Populations of Ceratocystis fimbriata on Colocasia esculenta and other hosts in the Mata Atlântica region in Brazil

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Ceratocystis fimbriata is native to Brazil, where it is able to cause serious diseases on numerous hosts, especially on non-native plants. Because C. fimbriata is soilborne and not wind dispersed, highly differentiated populations are found in different regions of Brazil. The present study compared populations of C. fimbriata on taro, mango, eucalyptus and kiwifruit from the coastal Mata Atlântica region with native populations of the fungus from the Cerrado-transition region in Brazil by using 14 SSR markers and DNA sequences of ITS and mating type genes. Microsatellite and phylogenetic analyses were performed to test the hypothesis that populations on different hosts from the Mata Atlântica region are related to each other and are native to the region. The ITS sequences varied greatly among the taro isolates, with six sequences identified, from which two had not been previously reported. For mating type genes, four sequences were identified among the isolates on taro, mango, eucalyptus and kiwifruit. Phylogenetic analyses showed that Mata Atlântica populations formed a monophyletic group distinct from Cerrado-transition region populations, although earlier studies had shown that isolates from the two regions are interfertile and are considered as a single biological species. Microsatellite analysis revealed low gene diversity for each of the three Mata Atlântica populations on taro, mango and kiwifruit, suggesting that these populations had gone through genetic bottlenecks, probably by dispersal of select genotypes in vegetative propagation material. Also, microsatellite markers showed that two microsatellite genotypes from taro are widely spread in Brazil, probably by infected corms.

Keywords: ceratocystis wilt, genetic diversity, microsatellites, population genetics, taro

Introduction

Ceratocystis fimbriata is a well-known and important pathogen in Brazil that causes wilting and canker of cultivated woody plants. The fungus has a broad geographic and host range and is able to infect numerous plant families (CAB International, 2005). However, some populations of C. fimbriata in North and South America appear to be host-specialized and restricted to specific geographic regions. As an example, mango (Mangifera indica) has been cultivated in South and Central America for many years, but until recently it was reported as a host of C. fimbriata only in Brazil (Oliveira et al., 2015), even though populations of the fungus are found infecting other hosts such as coffee (Coffea spp.) and cacao (Theobroma cacao) in the same areas where mango are cultivated in the absence of disease (Marin et al., 2003; Engelbrecht et al., 2007). Also, studies on population genetics of C. fimbriata in Brazil have shown that certain fungal populations on Eucalyptus (Ferreira et al., 2011) and mango (Oliveira et al., 2015) are native to different areas in Brazil, and those populations show great genetic diversity and limited gene flow between populations. Genetic diversity of Ceratocystis species has been successfully assessed by using microsatellite markers (Steimel et al., 2004) that were able to distinguish putatively native populations of the pathogen, as well as introduced populations of C. cacofunnela (Engelbrecht et al., 2007), C. platani (Engelbrecht et al., 2004; Ocasio-Morales et al., 2007) and C. fimbriata (Ferreira et al., 2010, 2011; Harrington et al., 2015; Li et al., 2016).

Besides woody plants, C. fimbriata can also cause black rot of sweet potato (Ipomoea batatas), taro (Colocasia esculenta) (Harrington et al., 2005; Thorpe et al., 2005) and Peruvian carrot (Arracacia xanthorrhiza) (Melo et al., 2016). In Brazil, the disease was found causing a postharvest black rot of edible corms of taro in supermarkets in the states of São Paulo, Rio de Janeiro, Bahia, Rondônia and Distrito Federal (Harrington et al., 2005). The genotypes of C. fimbriata on taro found in Brazil have limited variability in ITS sequence, and they differ from genotypes of C. fimbriata found on taro in China and Hawaii (Thorpe et al., 2005; Harrington et al., 2015; Li et al., 2016). Despite the purported similarity of ITS sequences of C. fimbriata isolates on taro collected in Yunnan, China, to taro isolates in Brazil (Huang et al., 2008), microsatellite alleles and DNA sequences of mating type genes showed that the Yunnan
taro isolates are more similar to natural populations of *C. fimbriata* on *Eucalyptus* in Brazil (Harrington et al., 2014, 2015; Li et al., 2016).

