 6). Furthermore, we observed a rapid exponential decrease of r^2 in the first kb for LD. At an inter-marker distance of 3kb the r^2 value was <0.37. In parallel, we observed a concomitant rapid and exponential increase in the proportion of markers passing the 4-gametes test, which was >0.38 at the same inter-marker distance. Hence, while *G. rostochiensis* appears to display all the expected characteristics of meiotic recombination, this was not the case for *M. incognita*. This confirms at

287 a whole genome-scale the lack of evidence for meiosis previously observed at the cytological
288 level in *M. incognita*.
289

290 Addition of further geographical isolates does not increase the genomic diversity 291 and confirms the lack of association between genetic distance and biological 292 traits

293 To investigate more widely the diversity of *M. incognita* isolates in relation to their mode
294 of reproduction and other biological traits, we included whole-genome sequencing data for
295 additional geographical isolates recently published (Szitenberg et al. 2017). These genome data
296 included one isolate from Ivory Coast, one from Libya, one from Guadeloupe (French West
297 Indies) and five from the USA (Szitenberg et al. 2017) (Figure 1). We pooled these eight new
298 isolates with the eleven Brazilian isolates produced as part of this analysis as well as the *M.*
299 *incognita* Morelos strain (reference genome) and performed a new PCA with the same
300 methodology. Astonishingly, adding these new isolates recovered the same separation in three
301 distinct clusters (A, B and C) (Figure 7). All the new isolates from additional and more diverse
302 geographical origins fell in just two of the previous Brazilian clusters (A and C). Cluster A that
303 previously contained R2-1 alone, now encompasses the Ivory Coast, Libyan and Guadeloupe
304 isolates. Cluster C that previously contained eight of the Brazilian isolates and covered all the
305 host races now includes the five US isolates as well as the Mexican isolate (Morelos, reference
306 genome). Cluster B remains so far Brazilian-specific with only R3-2 and R4-4 in this cluster.
307 Addition of these new geographical isolates (Szitenberg et al. 2017) did not substantially increase
308 the number of detected variable positions in the genome. Analyses ran with this whole set of
309 available *M. incognita* isolates also further supported the lack of association of SNP-based
310 clusters with biological traits such as host races, nature of the host of origin and geographical
311 distribution (Text S1, Fig. S7).
312

313 Discussion

314 Is the parasitic success of *M. incognita* an evolutionary paradox? This proposition would
315 be true only if *M. incognita* is adaptive despite having a fully parthenogenetic reproduction. Our
316 results seem to support these two aspects.
317 It was already evident that *M. incognita* has an adaptive potential. Indeed, experimental
318 evolution assays have shown the ability of *M. incognita* to overcome resistance conferred by the
319 Mi gene in tomato in a few generations (Castagnone-Sereno et al. 1994; Castagnone-Sereno
320 2006). Naturally virulent *M. incognita* populations (i.e. not controlled by the resistance gene) have

321 also been observed in the fields and probably emerged from originally avirulent populations
322 (Verdejo-Lucas et al. 2012; Tzortzakakis et al. 2014; Barbary et al. 2015), although it is unknown
323 if this resistance breaking is as rapid as under controlled lab conditions. However, adapting from
324 a compatible host plant to another very different incompatible plant is certainly more challenging
325 that breaking down a resistance gene in a same plant. Here, we showed that the different host
326 races defined in *M. incognita* as a function of patterns of (in)compatibilities with different plants
327 do not follow a phylogenetic signal. This could represent multiple independent adaptations or
328 gradual loss of compatibilities from a hyper-polyphagous ancestor. To address this question, we
329 have reconstructed host compatibilities at each ancestral node based on the SNP-based
330 phylogenetic classification of the *M. incognita* isolates (Fig. S8). This maximum-likelihood
331 reconstruction shows that the hypothesis of a hyper-polyphagous ancestor that then progressively
332 lost ability to either parasitize Cotton or Tobacco is not realistic. The ancestral reconstruction
333 rather suggests multiple independent gains and losses of the ability to parasitize these plants.
334 Consistent with this suggestion, host race switching within an isolate over time has already been
335 observed. Isolates of *M. incognita* race 2 and 3, which parasitize tobacco and cotton plants
336 respectively, switched to behaviour similar to race 3 and 2 after staying for eight months on coffee
337 plants (Rui Gomes Carneiro, personal communication). Together with the previously reported
338 ability to break down resistance gene in plants, the ability of *M. incognita* to loose and gain ability
339 to infect different plants highlights its adaptive potential.
340 The lack of sexual reproduction in *M. incognita* was so far only assumed based upon initial
341 cytogenetic observations (Triantaphyllou 1981; Triantaphyllou 1985) but never further supported
342 at whole-genome scale. Here, the different analyses we performed at the population genomics
343 level converge in supporting the lack of recombination and genetic exchanges in *M. incognita*.
344 The phylogenetic network analysis based on homozygous SNPs returned a bifurcating tree that
345 separated the different isolates and not a network. This suggests a lack of genetic exchange
346 between the isolates. In sexual 'recombining' species, the mitochondrial genome accumulates
347 mutations much faster than the nuclear genome. This is also true in the model nematode *C.*
348 *elegans* where the mitochondrial mutation rate is at least two orders of magnitude higher than the
349 nuclear mutation rate (Denver et al. 2004; Denver et al. 2009). The higher mitochondrial
350 accumulation of mutations is supposed to be the combined result of extremely rare or total lack
351 of recombination, the low effective population size and the effectively haploid inheritance in
352 mitochondria (Neiman Maurine and Taylor Douglas R 2009). In *M. incognita*, as opposed to *C.*
353 *elegans*, we found that the percentage of variable positions in the mitochondrial genome is only
354 one order of magnitude higher than in the nuclear genome. This suggests that the nuclear genome
355 evolves at a comparable rate to the mitochondrial genome and reinforces the idea that the nuclear
356 genome is mostly effectively haploid and non-recombining. Theoretically, the efficacy of

357 selection should be lower in non-recombining species than recombining ones. We showed that
358 the ratio of diversity at non-synonymous sites / diversity at synonymous sites (π_n/π_s) was indeed
359 ~3 times higher in *M. incognita* than in two outcrossing Caenorhabditis species. Finally, the
360 proportion of markers passing the 4-gametes test and linkage disequilibrium did not show the
361 exponential decrease, respectively increase, with physical distance as expected under
362 recombination. In contrast, a rapid exponential decrease of linkage disequilibrium was recently
363 observed and considered as an evidence for recombination in the bdelloid rotifer *Adineta vaga*
364 (Vakhrusheva et al. 2018). Collectively, these results strongly suggest absence (or extremely rare)
365 recombination and support the mitotic parthenogenetic reproduction of *M. incognita*.
366 Overall, we provided here additional evidence for adaptability and the first whole-genome level
367 assessment for the lack of recombination in *M. incognita*, consolidating this species as a main
368 model to study the paradox of adaptability and parasitic success in the absence of sexual
369 reproduction.
370 The adaptability of *M. incognita* despite its obligatory asexual reproduction and the lack
371 of phylogenetic signal underlying the host races have important practical implications at the
372 agricultural level. Characterizing populations that differ in their ability to infest a particular host
373 (that carries specific resistance genes) is of crucial importance for growers and agronomists.
374 Indeed, the main *Meloidogyne* spp. control strategies consist in deploying resistant cultivars and
375 appropriate crop rotation against a specific given race. If the identity of a population is unknown,
376 the crop selected for use in a management scheme may cause dramatic increases in nematode
377 populations (Hartman and Sasser 1985). However, the adaptability of *M. incognita* casts serious
378 doubts on the durability of such strategies and must be taken into account in rotation schemes.
379 Furthermore, the biological reality of host races themselves is challenged by the lack of
380 underlying genetic signal. Actually, the initial host race concept, has never been universally
381 accepted, in part because it covered only a small portion of the whole potential variation in
382 parasitic ability (Moens et al. 2009). Although *M. incognita* was already known to parasitize
383 hundreds of host plants, only six different host standards were used to characterise four races.
384 New host races might be defined in the future when including additional hosts in the differential
385 test. Furthermore, using the same six initial host plant species, two additional *M. incognita* races
386 that did not fit into the previously published race scheme have already been described (Robertson
387 et al. 2009). Although the terminology 'races' of *Meloidogyne* spp. has been recommended not
388 to be used since 2009 (Moens et al. 2009), several papers related to *M. incognita* diversity of host
389 compatibility or selection of resistant cultivars are still using this term; including on coffee (Lima
390 et al. 2015; Peres et al. 2017); cotton (Mota et al. 2013; da Silva et al. 2014) or soybean (Fourie
391 et al. 2006). This reflects the practical importance to differentiate *M. incognita* populations
392 according to their different ranges of host compatibilities. However, because these variations in

393 host ranges are not monophyletic and thus do not follow shared common genetic ancestry, we
394 recommend to abandon the term 'race'.

