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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. cacaofunesta* (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

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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 deliciosa* (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′-CTTGGTCATTTAGAG GAAGTAA-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).

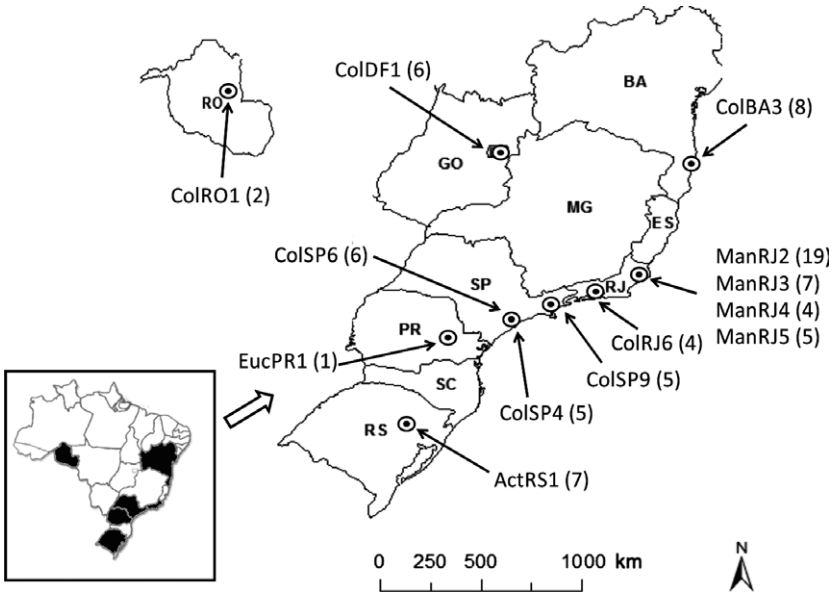

Figure 1 Map of Brazil showing the locations of the sampled *Ceratocystis fimbriata* populations. The first three letters of each population name indicate the host (*Colocasia*, *Eucalyptus*, *Actinidia*, *Mangifera*) and the last two letters indicate the state of origin (Bahia, Rondônia, São Paulo, 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 CFMAT1-F (5′-CAGCCTCGATTGAKGGTATG A-3′) and CFMAT1-R (5′-GGCATTTTTACGCTGGTTAG-3′) were used to amplify and sequence a region of the *MAT1-1-2* gene about 1000 bp long (Harrington *et al.*, 2014). The primers X9978a (5′-GCTAACCTTCACGCCAATTTTGCC-3′) and CFM2-1F (5′-AGTTACAAGTGTTCCCAAAAG-3′) were used to amplify and sequence a region of the *MAT1-2-1* 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 searches 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 Akaike 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, CfCAA9, CfCAA10, CfCAA15, CfCAA38, CfCAA80, CfCAT1, CfCAT1200, CfCAG5, CfCAG15, CfCAG900, CfGACA60 and CfGACA650) 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; Ocasio-Morales *et al.*, 2007), *C. pirilliforms* (Nkuekam *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 (CfCAT3K and CfCAT9X) 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 ColRJ6 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

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

						Microsatellite alleles				
								Nei's gene diversity (*H*)		
Group	Host	Population[a]	State	City	No. isolates	No. genotypes	Genotypic diversity (*G*)[b]	All isolates	Clone-corrected[c]	ITS rDNA genotypes[d]
Mata Atlântica	*Colocasia*	ColRJ6	Rio de Janeiro	Rio de Janeiro	4	3	3.00	0.0893	0.0952	11
		ColSP4	São Paulo	Tapiraí	5	3	2.80	0.1257	0.1587	9, 12, 13
		ColSP6	São Paulo	Sorocaba	6	3	2.60	0.0516	0.0635	12, 13
		ColSP9	São Paulo	Ubatuba	5	2	2.00	0.0343	0.0357	13
		ColBA3	Bahia	Porto Seguro	8	3	2.00	0.0424	0.0635	11, 12b
		ColDF1	Distrito Federal	Brasília	6	2	1.93	0.0317	0.0357	13
	Mangifera	ManRJ3	Rio de Janeiro	São Fidelis	7	4	2.97	0.0641	0.0804	14d
		ManRJ4	Rio de Janeiro	Itaocara	4	2	2.00	0.1339	0.1786	9, 14d, 14i
		ManRJ2	Rio de Janeiro	São Fidelis	19	4	1.63	0.0973	0.1875	ND
		ManRJ5	Rio de Janeiro	Itaocara	5	1	1.00	0.0000	0.0000	14d
	Actinidia	ActRS1	Rio Grande do Sul	Farroupilha	7	2	1.85	0.0317	0.0317	ND
Cerrado-transition	*Eucalyptus*	EucMG1	Minas Gerais	Curvelo	18	14	3.74	0.3086	0.3258	ND
		EucBA1	Bahia	Eunápolis	26	13	3.27	0.2079	0.2697	ND
		EucBA1	Bahia	Caravelas	6	4	3.20	0.2262	0.2500	ND
	Mangifera	ManCE1	Ceará	Brejo Santo	11	7	3.48	0.2680	0.3149	8a, 8b, 10, 15a
		ManPB1	Paraíba	Conde	10	6	3.20	0.3814	0.3889	4a, 8a, 10c
		ManSP1	São Paulo	Limeira	11	4	2.58	0.2267	0.2946	4, 6, 10, 14a

