ARTICLE (Discoveries)

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

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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 SNV 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 nematode 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 whole 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 genome (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 whole genome level study conclusively 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 three genome copies most likely because of hybridization events. 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 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 SNV markers
118 between isolates, (ii) the level of variation between isolates at the whole genome level, and (iii)
119 whether these variations follow a phylogenetic signal underlying life history traits such as the host
120 compatibility patterns, the geographic distribution or the current host crop plant.

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

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

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

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

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

152 We generated paired-end genome reads (~76 million per isolate) which covered the
153 ~184Mb *M. incognita* genome assembly (Blanc-Mathieu et al. 2017) at a depth > 100X (Table
154 S2) for each isolate. Variant calling, performed in regions with at least 10x coverage per sample,
155 identified 338,960 polymorphic positions (~0.19% of the total number of non-ambiguous
156 nucleotides). Around 20% of these positions corresponded to 1/1 SNV, fixed within each isolate
157 but variable between isolates and the reference genome. We examined the distribution of base
158 coverage of SNV fixed within all isolates (1/1 fixed SNV) and SNV that presented variations
159 within at least one isolate (0/1 SNV). We observed that the 0/1 SNV, which were variable within
160 isolates, showed a peak of distribution at ~twice the coverage of the peak for fixed 1/1 SNV in
161 the 11 isolates (Fig. S1). This parallels the distribution of base coverage in the *M. incognita*
162 reference genome scaffolds which shows a major peak at ~65X and a second minor peak at ~130X
163 (twice the coverage; Fig. S2). These genome regions at double coverage were considered as
164 representing highly similar pairs of homoeologous genome copies that were collapsed during the
165 assembly (Blanc-Mathieu et al. 2017). Although these regions are minority in the genome
166 assembly, they seem to be responsible for many 0/1 SNV (presenting within isolate variations).
167 The SNV in these minority regions of double coverage probably result from genome reads of two
168 homoeologous regions mapped to a single collapsed region in the reference assembly. Hence,
169 most of these 0/1 SNV might not represent variations between individuals within an isolate but
170 between the few collapsed homoeologous regions. Because the reference genome is mostly
171 assembled in haploid status (Fig. S2), and the nature of 0/1 SNV is unsure, we will utilise only
172 1/1 SNV fixed within isolates for all downstream analyses. Although this precludes analyses of
173 variations between individuals within isolates this allows a comparison of variations between
174 isolates based on >66,000 solid fixed markers.
175

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 SNV fixed 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 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 2). 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 fixed SNV 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 method 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 SNVs before conducting LD and 4-gametes tests. The results were totally contrasted between *M. incognita* and *G. rostochiensis* (Figure 3). In *G. rostochiensis*, the LD and 4-gametes curves started at relatively lower (<0.7) and higher (>0.15) values, respectively. 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

212 a whole genome-scale the lack of evidence for meiosis previously observed at the cytological
213 level in *M. incognita*.
214

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

216 Each isolate showed a different level of divergence from the reference genome with R1-
217 2 having the highest number of fixed SNV (41,518) and R1-6 having the least (17,194) variants
218 (Figure 4). The R3-4 isolate originated from a pool of four populations. However, the low number
219 of SNV compared to the reference indicates either that the genomes of these four populations
220 were very close or that a specific population displaced the other three (Figure 4). Thus, the R3-4
221 isolate was analysed exactly as the other isolates. Overall, the percentages of fixed SNV on the
222 nuclear genomes of the eleven isolates, compared to the Morelos reference strain, ranged between
223 0.01 % and 0.02 %. In comparison, the percentages of SNV in the mitochondrial genome ranged
224 between 0.04% and 0.18%.
225 Interestingly, race-specific variants exist only for race 2, which exhibited 30 race-specific
226 variations. This is possibly due to the fact that race 2 is represented by only two isolates (vs. 3 for
227 the rest of the isolates). The vast majority (~78%) of SNV were outside of coding regions; only
228 14,704 variable positions fell in coding regions and covered 7,259 out of 43,718 predicted protein-
229 coding genes. In these coding regions, 8,179 were synonymous substitutions, 3,854 SNV yielded
230 non-synonymous substitution, 93 nonsense mutations and the rest other disruptive mutations.
231 From the SNV falling in coding regions, we constructed a multiple alignment and
232 measured nucleotide diversity at synonymous (π_s) and non-synonymous (π_n) sites for the 11
233 isolates as well as the π_n/π_s ratio as a measure of the efficiency of selection. Consistent with the
234 overall low number of SNV, the π_s value across the 11 isolates was low (1.29 10^{-03}). This is one
235 order of magnitude lower than the values measured for two outcrosser nematodes from the
236 *Caenorhabditis* genus (Romiguier et al. 2014), *C. doughertyi* (formerly sp. 10: 4.93 10^{-02}) and *C.*
237 *brenneri* (3.22 10^{-02}). A similar difference of one order of magnitude was also observed for the
238 diversity at non-synonymous sites with a π_n value of 1.66 10^{-04} for *M. incognita* and values
239 reaching 2.53 10^{-03} and 1.28 10^{-03} for *C. doughetryi* and *C. brenneri*, respectively. However, the
240 π_n/π_s ratio was substantially higher for *M. incognita* (0.129) than for the two outcrossing
241 Caenorhabditis (0.051 and 0.040 for *C. doughetryi* and *C. brenneri*, respectively). These results
242 suggest a lower efficacy of selection in the obligate parthenogenetic *M. incognita* than in these
243 two outcrossing *Caenorhabditis* nematodes.

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

Using principal component analysis on the whole set of fixed SNV, we showed that the eleven *M. incognita* isolates formed three distinct clusters, which we named A, B and C (Figure 5). Cluster A is represented by isolate R1-2 alone, which has the highest number of variants. Cluster B is constituted by R3-2 and R4-4. The rest of the isolates fall in a single dense cluster C of overall low variation. There was no significant association between the clusters and the host race status (Fisher's exact test p-value=1, Sup. Text, Table S3). This implies that isolates of the same host race are not more similar to each other than isolates of different host races. There was also no significant association between the SNV-based clusters and the original host plant from which the nematodes have been collected (Fisher's exact test p-value=0.69, Sup. Text, Table S3). Interestingly, the four different host races are all represented in one single cluster (C). Within this cluster, the total number of variable positions was 29,597. Meaning that the whole range of host-race diversity is present in a cluster that represents only 44% of the total existing genomic variation. We also conducted an isolation by distance (IBD) analysis, which showed no correlation between the genetic distance and the geographical distance (Fig. S3).