395 Another main question relates to the level of intra-specific genome polymorphism
396 required to cover the different ranges of host compatibilities in *M. incognita* and their ability to
397 survive in different environments, despite their clonal reproduction. In this study, we found that
398 the cumulative homozygous divergence across the eleven isolates from Brazil and the reference
399 genome (sampled initially from Mexico) reached ~0.02% of the nucleotides. Addition of isolates
400 from Africa, the West Indies and the USA did not increase the maximal divergence. This
401 relatively low divergence is rather surprising, considering the variability in terms of distinct
402 compatible host spectra (host races). Host-specific SNPs were found only for Race 2 and no
403 functional consequence for these SNPs could be found, as they did not fall in predicted coding or
404 evident regulatory regions. Furthermore, the existence of race-specific SNPs themselves is even
405 questionable as addition of other isolates might disqualify the few Race 2-specific SNPs in the
406 future.

407 Collectively, our observations indicate that *M. incognita* is versatile and adaptive despite
408 its clonal mode of reproduction. The relatively low divergence at the SNP level suggests
409 acquisition of point and short scale mutations followed by selection of the fittest haplotype is
410 probably not the main or at least not the sole player in the adaptation of this species to different
411 host plants and environments. Other mechanisms such as epigenetics, copy number variations,
412 movement of transposable elements or large-scale structural variations could be at play in the
413 surprising adaptability of this clonal species. Consistent with this idea, convergent gene copy
414 number variations (CNV), have recently been shown to be associated with adaptation to a
415 resistance gene-bearing plant in *M. incognita* (Castagnone-Sereno et al. 2019). Interestingly, the
416 parthenogenetic marbled crayfish has multiplied by more than 100 its original area of repartition
417 across Madagascar, adapting to different environments despite showing a surprisingly low
418 number of nucleotide variation (only ~400 SNPs on a ~3Gb genome representing a proportion of
419 variable positions of $1.3\ 10^{-7}$ only). This also led the authors to suggest that mechanisms other
420 than acquisition of point mutations and selection of the fittest haplotype must be involved
421 (Gutekunst et al. 2018).

422 Previously, we have shown that the genome structure of *M. incognita* itself, could
423 participate in its versatility. Indeed, being allopolyploid, *M. incognita* has >90% of its genes in
424 multiple copies. The majority of these gene copies show diverged expression patterns one from
425 the other and signs of positive selection between the gene copies have been identified (Blanc-
426 Mathieu et al. 2017). How the expression patterns of these gene copies vary across different
427 geographical isolates with different host compatibilities would be interesting to explore in the
428 future.

429

Material and Methods

Purification, species identification of *M. incognita* isolates and determination of host races.

The *M. incognita* isolates involved in this study (Table 1) originate from populations collected from different crops and geographically distant origins in Brazil (Figure 1). For each isolate, one single female and its associated egg mass were retrieved as explained in (Carneiro & Almeida 2001). To determine the species (here *M. incognita*), we used esterase isozyme patterns on the female (Carneiro et al. 2000). The single egg mass was used for tomato plant infection and multiplication. We reproduced egg-mass isolates on tomato plants (*Solanum lycopersicum* L. cv. Santa Clara) under greenhouse conditions at a temperature of 25-28 ° C. After three months, we confirmed the *M. incognita* species using esterase phenotypes (Carneiro and Almeida 2001). Once enough nematodes were multiplied, a pool was collected and we performed the North Carolina Differential Host Test (NCDHT) (Hartman and Sasser 1985) with the following plants: cotton cv. Deltapine 61, tobacco cv. NC95, pepper cv. Early California Wonder, watermelon cv. Charleston Gray, peanut cv. Florunner and tomato cv. Rutgers to determine the host race status. We inoculated these plants with 5,000 eggs and J2 of *M. incognita* and maintained them under glasshouse conditions at 25-28° C for three months, with watering and fertilisation as needed. Two months after inoculation, the root system was rinsed with tap water, and egg masses were stained with Phloxine B (Hartman and Sasser 1985) to count the number of galls and eggs masses separated for each root system. We assigned a rating index number according to the scale: 0 = no galls or egg masses; 1 = 1-2 galls or egg masses; 2 = 3-10 galls or egg masses; 3 = 11-30 galls or egg masses; 4 = 31-100 galls or egg masses; and 5 > 100 galls or egg masses per root system (Table 1). Host–plants types that have an average gall and egg mass index of 2 or less are designated non-host (-). The other plants (index ≥ 3) are designated hosts (+). We categorised *M. incognita* host races based on their ability to parasitize tobacco and cotton (Table 1). Classically, the index for Rutgers tomato (susceptible control) is higher than 4 (+) (Hartman & Sasser, 1985). The rest of the population was kept for multiplication on tomato plants to produce enough nematodes for sequencing (typically >1 million individuals pooled together).

458

DNA preparation and SCAR test

For each characterized nematode isolate, we extracted and purified the genomic DNA from pooled eggs with the supplement protocol for nematodes of the QIAGEN Gentra® Puregene® Tissue Kit with the following modifications: incubation at 65 ° C in the cell lysis buffer for 30 min

463 and incubation at 55 ° C with proteinase K for 4h. We verified DNA integrity on 0.8% agarose
464 gel and the DNA quantification on Nanodrop. We confirmed isolate species purity by SCAR-
465 PCR (Zijlstra et al. 2000; Randig et al. 2002) using the SCAR primers specified in Table S6 for
466 the RKN *M. javanica, M. paranaensis, M. incognita,* and *M. exigua*.
467

468 ## Sequencing library preparation

469 We assessed input gDNA quantity using Qubit and normalised the samples to 20ng/ul as
470 described in TruSeq®DNA PCR-Free Library Prep Reference Guide (#FC-121-3001, Illumina)
471 prior fragmentation to 350bp with Covaris S2. We assessed the quality of fragments after size
472 selection and size control of the final libraries using High Sensitivity DNA Labchip kit on an
473 Agilent 2100 Bioanalyzer.
474

475 ## Whole genome sequencing

476 We quantified isolated sample libraries with KAPA library quantification kit (#7960298001,
477 Roche) twice with two independent dilutions at 1:10,000 and 1:20,000. We calculated the average
478 concentration of undiluted libraries and normalised them to 2nM each then pooled them for
479 sequencing step.
480 We generated high-coverage genomic data for the 11 *M. incognita* isolates by 2x150 bp paired-
481 end Illumina NextSeq 500 sequencing with High Output Flow Cell Cartridge V2 (#15065973,
482 Illumina) and High Output Reagent Cartridge V2 300 cycles (#15057929, Illumina) on the UCA
483 Genomix sequencing platform, in Sophia-Antipolis, France. We performed two runs to balance
484 the read's representation among the isolates and obtain homogeneity of coverage for the different
485 samples (Table S2).
486