Phylogenetic analyses of isolates from certain populations of *C. fimbriata* on mango in eastern Rio de Janeiro (Ferreira et al., 2010; Harrington et al., 2011; Oliveira et al., 2015), taro in São Paulo (Harrington et al., 2005, 2011; Ferreira et al., 2010), kiwifruit in Rio Grande do Sul (Piveta et al., 2016) and one isolate on *Eucalyptus* in Paraná (Harrington et al., 2011) suggest that these populations are related to each other and may represent a larger population native to the coastal region of the Mata Atlântica forests in Brazil (Silveira et al., 2006; Ferreira et al., 2010; Harrington et al., 2011, 2014; Oliveira et al., 2015). Thus, the present work aimed to test the hypothesis that populations of *C. fimbriata* on taro in Brazil are native to the coastal Mata Atlântica region and are closely related to other fungal populations on mango from eastern Rio de Janeiro and kiwifruit from Rio Grande do Sul. The study used ITS rDNA sequences, sequences of mating type genes, and microsatellite analyses to determine if these populations are related to each other and are native to the Mata Atlântica or introduced.

**Materials and methods**

**Fungal isolates and DNA extraction**

Representative isolates of Brazilian populations of *C. fimbriata* used in previous studies on mango (Ferreira et al., 2010; Oliveira et al., 2015), *Actinidia delicosa* (kiwifruit; Piveta et al., 2016) and *Eucalyptus* spp. (eucalyptus; Harrington et al., 2011) were compared with isolates on taro (Harrington et al., 2005; Thorpe et al., 2005; Ferreira et al., 2010). Besides the 12 isolates of *C. fimbriata* on taro (Ferreira et al., 2010), 35 new isolates were studied. The isolates were collected from infected edible corms found in grocery stores or local vendors in the states of São Paulo, Rio de Janeiro, Bahia, Rondônia and Distrito Federal (Fig. 1). The fungus was baited from infected corms by placing pieces of the infected tissue between two discs of carrot root. Ascospore masses from perithecia formed on the carrot discs were transferred to agar media for purification. After 10 days, pure cultures were stored at Iowa State University in 15% glycerol at −80 °C. Only one isolate per corm was stored and used in genetic analyses. For population studies, each population consisted of at least four isolates from individual corms in a bin sold by a single vendor. Populations from other hosts were from a single plantation, city or nursery (Ferreira et al., 2010, 2017; Oliveira et al., 2015; Piveta et al., 2016).

For DNA extraction, the isolates were grown on MYEA (2% malt extract, 0.2% yeast extract, 2% agar) for about 10 days at room temperature (c. 23 °C). The DNA was extracted using PrepMan Ultra (PE Biosystems).

**ITS barcoding and mating type gene sequences**

For the ITS rDNA region, sequences of new isolates were generated by using PCR followed by direct DNA sequencing of the PCR products with primers ITS1-F (5′-CTTGGATCAGAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), with the following cycling conditions: 85 °C for 2 min, 95 °C for 95 s; then 36 cycles of 58 °C for 1 min, 72 °C for 80 s and 95 °C for 70 s; then 52 °C for 1 min and 72 °C for 15 min (Harrington et al., 2011).