To assess the levels of separation vs. past genetic exchanges between these clusters, we calculated fixation index values (F_{ST}). Weighted F_{ST} values between clusters were all >0.83, suggesting a lack of genetic connections between the clusters (Table S4). Using the mean F_{ST} values, in contrast, while we observed a mean F_{ST} >0.98 between clusters A and B, indicating a lack of genetic connection between R1-2 and cluster B, the F_{ST} values were much lower between A and C (0.35) and between B and C (0.52). This would suggest isolates from clusters A and B both result from a past bottlenecked dispersal and propagation from some isolates in cluster C. We also conducted the same π_n/π_s analysis than the one performed at the whole species level for each cluster of the PCA containing at least 2 isolates. These cluster-specific statistics yielded similar π_n/π_s ratio than the one observed at whole species level (Cluster C: π_s 3.8 10^{-04}, π_n 5.36 10^{-05}, π_n/π_s 0.141; Cluster B: π_s 2.08 10^{-05}, π_n 2.64 10^{-06}, π_n/π_s 0.127).

Phylogenetic networks confirm the lack of association of SNV with biological traits and support clonal evolution

Using a phylogenetic network analysis based on SNV present in coding regions, we could confirm the same three clusters (Figure 6). This further supports the absence of phylogenetic signal underlying the host races (patterns of host compatibilities). Interestingly, this network analysis based on fixed SNV yielded a bifurcating tree and not a network. This result suggests a lack of genetic exchanges between the isolates, as expected from a clonal

species. To confirm this result, we conducted separate phylogenetic analyses for each of the 14 longest scaffolds with sufficient number of phylogenetically informative variable positions and the mitochondrial genome. All these analyses showed a clear separation between the three clusters (A, B, and C) with some minor polytomies within cluster C (Fig. S5 and Fig; S6).

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

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

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

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

315 # Discussion

316 Is the parasitic success of *M. incognita* an evolutionary paradox? This proposition would
317 be true only if *M. incognita* is adaptive despite having a fully parthenogenetic reproduction. Our
318 results support these two aspects.

319 The lack of sexual reproduction in *M. incognita* was so far only assumed based upon initial
320 cytogenetic observations (Triantaphyllou 1981; Triantaphyllou 1985) but never further supported
321 at whole-genome scale. Here, the different analyses we performed at the population genomics
322 level converge in supporting the lack of recombination and genetic exchanges in *M. incognita*.
323 The phylogenetic network analysis based on fixed SNVs returned a bifurcating tree that separated
324 the different isolates and not a network. This suggests a lack of genetic exchange between the
325 isolates. In sexual 'recombining' species, the mitochondrial genome accumulates mutations much
326 faster than the nuclear genome. This is also true in the model nematode *C. elegans* where the
327 mitochondrial mutation rate is at least two orders of magnitude higher than the nuclear mutation
328 rate (Denver et al. 2004; Denver et al. 2009). The higher mitochondrial accumulation of mutations
329 is supposed to be the combined result of extremely rare or total lack of recombination, the low
330 effective population size and the effectively haploid inheritance in mitochondria (Neiman
331 Maurine and Taylor Douglas R 2009). In *M. incognita*, as opposed to *C. elegans*, we found that
332 the percentage of variable positions in the mitochondrial genome is only one order of magnitude
333 higher than in the nuclear genome. This suggests that the nuclear genome evolves at a comparable
334 rate to the mitochondrial genome and reinforces the idea that the nuclear genome is mostly
335 effectively haploid and non-recombining. Theoretically, the efficacy of selection should be lower
336 in non-recombining species than recombining ones. We showed that the ratio of diversity at non-
337 synonymous sites / diversity at synonymous sites (π_n/π_s) was indeed one order of magnitude
338 higher in *M. incognita* than in two outcrossing Caenorhabditis species. Finally, the proportion of
339 markers passing the 4-gametes test and linkage disequilibrium did not show the exponential
340 increase, respectively decrease, with physical distance as expected under recombination. In
341 contrast, a rapid exponential decrease of linkage disequilibrium was recently observed and
342 considered as an evidence for recombination in the bdelloid rotifer *Adineta vaga* (Vakhrusheva
343 et al. 2018). Collectively, these results strongly suggest absence (or extremely rare) recombination
344 and support the mitotic parthenogenetic reproduction of *M. incognita*.

345 Despite its clonal reproduction, it was already evident that *M. incognita* has an adaptive
346 potential. Indeed, experimental evolution assays have shown the ability of *M. incognita* to
347 overcome resistance conferred by the Mi gene in tomato in a few generations (Castagnone-Sereno
348 et al. 1994; Castagnone-Sereno 2006). Naturally virulent *M. incognita* populations (i.e. not
349 controlled by the resistance gene) have also been observed in the fields and probably emerged
350 from originally avirulent populations (Verdejo-Lucas et al. 2012; Tzortzakakis et al. 2014;