487 ## Variant Detection

488 We trimmed and cleaned the reads from each library with cutadapt tool (Martin 2011) to remove
489 adapter sequences and bases of a quality inferior to 20. We aligned the clean reads to the *M.*
490 *incognita* reference genome (Blanc-Mathieu et al. 2017), using the BWA-MEM software package
491 (Li 2013). This reference genome is described as triploid with highly diverged genome copies as
492 a result of hybridization. Most of the duplicated regions have been correctly separated during
493 genome assembly, according to genome assembly size (183.53 Mb) that is in the range of the
494 estimated total DNA content in cells via flow cytometry (189±15Mb) (Blanc-Mathieu et al.
495 2017)). Hence, the genome was considered in this analysis as mostly haploid. However, the
496 distribution of per-base coverage on the genome assembly presented a 2-peaks distribution with
497 a second minor peak at ~twice the coverage of the main peak (Fig. S2). Genome regions of double

498 coverage most likely represent collapsed homoeologous loci of lower divergence and will
499 systematically be responsible for 'artefactual' heterozygous SNPs as the reads from the two
500 homoeologous copies will map a single collapsed region in the reference genomes. To avoid
501 confusion between SNPs representing variations between individuals from those being artefacts
502 due to collapsed homoeologous regions, heterozygous (0/1) SNPs were discarded from the
503 analysis and only homozygous (1/1) SNPs were considered.
504 We used SAMtools (Li et al. 2009) to filter alignments with MAPQ lower than 20, sort the
505 alignment file by reference position, and remove multi-mapped alignments.
506 We used the FreeBayes variant detection tool (Garrison and Marth 2012) to call SNPs
507 and small-scale insertions/deletions, incorporating all the library alignment files simultaneously
508 and produced a variant call file (VCF). We filtered the resulting VCF file with the vcffilter
509 function of vcflib (Anon 2018) , retaining the positions that had more than 20 Phred-scaled
510 probability (QUAL) and a coverage depth (DP) > 10. To conduct the same analyses on the genome
511 of the meiotic diploid nematode *Globodera pallida*, we first phased the SNPs to haplotypes using
512 WhatsHap (Martin et al. 2016) because the genome assembly mainly consist of collapsed paternal
513 and maternal haplotypes.
514

515 Genetic diversity between isolates, clusters and efficacy of purifying selection

516 We used SNPGenie (Nelson et al. 2015) to estimate the nucleotide variability at non-synonymous
517 and synonymous sites as well as efficacy of purifying selection (π_N , π_S and π_N/π_S) for each isolate
518 using as input the reference genome, the gene annotation file, and the variant call file. We
519 calculated fixation index (F_{ST}) for the three clusters using vcftools (Danecek et al. 2011).
520

521 Genetic tests for detection of recombination

522 We used custom made scripts (cd. Data Availability section) to calculate the proportion of
523 markers passing the 4-gametes test and Linkage Disequilibrium (LD) r^2 values as a function of
524 inter-marker distance along the *M. incognita* genome scaffolds.
525

526 Principal component analysis

527 We performed a principal component analysis (PCA) to classify the isolates according to their
528 SNP patterns and mapped the race characteristics, geographic location, or current host plants on
529 this classification. We used the filtered VCF file as input in the statistical package SNPRelate
530 (Zheng et al. 2012) to perform the PCA with default parameters.

Phylogenetic analysis

Based on the VCF file and the *M. incognita* gene predictions (Blanc-Mathieu et al. 2017), we selected 85,413 positions that contained synonymous or non-synonymous mutations (i.e. in coding regions). We aligned these positions and then used them as an input in SplitsTree4 with default parameters. The resulting network produced a bifurcating tree that was identical to the one obtained with RAxML-NG using GTR+G+ASC_LEWIS model. The bifurcating tree was used as input to PastML (Ishikawa et al. 2018) for reconstruction of the ancestral states of ability to parasitize tobacco and cotton (Fig. S8). Phylogenetic inferences for the largest scaffolds containing at least 20 SNPs and the mitochondrial genome were conducted with RAxML-NG (Kozlov et al. 2019) using the GTR+G substitution model (except for scaffolds 10 and 20 for which the K80+G model was used because not enough phylogenetically informative positions were available).

Test for association between biological traits and genetic clusters

We used a Fisher's exact test in R to assess whether there was a significant association between the SNP-based clusters and the host races or the crop species from which the isolates were originally collected. We also conducted an Isolation By Distance (IBD) analysis using the adegenet R package (Jombart and Ahmed 2011) to check how well the genetic distances correlate with geographic distances between the sampling points of the isolates. Geographic distances were calculated from exact sampling locations, when available, or centre points if the region was known but not the exact sampling location. Sample R3-4 was excluded from this analysis since it was a mix of samples pooled together from different geographical locations. L27 was also excluded since the sampling location was unknown.

Mitochondrial genome analysis

We sub-sampled genomic clean reads to 1% of the total library for each *M. incognita* isolate. Then, we assembled them independently using the plasmidSPAdes assembly pipeline (Antipov et al. 2016). We extracted the mitochondrial contigs based on similarity to the *M. incognita* reference mitochondrial genome sequence (NCBI ID: NC_024097). In all cases, the mitochondrion was assembled in one single contig. We identified the two repeated regions (63 bp repeat and 102 bp repeat), described in (Okimoto et al. 1991) and we calculated the number of each repeat present in these regions.

Acknowledgements

This work was supported by the Agence Nationale de la Recherche program (grant number ANR-13-JSV7-0006—ASEXEVOL), the Insitut National de la Recherche Agronomique program (grant number Accueil de Chercheur Etranger SPE 2016). GK has received the support of the EU in the framework of the Marie-Curie FP7 COFUND People Programme, through the award of an AgreenSkills+ fellowship (under grant number 609398), EM received a post-doc fellowship from the Consórcio Pesquisa Café, EVSA received funding from Embrapa for sequencing at UCA Genomix platform. We thank the SPIBOC Bioinformatics platform for the data hosting.

Figure Legends

Figure 1. World map showing geographical origins for all samples used in the study.

Expanded map of Brazil showing the states where the 11 isolates that were sequenced in this study were collected. Each state is highlighted with a different colour. The countries listed in the literature for other sequenced genomes are completely coloured. The cultures from which the samples were isolated are illustrated by photographs, which are pointed by arrows coming from the name of the respective isolate. Only 3 of the isolates described previously in the literature have their culture of origin reported. The names of the Brazilian isolates are in 4 different colour sources for each race (race 1 in green, 2 in red, 3 in grey and 4 in blue). The names of the isolates of the literature are written in white or black.

Figure 2. Distribution of the number of variants per race and isolate.

Number of variants per isolate and isolate-specific variants for the 11 Brazilian isolates.

Figure 3. PCA analysis of the different *M. incognita* isolates groups them into three clusters (A, B, and C).

The geographic origins are associated to coloured shapes*:* black circle: Paraná, orange diamond: Santa Catarina, green square: São Paulo, red triangle: Mato Grosso, blue star: pool. Host plant representative pictures are displayed next to the isolates: soybean pod (R1-2 and R3-2); cotton flower (R3-1, R3-4, R4-4, and R4-1); coffee grain (R2-6); cucumber vegetable (R1-3); tobacco leaves (R1-6 and R2-1); and watermelon fruit slice (R4-3).

Figure 4. Phylogenetic network for *M. incognita* isolates based on SNPs present in coding sequences.

The phylogenetic network based only on changes in coding sequences shows the same grouping into 3 distinct groups.

Figure 5. Linkage Disequilibrium (red) and 4-gametes test of *M. incognita* isolates.

Based on SNPs that were homozygous within isolates: proportion of pair of markers that pass the 4-gametes test (blue) and linkage disequilibrium measured as r2 between markers (red), both as a function of the inter-markers distance on *M. incognita* scaffolds.

Figure 6. Linkage Disequilibrium (red) and 4 gametes test (blue) for phased SNPs in *Globodera rostochiensis* isolates.

The r^2 correlation between markers, indicating linkage disequilibrium (LD) is given in the red (upper) plot, as a function of the physical distance between the markers. The proportion of pairs of two-state markers that pass the 4-gamete test is given in the blue (lower) plot as a function of the distance between the markers.

Figure 7. PCA analysis of all known *M. incognita* genomes.