**Figure 1** Map of Brazil showing the locations of the sampled *Ceratocystis fimbriata* populations. The first three letters of each population name indicate the host (*Colocasia, Eucalyptus, Actinidia, Mangifera*) and the last two letters indicate the state of origin (Bahia, Rondônia, São Paulo, Rio de Janeiro, Distrito Federal, Paraná and Rio Grande do Sul). Each sampled population in a state was distinctly numbered, and numbers in parenthesis indicate the number of isolates from the population.
For the MAT1-1-2 (MAT-1) and MAT1-2-1 (MAT-2) genes, new sequences of representative isolates were generated by using PCR followed by direct DNA sequencing of the PCR products. The primers CMF1A-F (5'- CAGCCTGATTGAGATGCCATG-3') and CMF1A-R (5'- GGACATTTATACCCGTTTAT-3') were used to amplify and sequence a region of the MAT1-1 gene about 1000 bp long (Harrington et al., 2014). The primers X9978a (5'- GTAAAACCTCAGCGCAATTTTGCC-3') and CFM2-1F (5'- AGTTACAAGTGTTCCCAAAAG-3') were used to amplify and sequence a region of the MAT1-2 gene about 1150 bp long (Harrington et al., 2014). The thermocycler settings for amplifying the MAT-1 and MAT-2 regions consisted of: initial denaturation at 94 °C for 2 min; 36 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min; and a final extension of 72 °C for 10 min.

The PCR products were purified using illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare) and sequenced at the Iowa State University DNA Facility.

Phylogenetic analysis

Sequence datasets for the three genetic regions (ITS, MAT-1 and MAT-2) were separately aligned in MAFFT ONLINE v. 7.0 (Katoh & Toh, 2010), using the FFT-NS-i (Slow; iterative refinement method) alignment strategy with the 200PAM/K = 2 scoring matrix and a gap opening penalty of 1.53 with an offset value of 0.0. Aligned sequences were then manually corrected when necessary using MEGA v. 5 (Tamura et al., 2011).

Ceratocystis variospora from Prunus was used as an outgroup taxon in the combined alignment of the MAT-1 and MAT-2 regions. For maximum parsimony analysis, PAUP v. 4.0b10 (Swofford, 2002) was used and gaps were treated as a fifth base, all characters had equal weight, and the heuristic search used simple stepwise addition and tree-bisection-reconnection. Bootstrap confidence values were calculated using 1000 replicates in PAUP. The likelihood values were calculated and the best model of nucleotide substitution for each gene was selected according to Akaiki information criterion (AIC) using MrMODELTEST v. 2.3 (Nylander, 2004). Bayesian inference (BI) used MrBayes v. 3.1.1 (Ronquist & Huelsenbeck, 2003) with the algorithm of Markov chain Monte Carlo (MCMC) with two sets of four chains (one cold and three heated) and the stop-rule option, stopping the analysis at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 1000; the first 25% of trees were removed.

Microsatellite markers

Fourteen loci were analysed (CfAAG8, CfAAG9, CICAA9, CICAA10, CICAA15, CICAA38, CICAA80, CICAT1, CICAT1200, CICAG3, CICAG15, CICAG900, CGIGAC60 and CGIGAC650) with the microsatellite primers designed by Steimel et al. (2004). These microsatellite markers were mapped onto the C. fimbriata genome (Simpson et al., 2013) and used in previous population studies on C. cacaofunesta (Engelbrecht et al., 2007), C. platani (Engelbrecht et al., 2004; Osacio-Morales et al., 2007), C. puripulvis (Nkueckam et al., 2009), and C. fimbriata (van Wyk et al., 2006; Ferreira et al., 2010, 2011, 2017; Harrington et al., 2015; Oliveira et al., 2015; Li et al., 2016). Of the 16 loci used in some of the earlier studies, two (CICAT3K and CICAT9X) were not used because their alleles could not be consistently resolved with some isolates. For each primer pair specific to the flanking regions of 14 simple sequence repeat regions, one of the primers was fluorescently labelled. PCR amplifications of all microsatellite loci were performed using a 96-well thermal cycler (PTC-100; MJ Research Inc.) following the conditions described previously (Ferreira et al., 2010). The PCR products were electrophoresed using a four-capillary ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems Inc.). Band sizes of the products were determined using marker standards and PEAK SCANNER v. 1.0 (Applied Biosystems Inc.). Each product length (within 1 bp) was considered to be a different allele. Most of the microsatellite loci contained trinucleotide repeats, and most alleles of a given locus differed by increments of 3 bp.