351 Barbary et al. 2015), although it is unknown if this resistance breaking is as rapid as under
352 controlled lab conditions. However, adapting from a compatible host plant to another very
353 different incompatible plant is certainly more challenging than breaking down a resistance gene
354 in a same plant. Here, we showed that the different host races defined in *M. incognita* as a function
355 of patterns of (in)compatibilities with different plants do not follow a phylogenetic signal. This
356 would imply multiple independent gains and losses of parasitic abilities to arrive at the current
357 phylogenetic distribution of host compatibility patterns (i.e. host races). Whether these multiple
358 gains and losses occurred from a hyper-polyphagous common ancestor or an ancestor with a more
359 restricted host range remains to be clarified. To address this question, we have reconstructed host
360 compatibilities at each ancestral node based on the SNP-based phylogenetic classification of the
361 *M. incognita* isolates (Fig. S8). This reconstruction showed that the two hypotheses concerning
362 the host range status of the last common ancestor were equally likely. Addition of other isolates
363 characterized for their host race might allow to favour one or the other hypothesis in the future.
364 Consistent with multiple gains and losses of parasitic abilities, host race switching within an
365 isolate over time has already been observed. Isolates of *M. incognita* race 2 and 3, which parasitize
366 tobacco and cotton plants respectively, switched to behaviour similar to race 3 and 2 after staying
367 for eight months on coffee plants (Rui Gomes Carneiro, personal communication). Together with
368 the previously reported ability to break down resistance gene in plants, the ability of *M. incognita*
369 to loose and gain ability to infect different plants highlights its adaptive potential.
370 Overall, we provided here additional evidence for adaptability and the first whole-genome level
371 assessment for the lack of recombination in *M. incognita*, consolidating this species as a main
372 model to study the paradox of adaptability and parasitic success in the absence of sexual
373 reproduction.
374 The adaptability of *M. incognita* despite its obligatory asexual reproduction and the lack
375 of phylogenetic signal underlying the host races have important practical implications at the
376 agricultural level. Characterizing populations that differ in their ability to infest a particular host
377 (that carries specific resistance genes) is of crucial importance for growers and agronomists.
378 Indeed, the main *Meloidogyne* spp. control strategies consist in deploying resistant cultivars and
379 appropriate crop rotation against a specific given race. If the identity of a population is unknown,
380 the crop selected for use in a management scheme may cause dramatic increases in nematode
381 populations (Hartman and Sasser 1985). However, the adaptability of *M. incognita* casts serious
382 doubts on the durability of such strategies and must be taken into account in rotation schemes.
383 Furthermore, the biological reality of host races themselves is challenged by the lack of
384 underlying genetic signal. Actually, the initial host race concept, has never been universally
385 accepted, in part because it covered only a small portion of the whole potential variation in
386 parasitic ability (Moens et al. 2009). Although *M. incognita* was already known to parasitize

387 hundreds of host plants, only six different host standards were used to characterise four races.
388 New host races might be defined in the future when including additional hosts in the differential
389 test. Furthermore, using the same six initial host plant species, two additional *M. incognita* races
390 that did not fit into the previously published race scheme have already been described (Robertson
391 et al. 2009). Although the terminology 'races' of *Meloidogyne* spp. has been recommended not
392 to be used since 2009 (Moens et al. 2009), several papers related to *M. incognita* diversity of host
393 compatibility or selection of resistant cultivars are still using this term; including on coffee (Lima
394 et al. 2015; Peres et al. 2017); cotton (Mota et al. 2013; da Silva et al. 2014) or soybean (Fourie
395 et al. 2006). This reflects the practical importance to differentiate *M. incognita* populations
396 according to their different ranges of host compatibilities. However, because these variations in
397 host ranges are not monophyletic and thus do not follow shared common genetic ancestry, we
398 recommend abandoning the term 'race'.

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

413 Collectively, our observations indicate that *M. incognita* is versatile and adaptive despite
414 its clonal mode of reproduction. The relatively low divergence at the SNV level suggests
415 acquisition of point and short scale mutations followed by selection of the fittest haplotype is
416 probably not the main or at least not the sole player in the adaptation of this species to different
417 host plants and environments. Other mechanisms such as epigenetics, copy number variations,
418 movement of transposable elements or large-scale structural variations could be at play in the
419 surprising adaptability of this clonal species. Consistent with this idea, convergent gene copy
420 number variations (CNV), have recently been shown to be associated with adaptation to a
421 resistance gene-bearing plant in *M. incognita* (Castagnone-Sereno et al. 2019). Interestingly, the
422 parthenogenetic marbled crayfish has multiplied by more than 100 its original area of repartition

423 across Madagascar, adapting to different environments despite showing a surprisingly low
424 number of nucleotide variation (only ~400 SNV on a ~3Gb genome representing a proportion of
425 variable positions of 1.3 10^{-7} only). This also led the authors to suggest that mechanisms other
426 than acquisition of point mutations and selection of the fittest haplotype must be involved
427 (Gutekunst et al. 2018).

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

435

436 Material and Methods

437 Purification, species identification of *M. incognita* isolates and determination of 438 host races.

439 The *M. incognita* isolates involved in this study (Table 1) originate from populations collected
440 from different crops and geographically distant origins in Brazil (Figure 1). For each isolate, one
441 single female and its associated egg mass were retrieved as explained in (Carneiro & Almeida
442 2001). To determine the species (here *M. incognita*), we used esterase isozyme patterns on the
443 female (Carneiro et al. 2000). The corresponding single egg mass was used for tomato plant
444 infection and multiplication. We reproduced egg-mass isolates on tomato plants (*Solanum*
445 *lycopersicum* L. cv. Santa Clara) under greenhouse conditions at a temperature of 25-28 ° C. After
446 three months, we confirmed the *M. incognita* species using esterase phenotypes (Carneiro and
447 Almeida 2001). Once enough nematodes were multiplied, a pool was collected and we performed
448 the North Carolina Differential Host Test (NCDHT) (Hartman and Sasser 1985) with the
449 following plants: cotton cv. Deltapine 61, tobacco cv. NC95, pepper cv. Early California Wonder,
450 watermelon cv. Charleston Gray, peanut cv. Florunner and tomato cv. Rutgers to determine the
451 host race status. We inoculated these plants with 5,000 eggs and J2 of *M. incognita* and maintained
452 them under glasshouse conditions at 25-28° C for three months, with watering and fertilisation as
453 needed. Two months after inoculation, the root system was rinsed with tap water, and egg masses
454 were stained with Phloxine B (Hartman and Sasser 1985) to count the number of galls and eggs
455 masses separated for each root system. We assigned a rating index number according to the scale:
456 0 = no galls or egg masses; 1 = 1-2 galls or egg masses; 2 = 3-10 galls or egg masses; 3 = 11-30
457 galls or egg masses; 4 = 31-100 galls or egg masses; and 5 > 100 galls or egg masses per root