The isolates were regrouped based on SNP patterns confirming the same three clusters. Origin countries are indicated by flags (Brazil for R1-2, R1-3, R1-6, R2-1, R2-6, R3-1, R3-2, R3-4, R4-1, R4-3, R4-4; USA for L27, 557R, HarC, W1, VW6; Mexico for Morelos; Libya for A14; Ivory Coast for L9; Guadeloupe for L19).

Data Availability

All the sequence data generated during this study have been deposited at the NCBI under GEO accession GSE116847 and available at this URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE116847

The different scripts and R codes used to process the data are available on GitHub at the following URL:https://github.com/GDKO/gdk_scripts/tree/master/popgenvcf

Author contributions

EGJD, PC-S, GDK, EVSA and LD contributed to the design of the project; RMDGC, ACZM, EM contributed to collection of samples. Classification of samples into races was done by RMDGC, ACZM, EM; library preparations were performed by M-JA; GDK, EGJD, LD, EVSA, MBB and DKK contributed to the analysis and interpretation of data. EGJD and GDK wrote the manuscript with contributions of EVSA, RMDGC, PC-S, M-JA and LD. All authors approved the final manuscript.

629 # Tables

630 Table 1. Host race characterization of the 11 *Meloidogyne incognita* isolates used in this study

Race	ID[a]	Host Crop[b]	PID[c]	Geographic origin	Esterase phenotype	NCDHT note[d]						Reference
						tb	tm	wm	pt	pr	ct	
1	R1-2	soybean	INC15	Londrina - PR	I2	0	5	4	0	5	0	(Mattos et al. 2016)
	R1-3	cucumber	ND	Piracicaba - SP	I2	0	5	5	0	4	0	
	R1-6	tobacco	LGM39	Mercedes - PR	I2 / I1	0	5	5	0	4	0	(Filho et al. 2016)
2	R2-1	tobacco	LGM09	Sombrio - SC	I2	5	5	4	0	4	0	(Filho et al. 2016)
	R2-6	coffee	22B	São Jorge do Patrocínio - PR	I1	4	5	5	0	4	1	
3	R3-1	cotton	PR-3	Umuarama - PR	I2	0	5	5	0	5	5	(da Silva et al. 2014)
	R3-2	soybean	ND	Londrina - PR	I2	0	5	5	0	5	5	
	R3-4	cotton (pool)	PR-3 Umu, PR-3 Lon, MTS-R3, BA-R3	Umuarama - PR, Londrina - PR, Dourados - MS, L.E.Magalhães - BA	I2	0	5	5	0	5	5	(da Silva et al. 2014)
4	R4-1	cotton	MT-4	Campo Verde - MT	I2	5	5	5	0	5	5	(da Silva et al. 2014)
	R4-3	watermelon	NG	Londrina - PR	I2	5	5	5	0	5	5	
	R4-4	cotton	GEN 306	Vargem Grande do Sul - SP	I1	5	5	5	0	5	5	

631 a, isolate identification code
632 b, host plant crop (soybean, *Glycine max*; cucumber, *Cucumis sativus*; tobacco, *Nicotiana tabacum*; coffee, *Coffea arabica*; cotton, *Gossypium hirsutum*.; watermelon, *Citrullus vugaris*
633 c, population id
634 d, host range results for the North Carolina Differential Host Test (NCDHT), numbers represent gall index with 0 = no galls; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; and 5 = more
635 than 100 galls. (tb=tobacco 'NC95', tm=tomato 'Rutgers', wm=watermelon 'Charleston Gray', pt=peanut 'Florunner', pr=pepper 'Early California Wonder', ct=cotton 'Deltapine 61'

636 Table 2. Number of repeats per region (63nt and 102nt) in the mitochondrial
637 DNA of each isolate, decimals indicate truncated repeats

ID	63nt Region	102nt Region	Location	Host plant
R1-2	7.3	5.5	Londrina - PR	soybean
R1-3	7	13	Piracicaba - SP	cucumber
R1-6	1.2	7	Mercedes - PR	tobacco
R2-1	7	15.4	Sombrio - SC	tobacco
R2-6	7	9	São Jorge do Patrocínio - PR	coffee
R3-1	7	13	Umuarama - PR	cotton
R3-2	14	8.3	Londrina - PR	soybean
R3-4	6	13	Umuarama – PR Londrina – PR Dourados – MS L.E.Magalhães - BA	cotton
R4-1	6	14.7	Campo Verde - MT	cotton
R4-3	3	9	Londrina - PR	watermelon
R4-4	14	8.3	Vargem Grande do Sul - SP	cotton

638

639 Figure 1. World map showing geographical origins for all samples used in the study.

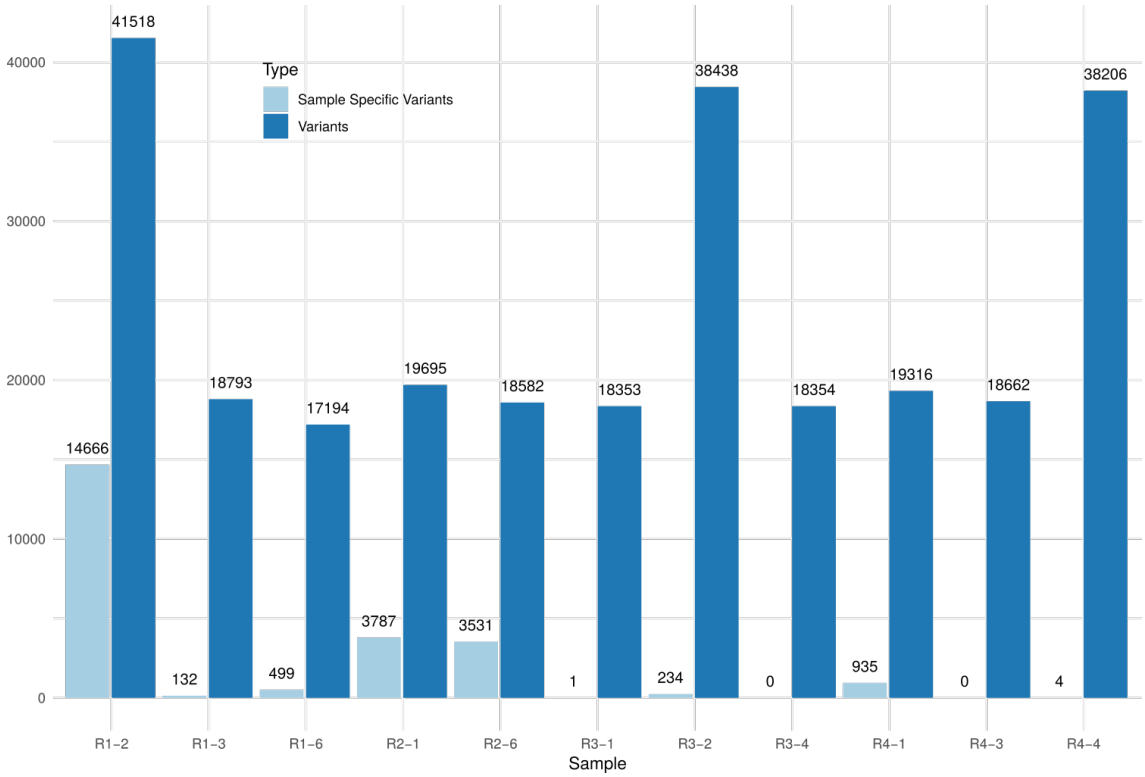


Figure 2. Distribution of the number of variants per race and isolate.