Microsatellite analysis

Nei’s gene diversity of microsatellite loci for each population was calculated with and without clone-corrected data using POPGENE v. 1.32 (Yeh & Boyle, 1997). Clone-corrected datasets were a subset of the population left after removing isolates that were genetically identical, that is, a genotype within a population was counted only once. The clone-corrected value for $H$ would be expected to be higher than the uncorrected value if the population was dominated by one or few genotypes, as might occur if a few genotypes were dispersed within the population through movement of infected taro corms.

Besides the simple calculation of genotypic diversity ($D$, number of genotypes found in the population divided by the number of isolates sampled), multilocus genotypic diversity was estimated with the Stoddart & Taylor’s $G$ index (Stoddart & Taylor, 1988). The maximum value of $G$ is limited by the number of isolates sampled in the smallest population; therefore, Stoddart & Taylor’s $G$ was scaled by the expected number of genotypes for the smallest sample size being compared (Grünwald et al., 2003). For individual plantations, the expected number of genotypes in a sample of four isolates (minimum value = 1.0 and maximum value = 4.0) was estimated based on rarefaction curves using the VEGAN package from CRAN in R v. 2.6.1 (R Core Development Team, 2007).

Relationship among populations was determined using UPGMA (unweighted pair group method with arithmetic mean) dendrograms constructed with POPULATIONS v. 1.2.30 (Langella, 2002). Bootstrap values for branches of the population trees were calculated from 1000 replicates. Relationships among genotypes were also examined using genetic distance matrices (Nei’s), UPGMA trees and 1000 bootstrap replications generated with POPULATIONS v. 1.2.30.

Results

ITS barcoding

Harrington et al. (2014) designated unique ITS sequences of C. fimbriata from the Latin American clade as ITS genotypes. Of the 40 taro isolates that were sequenced for ITS, six different ITS genotypes were identified (ITS9, ITS11, ITS11b, ITS12, ITS12b, ITS13), two of which were new (ITS11b and ITS12b; GenBank accession nos KX838966 and KX838967, respectively). The most common genotype found was ITS13, which was present in all Colocasia populations, with the exception of the Rio de Janeiro population ColJR6 and Bahia population ColBA3 (Table 1). These six genotypes were unique to taro isolates and not previously reported on any other host, with the exception of ITS9, which was reported on
mango isolates from eastern Rio de Janeiro (Harrington et al., 2011).

As found in earlier studies (Harrington et al., 2014), the ITS sequences of the taro isolates did not clearly group with ITS sequences of Brazilian isolates from other hosts, with the exception of the ITS9 sequence. Three ITS genotypes (ITS9, ITS14d and ITS14i) were identified among isolates of *C. fimbriata* on mango from the eastern region of Rio de Janeiro state (Harrington et al., 2011, 2014; Oliveira et al., 2015). Additionally, the ITS of isolates of *C. fimbriata* from populations on kiwifruit from Rio Grande do Sul (Piveta et al., 2016) did not group with those of taro and mango isolates from eastern Rio de Janeiro in maximum parsimony analysis (phylogenetic tree not shown).

**Phylogenetic analysis**

A combined dataset of sequences from the MAT-1/ MAT-2 region (Table 2) was used to generate a single most parsimonious tree of 467 steps (Fig. 2). The final aligned dataset of 62 taxa contained 2158 characters including gaps, of which 1718 were constant, 359 were parsimony uninformative, and 81 were parsimony informative. Evolution model HKY + G was selected and incorporated into the Bayesian analysis. The single most parsimonious tree had a very similar topology to the Bayesian tree. There were only two mating type genotypes identified among the 18 taro isolates that were sequenced for the mating type genes. The MAT5d sequence of isolate C1866 differed from that of the other taro isolates by only 1 bp. Three isolates of *C. fimbriata* obtained from a single plantation of kiwifruit in Rio Grande do Sul (Ferreira et al., 2017) had the same mating type sequence as isolate C1866 from taro (Fig. 2).