[a]Only populations that contain more than four isolates are shown.
[b]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).
[c]Clone correction removed isolates that had genotypes identical to other isolates from the same site.
[d]Genotype numbers follow the designations of Harrington *et al.* (2014). ND, not determined.

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). The C1866 sequence had the MAT-1 genotype 5b and the MAT-2 genotype 5a (Harrington *et al.*, 2014), which is similar to the mating genotype found in isolates on mango from eastern Rio de Janeiro, as well as the genotype found in an isolate on *Eucalyptus* in Paraná state (5c genotype) (Harrington *et al.*, 2014). Thus, isolates of *C. fimbriata* on taro, mango (eastern Rio de Janeiro), kiwifruit and *Eucalyptus* sp. clustered together in a separate group, referred to herein as the Mata Atlântica group, and that branch was supported by a moderate bootstrap value (83%) and high posterior probability value (1.00) (Fig. 2).

Microsatellite genotypes

In total, 90 isolates from the Mata Atlântica group were studied, and all 14 microsatellite loci were polymorphic,

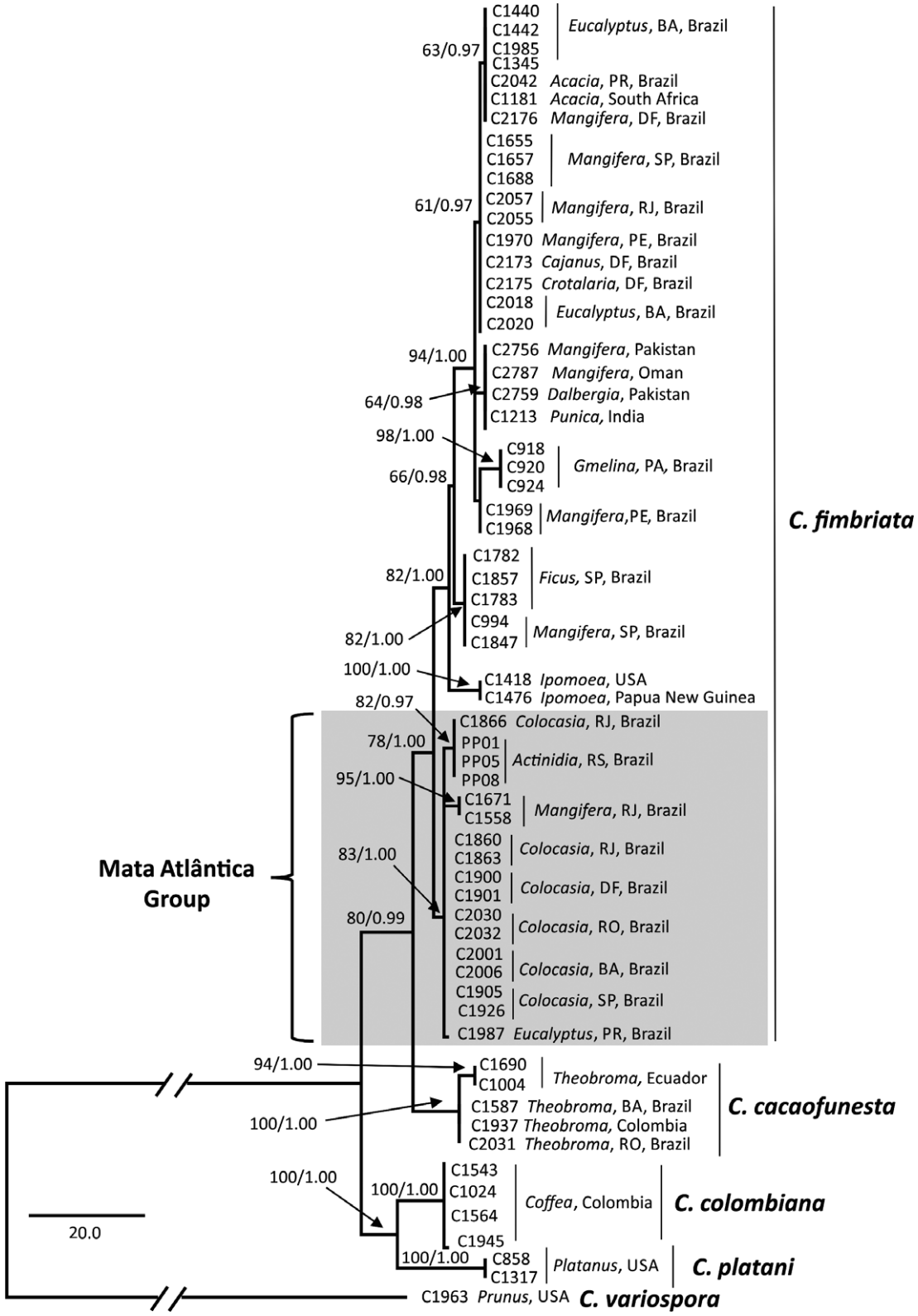

Figure 2 The single most parsimonious tree based on combined dataset of DNA sequences of MAT-1 and MAT-2 genes of representatives of the Latin American clade of the *Ceratocystis fimbriata* complex. Bootstrap values greater than 60% or posterior probability values greater than 0.9 are indicated on appropriate 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.

Table 2 Representative sequences of isolates of *Ceratocystis* species used for phylogenetic analysis of mating type genes

Species	Mating type genotype[a]	Hosts	Origin	Isolate numbers	*MAT1-1-2* GenBank accession	*MAT1-2-1* GenBank accession
C. fimbriata	4a	*Mangifera indica*, *Ficus carica*	São Paulo, Brazil	C994 (=CBS 600.70), C1847, C1782 (=CBS 115166), C1783, C1857	KF482987	HQ157551