458 system (Table 1). Host–plants types that have an average gall and egg mass index of 2 or less are
459 designated non-host (-). The other plants (index ≥ 3) are designated hosts (+). We categorised *M.*
460 *incognita* host races based on their ability to parasitize tobacco and cotton (Table 1). Classically,
461 the index for Rutgers tomato (susceptible control) is higher than 4 (+) (Hartman & Sasser, 1985).
462 The rest of the population was kept for multiplication on tomato plants to produce enough
463 nematodes for sequencing (typically >1 million individuals pooled together).
464

465 ## DNA preparation and SCAR test

466 For each characterized nematode isolate, we extracted and purified the genomic DNA from
467 pooled eggs with the supplement protocol for nematodes of the QIAGEN Gentra® Puregene®
468 Tissue Kit with the following modifications: incubation at 65 ° C in the cell lysis buffer for 30 min
469 and incubation at 55 ° C with proteinase K for 4h. We verified DNA integrity on 0.8% agarose
470 gel and the DNA quantification on Nanodrop. We confirmed isolate species purity by SCAR-
471 PCR (Zijlstra et al. 2000; Randig et al. 2002) using the SCAR primers specified in Table S5 for
472 the RKN *M. javanica, M. paranaensis, M. incognita,* and *M. exigua*.
473

474 ## Sequencing library preparation

475 We assessed input gDNA quantity using Qubit and normalised the samples to 20ng/ul as
476 described in TruSeq®DNA PCR-Free Library Prep Reference Guide (#FC-121-3001, Illumina)
477 prior fragmentation to 350bp with Covaris S2. We assessed the quality of fragments after size
478 selection and size control of the final libraries using High Sensitivity DNA Labchip kit on an
479 Agilent 2100 Bioanalyzer.
480

481 ## Whole genome sequencing

482 We quantified isolated sample libraries with KAPA library quantification kit (#7960298001,
483 Roche) twice with two independent dilutions at 1:10,000 and 1:20,000. We calculated the average
484 concentration of undiluted libraries and normalised them to 2nM each then pooled them for
485 sequencing step.
486 We generated high-coverage genomic data for the 11 *M. incognita* isolates by 2x150 bp paired-
487 end Illumina NextSeq 500 sequencing with High Output Flow Cell Cartridge V2 (#15065973,
488 Illumina) and High Output Reagent Cartridge V2 300 cycles (#15057929, Illumina) on the UCA
489 Genomix sequencing platform, in Sophia-Antipolis, France. We performed two runs to balance
490 the read's representation among the isolates and obtain homogeneity of coverage for the different
491 samples (Table S2).
492

493 ## Variant Detection

494 We trimmed and cleaned the reads from each library with cutadapt tool (Martin 2011) to remove
495 adapter sequences and bases of a quality inferior to 20. We mapped the clean reads to the *M.*
496 *incognita* reference genome (Blanc-Mathieu et al. 2017), using the BWA-MEM software package
497 (Li 2013). This reference genome is described as triploid with three equally highly diverged A, B
498 and C genome copies as a result of hybridization events. Most of the triplicated regions have been
499 correctly separated during genome assembly, according to genome assembly size (183.53 Mb)
500 that is in the range of the estimated total DNA content in cells via flow cytometry (189±15Mb)
501 (Blanc-Mathieu et al. 2017)). Hence, the genome was considered in this analysis as mostly
502 haploid. However, the distribution of per-base coverage on the genome assembly presented a 2-
503 peaks distribution with a second minor peak at ~twice the coverage of the main peak (Fig. S2).
504 Genome regions of double coverage most likely represent cases where two of the three
505 homoeologous loci have been collapsed during the assembly, probably due to lower divergence.
506 Such regions will systematically be responsible for 'artefactual' 0/1 SNV (presenting variations
507 within isolates) as the reads from the two homoeologous copies will map a single collapsed region
508 in the reference genomes. To avoid confusion between SNV representing true variations between
509 individuals within isolates from those being artefacts due to collapsed homoeologous regions, 0/1
510 SNV were discarded from the analysis and only 1/1 SNV fixed within isolates were considered.
511 We used SAMtools (Li et al. 2009) to filter alignments with MAPQ lower than 20, sort the
512 alignment file by reference position, and remove multi-mapped alignments.

513 We used the FreeBayes variant detection tool (Garrison and Marth 2012) to call SNV and
514 small-scale insertions/deletions, incorporating all the library alignment files simultaneously and
515 produced a variant call file (VCF). We filtered the resulting VCF file with the vcffilter function
516 of vcflib (Anon 2018) , retaining the positions that had more than 20 Phred-scaled probability
517 (QUAL) and a coverage depth (DP) > 10. To conduct the same analyses on the genome of the
518 meiotic diploid nematode *Globodera pallida*, we first phased the SNV to haplotypes using
519 WhatsHap (Martin et al. 2016) because the genome assembly mainly consist of collapsed paternal
520 and maternal haplotypes.

521

522 ## Genetic tests for detection of recombination

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

526

527 ## Genetic diversity between isolates, clusters and efficacy of purifying selection

528 We used bpppopstats from the Bio++ libraries (Guéguen et al. 2013) to estimate the nucleotide
529 variability at non-synonymous and synonymous sites as well as efficacy of purifying selection
530 (π_N , π_S and π_N/π_S) using a multiple alignment of the coding regions. We calculated fixation index
531 (F_{ST}) for the three clusters using vcftools (Danecek et al. 2011).
532

533 ## Principal component analysis

534 We performed a principal component analysis (PCA) to classify the isolates according to their
535 SNV patterns and mapped the race characteristics, geographic location, or current host plants on
536 this classification. We used the filtered VCF file as input in the statistical package SNPRelate
537 (Zheng et al. 2012) to perform the PCA with default parameters.
538

539 ## Phylogenetic analysis

540 Based on the VCF file and the *M. incognita* gene predictions (Blanc-Mathieu et al. 2017), we
541 selected 85,413 positions that contained synonymous or non-synonymous mutations (i.e. in
542 coding regions). We aligned these positions and then used them as an input in SplitsTree4 with
543 default parameters. The resulting network produced a bifurcating tree that was identical to the
544 one obtained with RAxML-NG using GTR+G+ASC_LEWIS model. The bifurcating tree was
545 used as input to PastML (Ishikawa et al. 2018) for reconstruction of the ancestral states of ability
546 to parasitize tobacco and cotton (Fig. S8). Phylogenetic inferences for the largest scaffolds
547 containing at least 20 SNV and the mitochondrial genome were conducted with RAxML-NG
548 (Kozlov et al. 2019) using the GTR+G substitution model (except for scaffolds 10 and 20 for
549 which the K80+G model was used because not enough phylogenetically informative positions
550 were available).
551