Table 1 Genetic diversity of populations of *Ceratocystis fimbriata* on *Colocasia esculenta*, *Mangifera indica*, *Actinidia deliciosa* and *Eucalyptus* spp. in Brazil based on 14 microsatellite loci

<table>
<thead>
<tr>
<th>Group</th>
<th>Host</th>
<th>Population</th>
<th>State</th>
<th>City</th>
<th>No. isolates</th>
<th>No. genotypes</th>
<th>Genotypic diversity (<em>G</em>)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Clone-corrected&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ITS rDNA genotypes&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Nei’s gene diversity (<em>H</em>)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mata Atlântica</td>
<td><em>Colocasia</em></td>
<td>ColRJ6</td>
<td>Rio de Janeiro</td>
<td>Rio de Janeiro</td>
<td>4</td>
<td>3</td>
<td>3.00</td>
<td>0.0893</td>
<td>0.0952</td>
<td>11</td>
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<tr>
<td></td>
<td></td>
<td>ColSP7</td>
<td>São Paulo</td>
<td>Tapirai</td>
<td>5</td>
<td>2</td>
<td>2.80</td>
<td>0.1257</td>
<td>0.1587</td>
<td>9, 12, 13</td>
</tr>
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<td></td>
<td></td>
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<td>Sorocaba</td>
<td>6</td>
<td>3</td>
<td>2.60</td>
<td>0.0516</td>
<td>0.0635</td>
<td>12, 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ColSP9</td>
<td>São Paulo</td>
<td>Ubatuba</td>
<td>5</td>
<td>2</td>
<td>2.00</td>
<td>0.0343</td>
<td>0.0357</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ColBA3</td>
<td>Bahia</td>
<td>Porto Seguro</td>
<td>8</td>
<td>3</td>
<td>2.00</td>
<td>0.0424</td>
<td>0.0695</td>
<td>11, 12b</td>
</tr>
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<td></td>
<td></td>
<td>ColDF1</td>
<td>Distrito Federal</td>
<td>Brasilia</td>
<td>6</td>
<td>2</td>
<td>1.93</td>
<td>0.0317</td>
<td>0.0357</td>
<td>13</td>
</tr>
<tr>
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<td><em>Mangifera</em></td>
<td>ManRJ3</td>
<td>Rio de Janeiro</td>
<td>São Fidelis</td>
<td>7</td>
<td>4</td>
<td>2.97</td>
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<td>14d</td>
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<td></td>
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<td>Itaocara</td>
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<td>2.00</td>
<td>0.1339</td>
<td>0.1786</td>
<td>9, 14d, 14i</td>
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<td></td>
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<td>São Fidelis</td>
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<td>0.0973</td>
<td>0.1875</td>
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<td></td>
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<td>Rio de Janeiro</td>
<td>Itaocara</td>
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<td>0.0000</td>
<td>0.0000</td>
<td>14d</td>
</tr>
<tr>
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<td>ActRS1</td>
<td>Rio Grande do Sul</td>
<td>Parrauhipilha</td>
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<tr>
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<td>EucMG1</td>
<td>Minas Gerais</td>
<td>Curvelo</td>
<td>18</td>
<td>14</td>
<td>3.74</td>
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<td>0.3258</td>
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<td></td>
<td>EucB1A</td>
<td>Bahia</td>
<td>Eunápolis</td>
<td>26</td>
<td>13</td>
<td>3.27</td>
<td>0.2079</td>
<td>0.2697</td>
<td>ND</td>
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<tr>
<td></td>
<td></td>
<td>EucB1A</td>
<td>Bahia</td>
<td>Caravelas</td>
<td>6</td>
<td>4</td>
<td>3.20</td>
<td>0.2262</td>
<td>0.2500</td>
<td>ND</td>
</tr>
<tr>
<td>Mangifera</td>
<td><em>Mangifera</em></td>
<td>ManCE1</td>
<td>Ceará</td>
<td>Brejo Santo</td>
<td>11</td>
<td>7</td>
<td>3.48</td>
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<td>0.3149</td>
<td>8a, 8b, 10, 15a</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Paraíba</td>
<td>Conde</td>
<td>10</td>
<td>6</td>
<td>3.20</td>
<td>0.3914</td>
<td>0.3889</td>
<td>4a, 8a, 10c</td>
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<td>São Paulo</td>
<td>Limeira</td>
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<td>4</td>
<td>2.58</td>
<td>0.2267</td>
<td>0.2946</td>
<td>4, 6, 10, 14a</td>
</tr>
</tbody>
</table>

<sup>a</sup>Only populations that contain more than four isolates are shown.