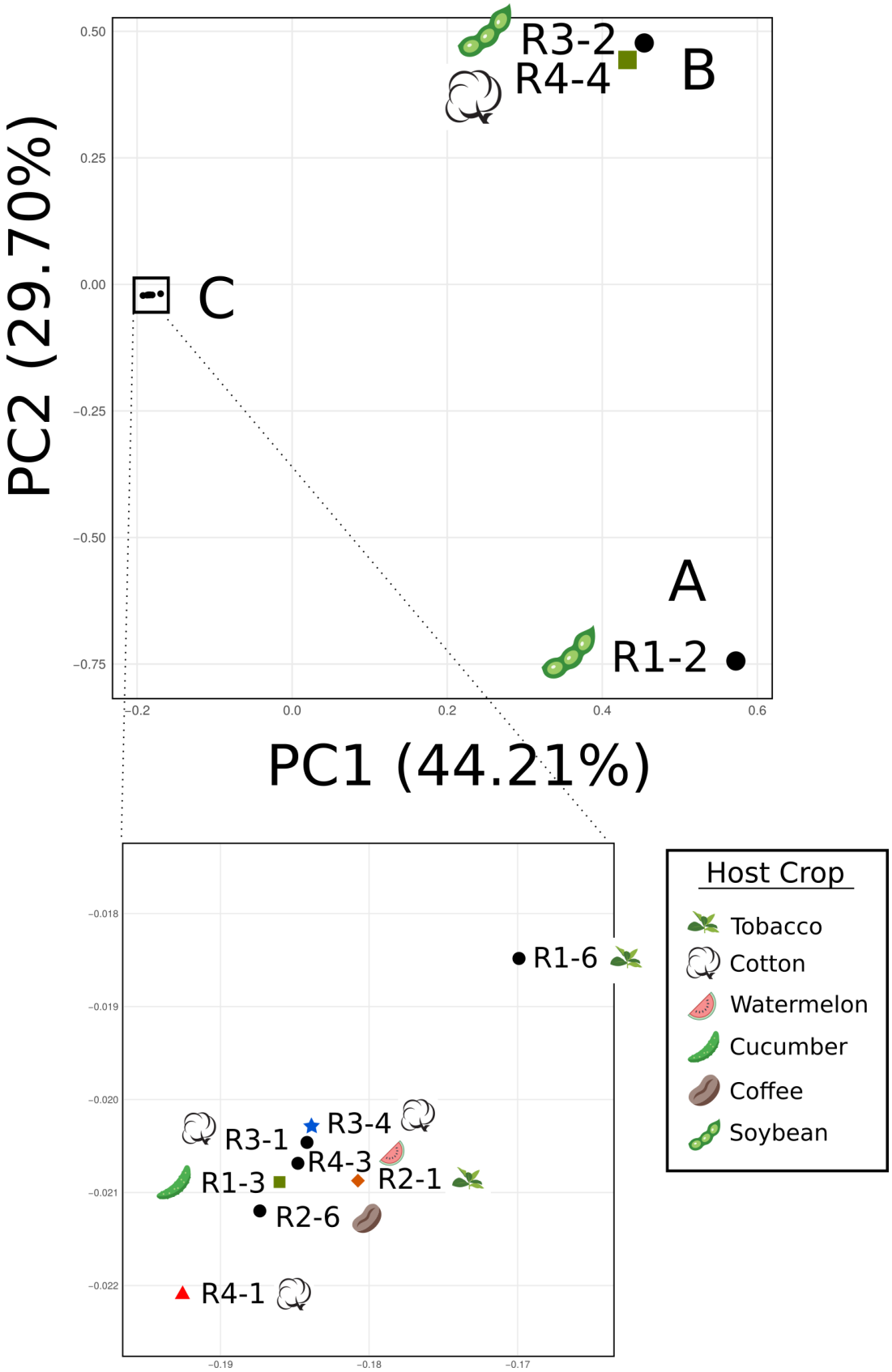


Figure 3. PCA analysis of the different *M. incognita* isolates groups them into three clusters (A, B, and C).

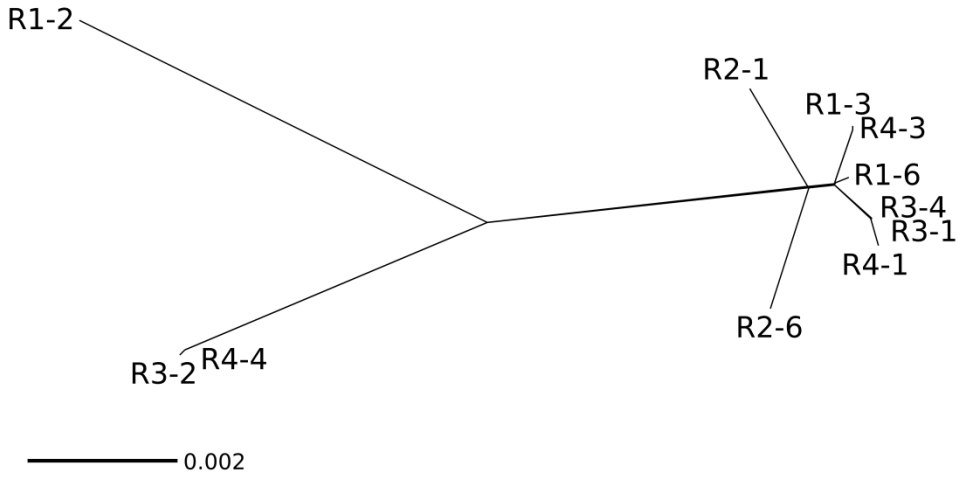


Figure 4. Phylogenetic network for *M. incognita* isolates based on SNPs present in coding sequences.