552 ## Test for association between biological traits and genetic clusters

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

562 Mitochondrial genome analysis

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

570

571 Acknowledgements

572 This work was supported by the Agence Nationale de la Recherche program (grant number ANR-
573 13-JSV7-0006—ASEXEVOL), the Insitut National de la Recherche Agronomique program
574 (grant number Accueil de Chercheur Etranger SPE 2016). GK has received the support of the EU
575 in the framework of the Marie-Curie FP7 COFUND People Programme, through the award of an
576 AgreenSkills+ fellowship (under grant number 609398), EM received a post-doc fellowship from
577 the Consórcio Pesquisa Café, EVSA received funding from Embrapa for sequencing at UCA
578 Genomix platform. We thank the SPIBOC Bioinformatics platform for the data hosting.

579

580 Figure Legends

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

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

591

592 Figure 2. Linkage Disequilibrium (red) and 4-gametes test of *M. incognita*
593 isolates.
594 Based on SNVs fixed within isolates: proportion of pair of markers that pass the 4-gametes test
595 (blue) and linkage disequilibrium measured as r2 between markers (red), both as a function of the
596 inter-markers distance on *M. incognita* scaffolds.
597
598 Figure 3. Linkage Disequilibrium (red) and 4 gametes test (blue) for phased
599 SNVs in *Globodera rostochiensis* isolates.
600 The r^2 correlation between markers, indicating linkage disequilibrium (LD) is given in the red
601 (upper) plot, as a function of the physical distance between the markers. The proportion of pairs
602 of two-state markers that pass the 4-gamete test is given in the blue (lower) plot as a function of
603 the distance between the markers.
604
605 Figure 4. Distribution of the number of variants per race and isolate.
606 Number of variants per isolate and isolate-specific variants for the 11 Brazilian isolates.
607
608 Figure 5. PCA analysis of the different *M. incognita* isolates groups them into
609 three clusters (A, B, and C).
610 The geographic origins are associated to coloured shapes*:* black circle: Paraná, orange diamond:
611 Santa Catarina, green square: São Paulo, red triangle: Mato Grosso, blue star: pool. Host plant
612 representative pictures are displayed next to the isolates: soybean pod (R1-2 and R3-2); cotton
613 flower (R3-1, R3-4, R4-4, and R4-1); coffee grain (R2-6); cucumber vegetable (R1-3); tobacco
614 leaves (R1-6 and R2-1); and watermelon fruit slice (R4-3).
615
616 Figure 6. Phylogenetic network for *M. incognita* isolates based on SNV present
617 in coding sequences.
618 The phylogenetic network based only on changes in coding sequences shows the same grouping
619 into 3 distinct groups.
620
621 Figure 7. PCA analysis of all known *M. incognita* genomes.
622 The isolates were regrouped based on SNV patterns confirming the same three clusters. Origin
623 countries are indicated by flags (Brazil for R1-2, R1-3, R1-6, R2-1, R2-6, R3-1, R3-2, R3-4, R4-
624 1, R4-3, R4-4; USA for L27, 557R, HarC, W1, VW6; Mexico for Morelos; Libya for A14; Ivory
625 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.

641 # Tables & Figures

642 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	

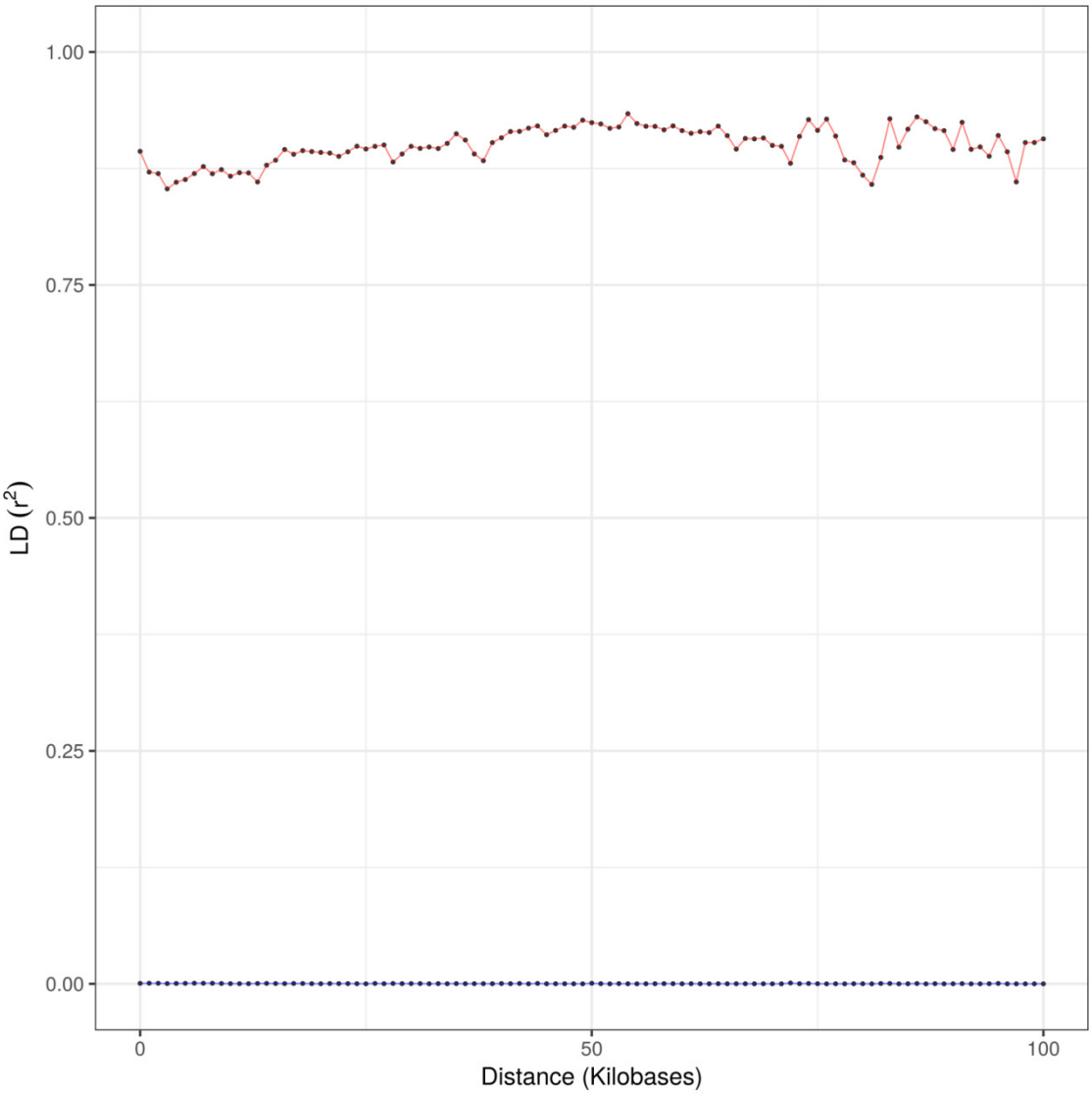
643 a, isolate identification code
644 b, host plant crop (soybean, *Glycine max*; cucumber, *Cucumis sativus*; tobacco, *Nicotiana tabacum*; coffee, *Coffea arabica*; cotton, *Gossypium hirsutum*.; watermelon, *Citrullus vugaris*
645 c, population id
646 d, host range results for the North Carolina 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
647 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'