<sup>b</sup>Stoddart & Taylor’s genotypic diversity, with rarefaction. Rarefaction gave estimated values for *G* of 1.0 (only one genotype in the population) to maximum value of 4.0 (all isolates of a different genotype).

<sup>c</sup>Clone correction removed isolates that had genotypes identical to other isolates from the same site.

<sup>d</sup>Genotype numbers follow the designations of Harrington et al. (2014). ND, not determined.

In total, 90 isolates from the Mata Atlântica group were studied, and all 14 microsatellite loci were polymorphic,
Figure 2 The single most parsimonious tree based on combined dataset of DNA sequences of MAT-1 and MAT-2 genes of representatives of the Latin American clade of the Ceratocystis fimbriata complex. Bootstrap values greater than 60% or posterior probability values greater than 0.9 are indicated on appropriate branches. Isolates with grey background represent those found in the Mata Atlântica region in Brazil. The tree was rooted to *C. variospora* (C1963) of the North American clade. Scale bar indicates number of base substitutions.
with exception of the locus CAG900 (Table 3). The most polymorphic locus was CAA38, with seven different alleles. The ranges of allele sizes in bp for the loci were 180–198 (AAG8), 391–403 (AAG9), 191–266 (CAA9), 125–128 (CAA10), 321–330 (CAA15), 168–250 (CAA38), 302–314 (CAA80), 265–286 (CAG15), 248–261 (CAT1), 187–221 (GACA60) and 213–219 (GACA6K). Thirteen microsatellite genotypes were identified among 47 isolates from mango (eastern Rio de Janeiro), two microsatellite genotypes among 35 isolates on taro, eight microsatellite genotypes among 47 isolates on banana (Junction, São Paulo, Brazil). The UPGMA tree constructed using the genotypes of the Mata Atlântica group showed that the isolates grouped according to their respective host of origin (Fig. 3).

Most of the taro grown for countrywide distribution comes from farms in the coastal region of São Paulo, although taro is also grown in the coastal areas of Rio de Janeiro. Two microsatellite genotypes were commonly found among the populations of C. fimbriata on taro from São Paulo and Rio de Janeiro. One of the common genotypes was found in six populations, and the other genotype was found in eight populations (Fig. 3). Isolates collected in Ouro Preto do Oeste (Rondônia) were from a grocery store that purchased the taro corms from a distribution centre in São Paulo, and the two isolates from this vendor had exactly the same genotype as found on corms from São Paulo. In Distrito Federal (Brasília), isolates were collected in a market from a taro grower who had obtained their original planting material from São Paulo, and the six isolates from this grower had the same common São Paulo genotype. Three distinct microsatellite genotypes were found among the eight taro isolates from a grocery store in Bahia (Fig. 3), but it could not be determined where the corms were grown.

### Genetic relatedness of populations

In order to compare populations from the Mata Atlântica region with populations that appeared to represent...
natural populations on mango and *Eucalyptus* in other regions of Brazil, a UPGMA tree based on allele frequencies was constructed (Fig. 4). A total of 17 populations were studied. Six *Mata Atlântica* populations on taro, four on mango and one on kiwifruit (Ferreira et al., 2017) were compared to three native populations on mango from northeastern and southeastern Brazil (Oliveira et al., 2015) and three native (soil-borne) populations on *Eucalyptus* from states of Bahia and Minas Gerais (Ferreira et al., 2010, 2011). Genotypes of isolates from the different host groups within the *Mata Atlântica* group were each unique and distinct from mango and *Eucalyptus* isolates from other regions in Brazil, with moderate bootstrap value (60%; Fig. 4).