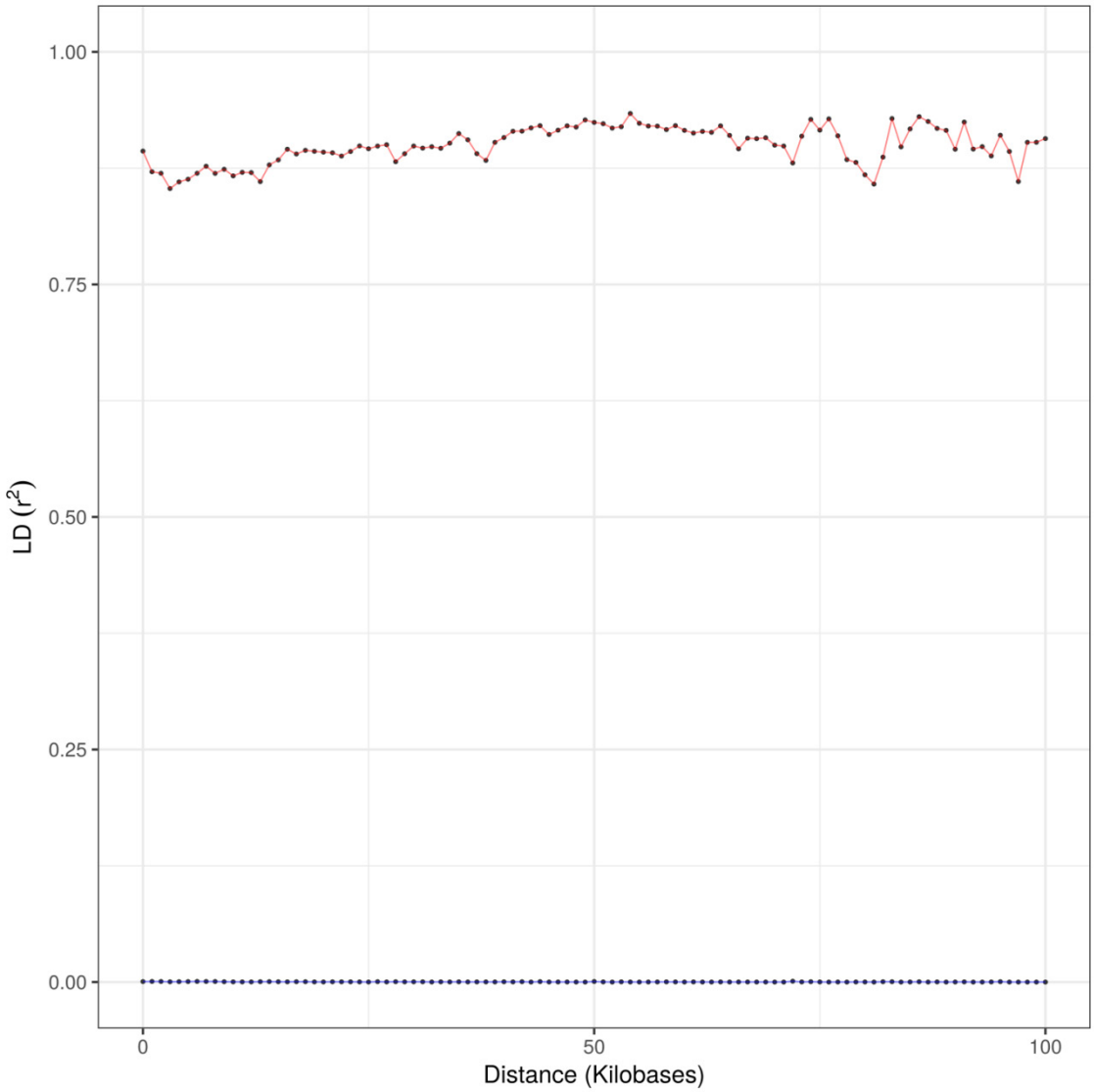
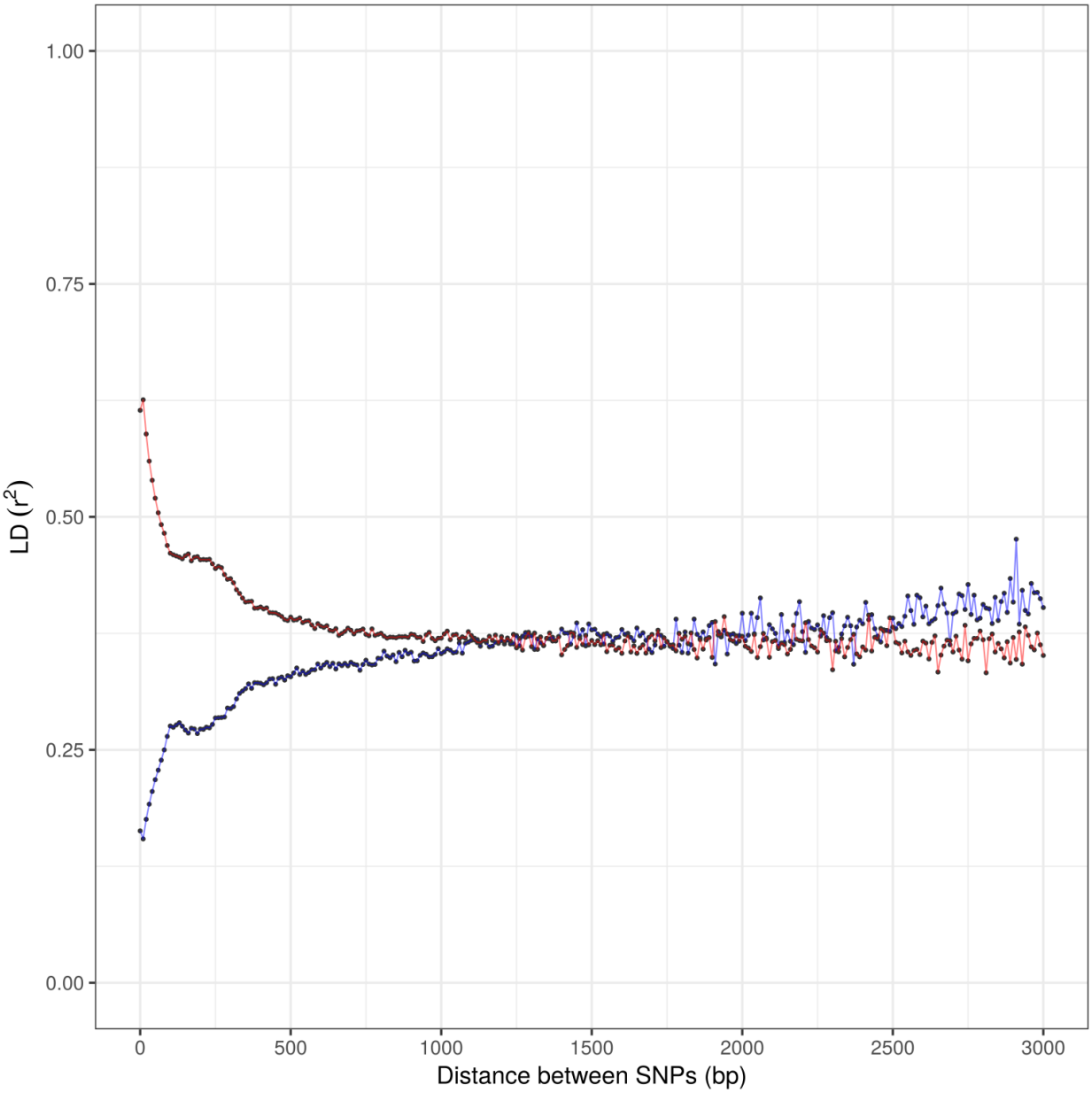


Figure 5. Linkage Disequilibrium (red) and 4-gametes test (blue) of *M. incognita* isolates.



654

655 Figure 6. Linkage Disequilibrium (red) and 4 gamete test (blue) for phased SNPs

656 in *Globodera rostochiensis* isolates.

657

658

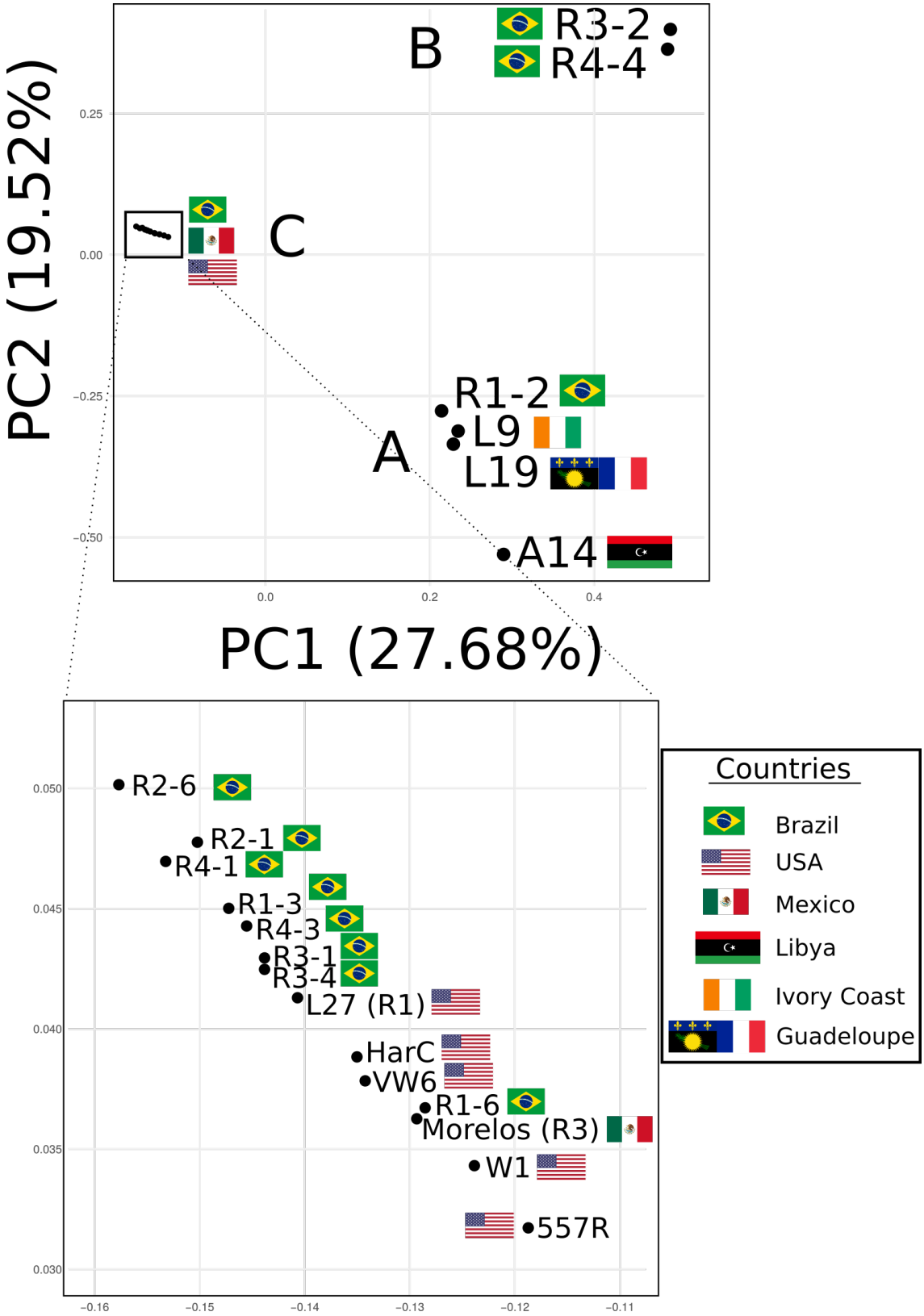


Figure 7. PCA analysis of all known *M. incognita* genomes.