648 Table 2. Number of repeats per region (63nt and 102nt) in the mitochondrial
649 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

650

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

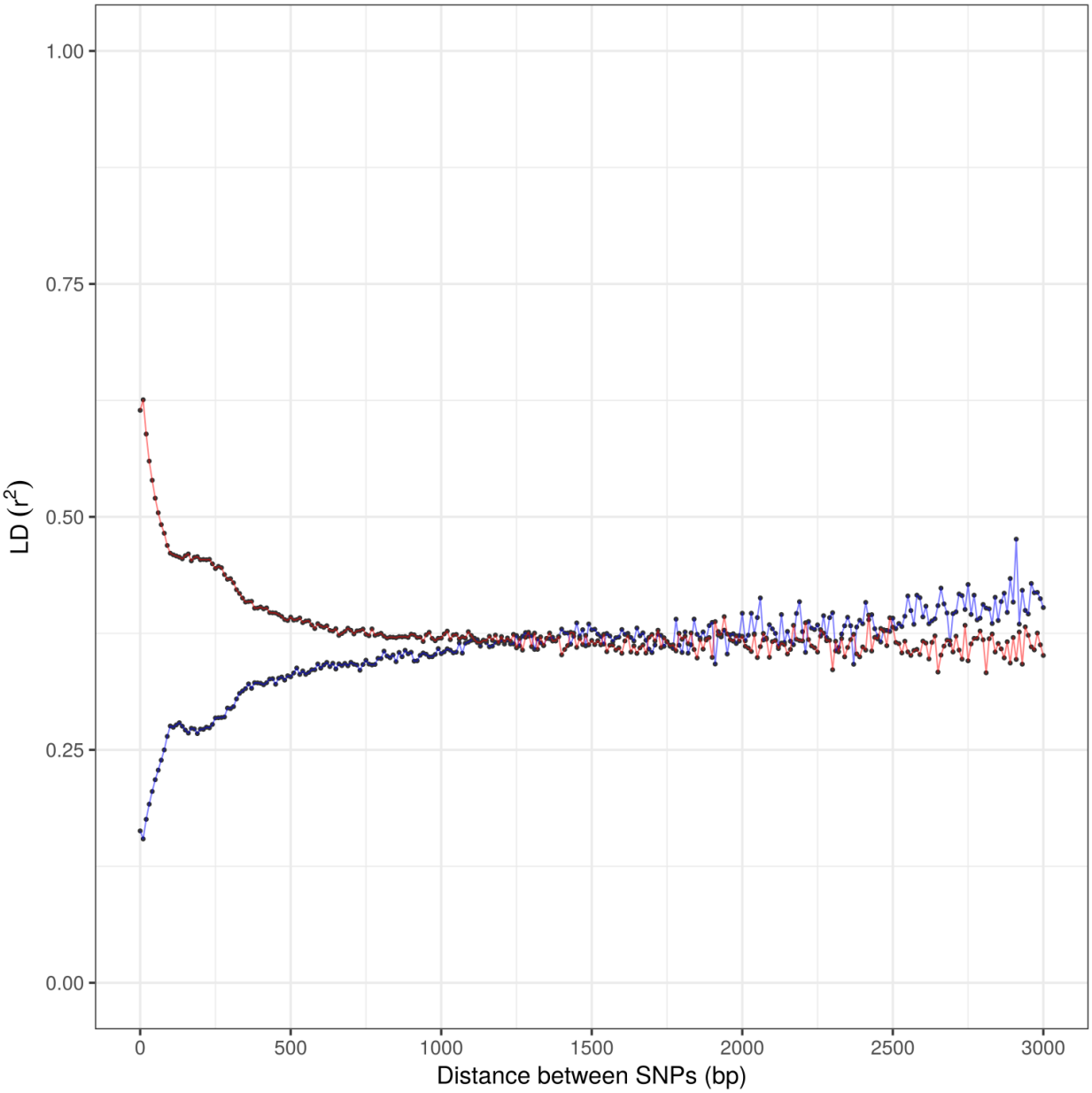


652

653 Figure 2. Linkage Disequilibrium (red) and 4-gametes test (blue) of *M. incognita*

654 isolates.

655



656

657 Figure 3. Linkage Disequilibrium (red) and 4 gamete test (blue) for phased SNVs

658 in *Globodera rostochiensis* isolates.