Nei’s gene diversity ($H$) was calculated for each of the 17 populations of *C. fimbriata* (Table 1). Low levels of gene diversity were found in most of the *Mata Atlântica* populations relative to natural populations on *Eucalyptus* and mango. Even when the populations were clone corrected, no substantial increase was noticed in gene diversity. Also low levels of genotypic diversity were identified in most of the *Mata Atlântica* populations.

**Discussion**

According to the phylogenetic analysis of mating type genes, the taro isolates and other isolates from the coastal *Mata Atlântica* region in Brazil clustered together in a monophyletic group within the Latin American Clade (LAC) of the *C. fimbriata* complex. If species were delimited solely as monophyletic groups, the *Mata Atlântica* group could be considered a distinct species, as suggested for other regional populations in the LAC (Fourie et al., 2014). Many of the new species in the LAC have been described based on variation in insertion-deletion (indel) regions in the ITS region, but typically these species names have been based on introduced populations that have gone through severe genetic bottlenecks or on isolates from a limited number of collection sites (Harrington et al., 2014; Oliveira et al., 2015; Li et al., 2016). Sequences of the ITS region serve as an important barcode for identification of fungi (Schoch et al., 2012), but evolutionary inferences based solely on the ITS region are often misleading, especially with the *C. fimbriata* complex (Fourie et al., 2014; Harrington et al., 2014). Even with multigene trees and microsatellite markers, the
distinctions between populations (or genotypes) and species has been controversial with this group of fungi (Fourie et al., 2014; Harrington et al., 2014; Oliveira et al., 2015). To be considered as phylogenetic species in the traditional sense, it is necessary to identify diagnostic phenotypic characters within a lineage (Harrington & Rizzo, 1999). Localized lineages would be considered as unique species if an isolated population acquired a fixed phenotypic character of ecological importance (Harrington et al., 2014). Aside from occurrence on exotic, cultivated hosts, no unique phenotypic character appears to provide suitable diagnosis to warrant species designation for the populations of *C. fimbriata* that appear to be native to the Mata Atlântica region.

Interfertility experiments (Ferreira et al., 2010; Oliveira et al., 2015) showed that isolates of *C. fimbriata* from the Mata Atlântica region in Brazil were interfertile with Brazilian isolates from other hosts and regions, as well as with sweet potato isolates, on which the name *C. fimbriata* is based (Halsted, 1890). In addition, mango isolates from the Mata Atlântica region were morphologically indistinguishable from other mango isolates of *C. fimbriata* in Brazil, Oman and Pakistan, as well as isolates from sweet potato (Oliveira et al., 2015). Thus, the Mata Atlântica group, most of the other South American populations, and the worldwide sweet potato strain are monophyletic, morphologically indistinguishable and form a single biological species, namely, *C. fimbriata* (Harrington et al., 2014; Li et al., 2016).

The Mata Atlântica group appears to comprise different host-associated populations in the coastal region of what could be considered the Serra do Mar range. Geographic restrictions to gene flow may be expected in natural populations of *C. fimbriata*, because the fungus is soilborne, insect dispersed and not wind dispersed (Hinds, 1972; Harrington et al., 2011; Harrington, 2013). Thus, it would not be surprising that the analysis of mating type genes would distinguish the Mata Atlântica populations from *Eucalyptus* and mango populations, which are believed to be native to the Cerrado-transition region, from the interior of São Paulo to the northeast (Ferreira et al., 2010; Oliveira et al., 2015).

Three mating type sequences were previously reported (Harrington et al., 2014) in isolates from the Mata Atlântica group, namely the genotype 5a found in isolates from mango in eastern Rio de Janeiro, genotype 5b on taro isolates from São Paulo, and genotype 5c found in a single isolate collected on *Eucalyptus* in Paraná. A new mating type sequence (mating type genotype 5d) that differed from the others in a single base substitution was identified in isolates on kiwifruit from Rio Grande do Sul (Ferreira et al., 2017), as well as in one isolate on taro from Rio de Janeiro.