662 # References

663 Abad P, Gouzy J, Aury J-M, Castagnone-Sereno P, Danchin EGJ, Deleury E, Perfus-
664 Barbeoch L, Anthouard V, Artiguenave F, Blok VC, et al. 2008. Genome sequence of
665 the metazoan plant-parasitic nematode Meloidogyne incognita. Nat. Biotechnol.
666 26:909–915.
667 Anon. 2018. vcflib: a simple C++ library for parsing and manipulating VCF files, +
668 many command-line utilities. vcflib Available from: https://github.com/vcflib/vcflib
669 Antipov D, Hartwick N, Shen M, Raiko M, Lapidus A, Pevzner PA. 2016.
670 plasmidSPAdes: assembling plasmids from whole genome sequencing data.
671 Bioinformatics 32:3380–3387.
672 Barbary A, Djian-Caporalino C, Palloix A, Castagnone-Sereno P. 2015. Host genetic
673 resistance to root-knot nematodes, Meloidogyne spp., in Solanaceae: from genes to the
674 field. Pest Manag. Sci. 71:1591–1598.
675 Baum TJ. 1994. Characterization and Phylogenetic Analysis of Four Root-Knot
676 Nematode Species Using DNA Amplification Fingerprinting and Automated
677 Polyacrylamide Gel Electrophoresis. Mol. Plant. Microbe Interact. 7:39.
678 Blanc-Mathieu R, Perfus-Barbeoch L, Aury J-M, Rocha MD, Gouzy J, Sallet E, Martin-
679 Jimenez C, Bailly-Bechet M, Castagnone-Sereno P, Flot J-F, et al. 2017. Hybridization
680 and polyploidy enable genomic plasticity without sex in the most devastating plant-
681 parasitic nematodes. PLOS Genet. 13:e1006777.
682 Blok VC, Powers TO. 2009. Biochemical and molecular identification. Available from:
683 https://nebraska.pure.elsevier.com/en/publications/biochemical-and-molecular-
684 identification
685 Carneiro RMDG, Almeida MR, Quénéhervé P. 2000. Enzyme phenotypes of
686 *Meloidogyne* spp. populations. Nematology 2:645–654.
687 Carneiro RMDG, Almeida MRA. 2001. Técnica de eletroforese usada no estudo de
688 enzimas dos nematóides de galhas para identificação de espécies. Nematol. Bras. 25:35–
689 44.
690 Castagnone-Sereno P. 2006. Genetic variability and adaptive evolution in
691 parthenogenetic root-knot nematodes. Heredity 96:282–289.
692 Castagnone-Sereno P, Danchin EGJ. 2014. Parasitic success without sex – the nematode
693 experience. J. Evol. Biol. 27:1323–1333.

694 Castagnone-Sereno P, Mulet K, Danchin EGJ, Koutsovoulos GD, Karaulic M, Da
695 Rocha M, Bailly-Bechet M, Pratx L, Perfus-Barbeoch L, Abad P. 2019. Gene copy
696 number variations as signatures of adaptive evolution in the parthenogenetic, plant-
697 parasitic nematode *Meloidogyne incognita*. Mol. Ecol.:mec.15095.
698 Castagnone-Sereno P, Wajnberg E, Bongiovanni M, Leroy F, Dalmasso A. 1994.
699 Genetic variation inMeloidogyne incognita virulence against the tomatoMi resistance
700 gene: evidence from isofemale line selection studies. Theor. Appl. Genet. 88:749–753.
701 Cenis JL. 1993. Identification of Four Major Meloidogyne spp. by Random Amplified
702 Polymorphic DNA (RAPD-PCR). Phytopathology:76–78.
703 Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE,
704 Lunter G, Marth GT, Sherry ST, et al. 2011. The variant call format and VCFtools.
705 Bioinformatics 27:2156–2158.
706 Denver DR, Dolan PC, Wilhelm LJ, Sung W, Lucas-Lledó JI, Howe DK, Lewis SC,
707 Okamoto K, Thomas WK, Lynch M, et al. 2009. A genome-wide view of
708 Caenorhabditis elegans base-substitution mutation processes. Proc. Natl. Acad. Sci.
709 106:16310–16314.
710 Denver DR, Morris K, Lynch M, Thomas WK. 2004. High mutation rate and
711 predominance of insertions in the Caenorhabditis elegans nuclear genome. Nature
712 430:679–682.
713 Eisenback JD, Hunt D. 2009. General morphology. In: R. N. Perry MM, editor. Root-
714 Knot Nematodes. Wallingford: cabi. p. 18–54.
715 Esbenshade PR, Triantaphyllou AC. 1985. Use of Enzyme Phenotypes for Identification
716 of Meloidogyne Species. J. Nematol. 17:6–20.
717 Eves-van den Akker S, Laetsch DR, Thorpe P, Lilley CJ, Danchin EGJ, Da Rocha M,
718 Rancurel C, Holroyd NE, Cotton JA, Szitenberg A, et al. 2016. The genome of the
719 yellow potato cyst nematode, Globodera rostochiensis, reveals insights into the basis of
720 parasitism and virulence. Genome Biol. [Internet] 17. Available from:
721 http://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-0985-1
722 Filho JV de A, Machado ACZ, Dallagnol LJ, Aranha Camargo LE. 2016. Root-Knot
723 Nematodes (Meloidogyne spp.) Parasitizing Resistant Tobacco Cultivars in Southern
724 Brazil. Plant Dis. 100:1222–1231.

725 Flot J-F, Hespeels B, Li X, Noel B, Arkhipova I, Danchin EGJ, Hejnol A, Henrissat B,
726 Koszul R, Aury J-M, et al. 2013. Genomic evidence for ameiotic evolution in the
727 bdelloid rotifer Adineta vaga. Nature 500:453–457.
728 Fourie H, McDonald AH, Waele DD. 2006. Host suitability of South African and
729 foreign soybean cultivars to Meloidogyne incognita race 2. South Afr. J. Plant Soil
730 23:132–137.
731 Garrison E, Marth G. 2012. Haplotype-based variant detection from short-read
732 sequencing. ArXiv12073907 Q-Bio [Internet]. Available from:
733 http://arxiv.org/abs/1207.3907
734 Glémin S, Galtier N. 2012. Genome Evolution in Outcrossing Versus Selfing Versus
735 Asexual Species. In: Anisimova M, editor. Evolutionary Genomics. Vol. 855. Totowa,
736 NJ: Humana Press. p. 311–335. Available from: http://link.springer.com/10.1007/978-1-
737 61779-582-4_11
738 Gutekunst J, Andriantsoa R, Falckenhayn C, Hanna K, Stein W, Rasamy J, Lyko F.
739 2018. Clonal genome evolution and rapid invasive spread of the marbled crayfish. Nat.
740 Ecol. Evol. 2:567–573.
741 Hartman KM, Sasser JN. 1985. Identification of *Meloidogyne* species on the basis of
742 differential host test and perineal-pattern morphology. In: Barker KR, Carter CC, Sasser
743 JN, editors. Advanced Treatise on Meloidogyne. Vol. II. Raleigh: North Carolina State
744 University. p. 69–77.
745 Hill WG, Robertson A. 1966. The effect of linkage on limits to artificial selection.
746 Genet. Res. 8:269–294.
747 Ishikawa SA, Zhukova A, Iwasaki W, Gascuel O. 2018. A Fast Likelihood Method to
748 Reconstruct and Visualize Ancestral Scenarios. bioRxiv:379529.
749 Jombart T, Ahmed I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide
750 SNP data. Bioinformatics 27:3070–3071.
751 Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MGK, Kikuchi T,
752 Manzanilla-López R, Palomares-Rius JE, Wesemael WML, et al. 2013. Top 10 plant-
753 parasitic nematodes in molecular plant pathology. Mol. Plant Pathol. 14:946–961.
754 Kondrashov AS. 1988. Deleterious mutations and the evolution of sexual reproduction.
755 Nature 336:435–440.

Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019. RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. bioRxiv:447110.

Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. ArXiv13033997 Q-Bio [Internet]. Available from: http://arxiv.org/abs/1303.3997

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079.

Lima EA, Furlanetto C, Nicole M, Gomes ACMM, Almeida MRA, Jorge-Júnior A, Correa VR, Salgado SM, Ferrão MAG, Carneiro RMDG. 2015. The Multi-Resistant Reaction of Drought-Tolerant Coffee "Conilon Clone 14" to Meloidogyne spp. and Late Hypersensitive-Like Response in Coffea canephora. Phytopathology 105:805–814.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12.

Martin M, Patterson M, Garg S, Fischer SO, Pisanti N, Klau GW, Schöenhuth A, Marschall T. 2016. WhatsHap: fast and accurate read-based phasing. bioRxiv:085050.

Mattos VS, Furlanetto C, Silva JGP, Santos DFD, Almeida MRA, Correa VR, Moita AW, Castagnone-Sereno P, Carneiro RMDG. 2016. *Meloidogyne* spp. populations from native Cerrado and soybean cultivated areas: genetic variability and aggressiveness. Nematology 18:505–515.

Moens M, Perry RN, Starr JL. 2009. Meloidogyne species: a diverse group of novel and important plant parasites. In: Root-knot Nematodes. CABI Publishing. p. 1–17. Available from: http://hdl.handle.net/1854/LU-866916

Mota FC, Alves GCS, Giband M, Gomes ACMM, Sousa FR, Mattos VS, Barbosa VHS, Barroso P a. V, Nicole M, Peixoto JR, et al. 2013. New sources of resistance to Meloidogyne incognita race 3 in wild cotton accessions and histological characterization of the defence mechanisms. Plant Pathol. 62:1173–1183.

Muller HJ. 1964. The Relation of Recombination to Mutational Advance. Mutat Res 106:2–9.

Neiman Maurine, Taylor Douglas R. 2009. The causes of mutation accumulation in mitochondrial genomes. Proc. R. Soc. B Biol. Sci. 276:1201–1209.

Nelson CW, Moncla LH, Hughes AL. 2015. SNPGenie: estimating evolutionary parameters to detect natural selection using pooled next-generation sequencing data. Bioinformatics 31:3709–3711.

Okimoto R, Chamberlin HM, Macfarlane JL, Wolstenholme DR. 1991. Repeated sequence sets in mitochondrial DNA molecules of root knot nematodes (Meloidogyne): nucleotide sequences, genome location and potential for host-race identification. Nucleic Acids Res. 19:1619–1626.

Peres ACJ, Salgado SML, Correa VR, Santos MFA, Mattos VS, Monteiro JMS, Carneiro RMDG. 2017. Resistance of genotypes against and under controlled and field conditions. Nematology 19:617–626.

Randig O, Bongiovanni M, Carneiro RMDG, Castagnone-Sereno P. 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. Genome 45:862–870.

Robertson L, Díez-Rojo MA, López-Pérez JA, Buena AP, Escuer M, Cepero JL, Martínez C, Bello A. 2009. New Host Races of Meloidogyne arenaria, M. incognita, and M. javanica from Horticultural Regions of Spain. Plant Dis. 93:180–184.

Romiguier J, Gayral P, Ballenghien M, Bernard A, Cahais V, Chenuil A, Chiari Y, Dernat R, Duret L, Faivre N, et al. 2014. Comparative population genomics in animals uncovers the determinants of genetic diversity. Nature 515:261–263.

Santos MFA dos, Furlanetto C, Almeida MRA, Carneiro MDG, Mota FC, Gomes ACMM, Silveira NOR, Silva JGP, Castagnone-Sereno P, Tigano MS, et al. 2012. Biometrical, biological, biochemical and molecular characteristics of Meloidogyne incognita isolates and related species. Eur. J. Plant Pathol. 134:671–684.

Sasser JN. 1952. Identification of root-knot nematodes (Meloidogyne spp.) by host reaction. Plant Dis. Report. 36:84–86.

Sasser JN, Eisenback JD, Carter CC, Triantaphyllou AC. 1983. The International Meloidogyne Project - Its Goals and Accomplishments. Annu. Rev. Phytopathol. 21:271–288.

da Silva E, da Silva Mattos V, Furlaneto C, Giband M, Barroso P, Moita A, Jorge-Junior A, Correa V, Castagnone-Sereno P, Carneiro R. 2014. Genetic variability and virulence of Meloidogyne incognita populations from Brazil to resistant cotton genotypes. Eur. J. Plant Pathol. 139:195–204.

820 Singh SK, Hodda M, Ash GJ. 2013. Plant-parasitic nematodes of potential
821 phytosanitary importance, their main hosts and reported yield losses. EPPO Bull.
822 43:334–374.
823 Szitenberg A, Salazar-Jaramillo L, Blok VC, Laetsch DR, Joseph S, Williamson VM,
824 Blaxter ML, Lunt DH. 2017. Comparative Genomics of Apomictic Root-Knot
825 Nematodes: Hybridization, Ploidy, and Dynamic Genome Change. Genome Biol. Evol.
826 9:2844–2861.
827 Triantaphyllou AC. 1981. Oogenesis and the chromosomes of the parthenogenetic Root
828 Knot Nematode Meloidogyne incognita. J. Nematol.:95–104.
829 Triantaphyllou AC. 1985. Cytogenetics, cytotaxonomy and phylogeny of root-knot
830 nematodes. In: Sasser JN, Carter CC, editors. An advanced treatise on Meloidogyne.
831 Vol. 1. Raleigh: North Carolina State University Graphics. p. 113–126.
832 Trudgill DL, Blok VC. 2001. Apomictic, polyphagous root-knot nematodes:
833 exceptionally successful and damaging biotrophic root pathogens. Annu. Rev.
834 Phytopathol. 39:53–77.
835 Tzortzakakis EA, Conceição I, Dias AM, Simoglou KB, Abrantes I. 2014. Occurrence
836 of a new resistant breaking pathotype of Meloidogyne incognita on tomato in Greece. J.
837 Plant Dis. Prot. 121:184–186.
838 Vakhrusheva OA, Mnatsakanova EA, Galimov YR, Neretina TV, Gerasimov ES,
839 Ozerova SG, Zalevsky AO, Yushenova IA, Arkhipova IR, Penin AA, et al. 2018.
840 Recombination in a natural population of the bdelloid rotifer Adineta vaga.
841 bioRxiv:489393.
842 Verdejo-Lucas S, Talavera M, Andrés MF. 2012. Virulence response to the Mi.1 gene
843 of Meloidogyne populations from tomato in greenhouses. Crop Prot. 39:97–105.
844 Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS. 2012. A high-
845 performance computing toolset for relatedness and principal component analysis of
846 SNP data. Bioinformatics 28:3326–3328.
847 Zijlstra C, Donkers-Venne DTHM, Fargette M. 2000. Identification of *Meloidogyne*
848 *incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region
849 (SCAR) based PCR assays. Nematology 2:847–853.