659

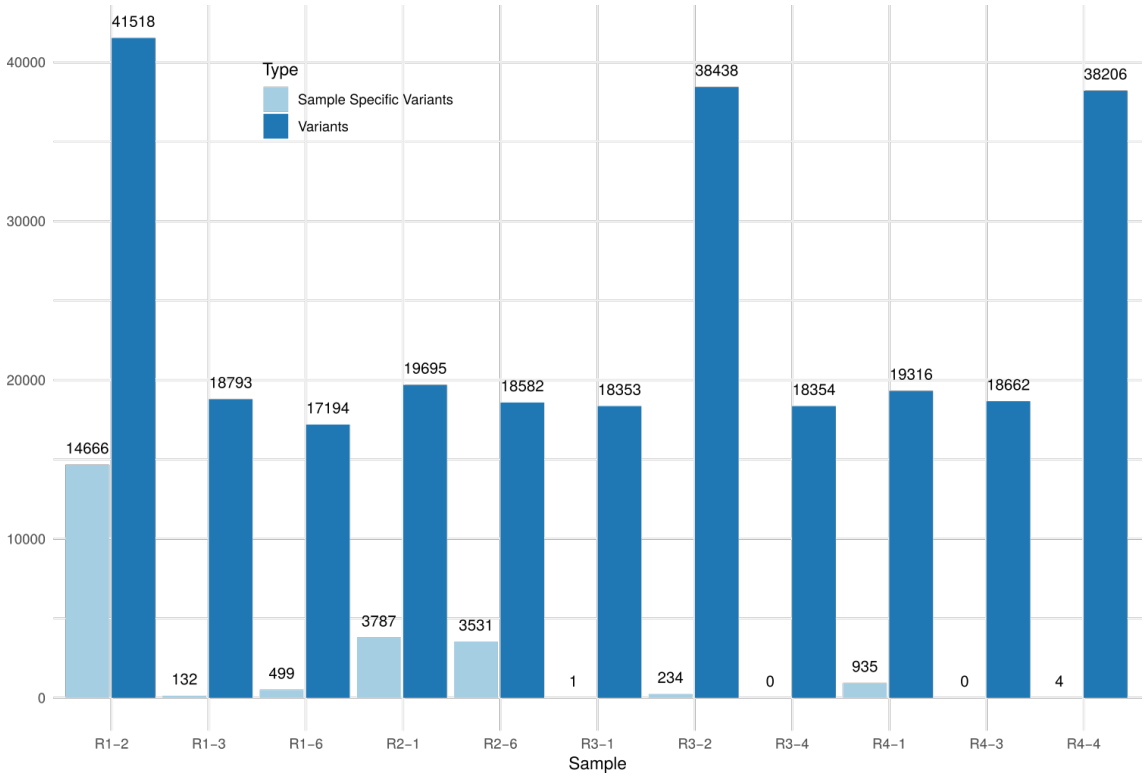


Figure 4. Distribution of the number of variants per race and isolate.

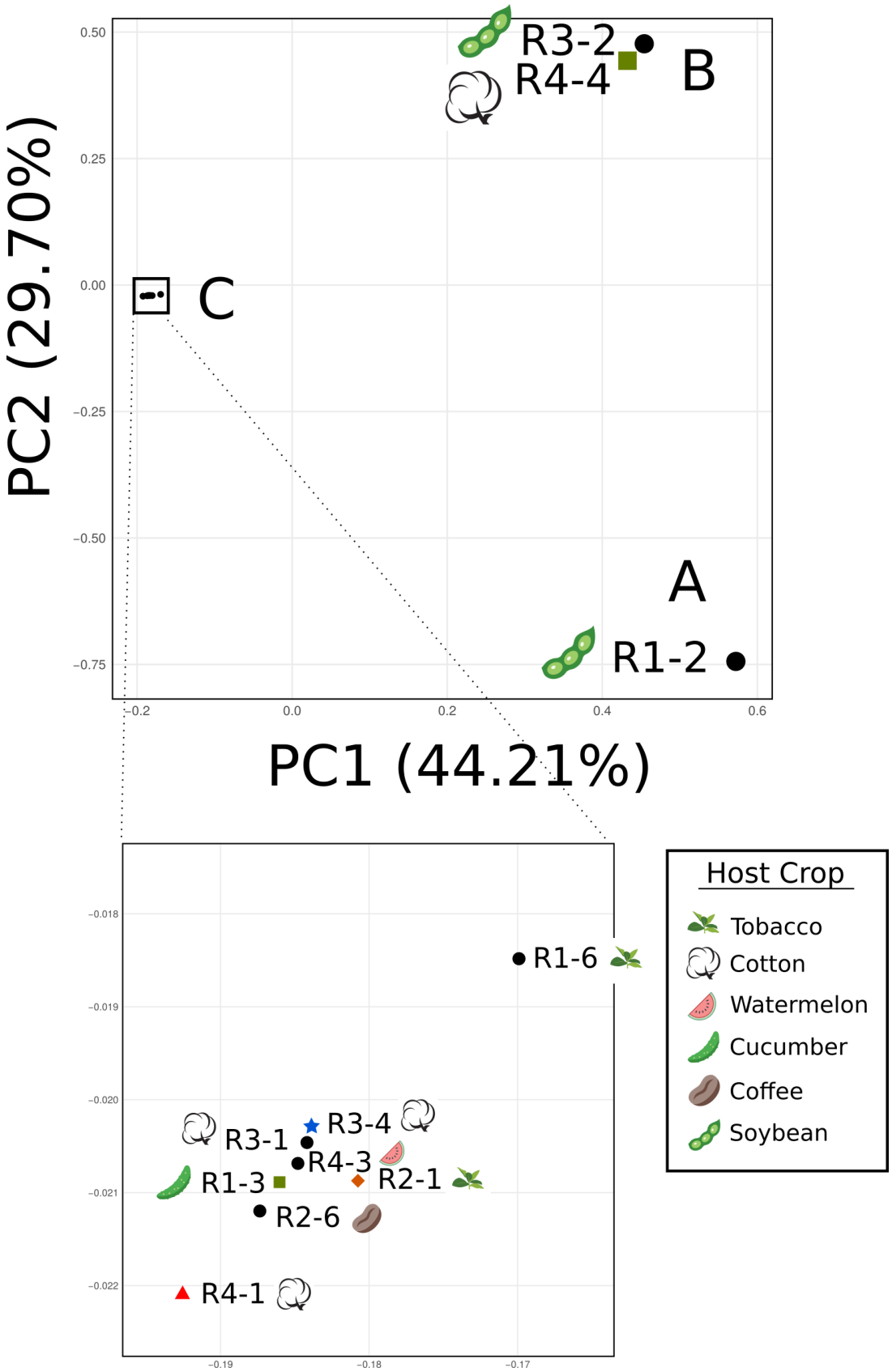


Figure 5. PCA analysis of the different *M. incognita* isolates groups them into three clusters (A, B, and C).