At a finer scale, microsatellite markers were able to distinguish Mata Atlântica populations on taro, mango (eastern Rio de Janeiro) and kiwifruit (Rio Grande do

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**Table 3** Microsatellite alleles based on approximate band sizes (in base pairs) of isolates of *Ceratocystis fimbriata* from Mata Atlântica populations, Brazil, on *Colocasia esculenta*, *Mangifera indica* and *Actinidia deliciosa.*

<table>
<thead>
<tr>
<th>Locus</th>
<th>No. of isolates</th>
<th>Population</th>
<th>AAG8</th>
<th>AAG9</th>
<th>CA9</th>
<th>CAA10</th>
<th>CAA15</th>
<th>CAA38</th>
<th>CAA80</th>
<th>CAG5</th>
<th>CAG15</th>
<th>CAG18</th>
<th>CAT1</th>
<th>CAT12</th>
<th>GACA60</th>
<th>GACA6K</th>
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<tbody>
<tr>
<td>ColRJ6</td>
<td>4</td>
<td>ColSP6</td>
<td>5</td>
<td>180</td>
<td>400</td>
<td>400</td>
<td>209</td>
<td>128</td>
<td>321</td>
<td>324</td>
<td>223</td>
<td>223</td>
<td>321</td>
<td>324</td>
<td>223</td>
<td>314</td>
</tr>
<tr>
<td>ColDF1</td>
<td>6</td>
<td>ColSP9</td>
<td>5</td>
<td>180</td>
<td>400</td>
<td>400</td>
<td>209</td>
<td>128</td>
<td>321</td>
<td>324</td>
<td>223</td>
<td>223</td>
<td>321</td>
<td>324</td>
<td>223</td>
<td>314</td>
</tr>
<tr>
<td>ColBA3</td>
<td>8</td>
<td>ColRJ5</td>
<td>5</td>
<td>180</td>
<td>197</td>
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<td>223</td>
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<td>324</td>
<td>223</td>
<td>314</td>
</tr>
<tr>
<td>ManRJ5</td>
<td>5</td>
<td>ManRJ2</td>
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<td>ActRJ5</td>
<td>4</td>
<td>186</td>
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<td>223</td>
<td>321</td>
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<td>223</td>
<td>314</td>
</tr>
</tbody>
</table>

*Only populations that contain more than four isolates are shown.*
Sul), as well as the isolate from *Eucalyptus* (Paraná). Natural populations of *C. fimbriata* may vary in their aggressiveness to various non-native hosts, presumably through selection pressure due to human activities (Baker *et al.*, 2003; Engelbrecht & Harrington, 2005; Silveira *et al.*, 2006; Harrington *et al.*, 2011). The most aggressive strains on a particular host are most likely to be sampled because they cause the most crop damage. In addition, vegetative propagation of a host such as taro or pruning a host such as mango could increase the proportion of aggressive genotypes in populations (Ferreira *et al.*, 2010; Harrington *et al.*, 2011; Oliveira *et al.*, 2015).

Generally, taro isolates from various locations around the world have appeared to be particularly aggressive on taro and other species of Araceae (Thorpe *et al.*, 2005; Harrington *et al.*, 2011). Mango isolates from Mata Atlântica (eastern Rio de Janeiro) may be particularly aggressive to *Annona* (*Ammona squamosa*) and mango (Baker *et al.*, 2003; Silveira *et al.*, 2006; Harrington *et al.*, 2011). Depending on the mango cultivar inoculated, isolates from eastern Rio de Janeiro can be more aggressive than mango isolates from other regions of Brazil (Oliveira *et al.*, 2015). Isolates from kiwifruit, including those of the *Mata Atlântica* populations had gone through genetic bottlenecks (Harrington *et al.*, 2011), explaining the dominance of two common genotypes in populations (Ferreira *et al.*, 2011, 2017; Harrington *et al.*, 2015; Li *et al.*, 2016). Introductions of the pathogen into new areas continues to be an important economic and ecological issue, and it calls for better sanitation and quarantine practices.

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