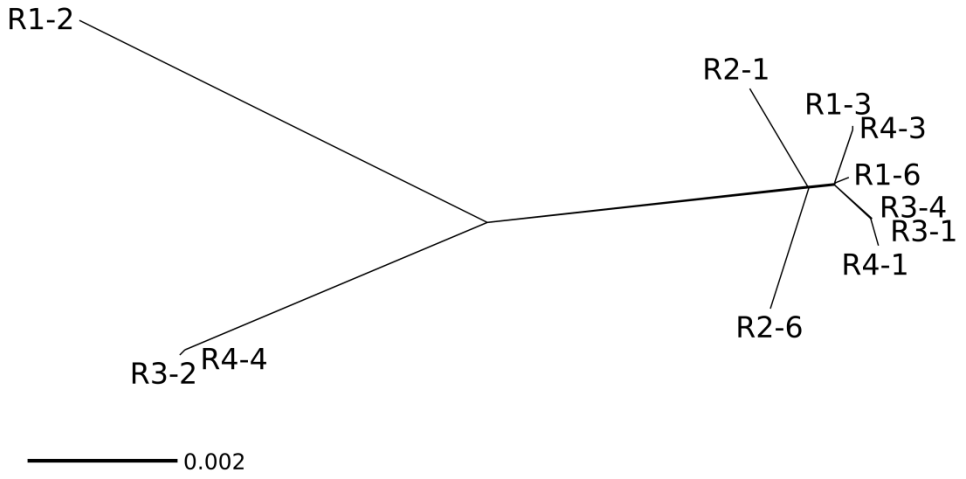
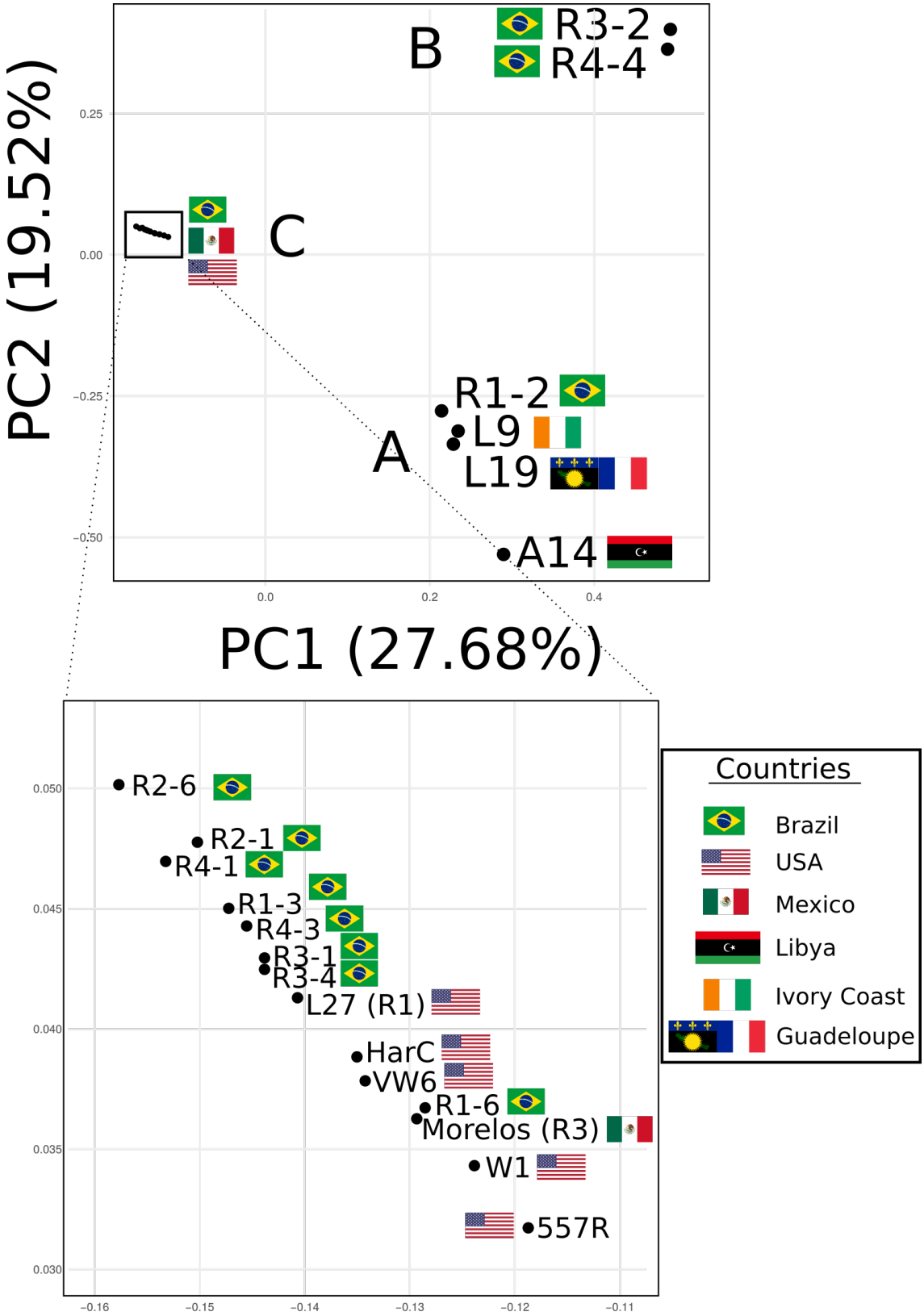


Figure 6. Phylogenetic network for *M. incognita* isolates based on SNV present in coding sequences.



669

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

671

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