	3a	*Eucalyptus* spp., *M. indica*, *Acacia* sp., *Acacia mearnsii*	Bahia, Distrito Federal, Paraná, Brazil; South Africa	C1442 (=CBS 115174), C1440, C1985, C1345, C2176, C2042, C1181	KF482985	HQ157550
	3b	*M. indica*, *Cajanus cajan*, *Eucalyptus* spp., *Crotalaria* sp.	Pernambuco, Rio de Janeiro, São Paulo, Bahia, Distrito Federal, Brazil	C1688 (= CBS 114721), C1970, C2055, C2057, C2173, C1655, C1657, C2018, C2020, C2175	KF482986	HQ157550
	7	*M. indica*, *Dalbergia sissoo*, *Punica granatum*	Pakistan; Oman	C2759 (=CBS 135868), C2787 (=CBS 135867), C2756, C1213	KF482991	KF482999
	2	*M. indica*	Pernambuco, Brazil	C1968, C1969	KF482984	HQ157553
	1	*Gmelina arborea*	Pará, Brazil	C918 (=CBS 115173), C920, C924	KF482983	HQ157549
	5a	*M. indica*	Rio de Janeiro, Brazil	C1558 (=CBS 115175), C1671	KF482988	HQ157552
	5d	*Actinidia deliciosa*, *Colocasia esculenta*	Rio de Janeiro, Rio Grande do Sul, Brazil	C1866, PP01, PP05, PP08	KX838968	HQ157552
	5b	*C. esculenta*	São Paulo, Bahia, Rondônia, Distrito Federal, Rio de Janeiro, Brazil	C1905 (=CBS 115171), C1926, C2001, C2006, C2030, C2032, C1900, C1901, C1860, C1863	KF482989	HQ157552
	5c	*Eucalyptus* spp.	Paraná, Brazil	C1987	KF482990	HQ157552
	8	*Ipomoea batatas*	Papua New Guinea; North Carolina, USA	C1476 (=ICMP 8579), C1418	KF482992	KF483000
C. cacaofunesta	—	*Theobroma cacoa*	Rondônia, Bahia, Brazil; Ecuador; Colombia	C1004 (=CBS 153.62), C1690, C1937, C1587, C2031	KF482993	KF483001
C. colombiana	—	*Coffea arabica*	Colombia	C1543 (=CBS 135861), C1024, C1564, C1945	KF482994	KF483002
C. platani	—	*Platanus accidentalis*	North Carolina, California, USA	C1317 (=CBS 115162), C858	KF482995	KF483003
C. variospora	—	*Prunus* sp.	Iowa, USA	C1963 (=CBS 135862)	KF482996	KF483004

[a]Mating type genotype designations from Harrington *et al.* (2014).

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 on taro, eight microsatellite genotypes among 35 isolates on mango (eastern Rio de Janeiro), two microsatellite genotypes among seven isolates from kiwifruit at the Pomar Pasa farm (Rio Grande do Sul). 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

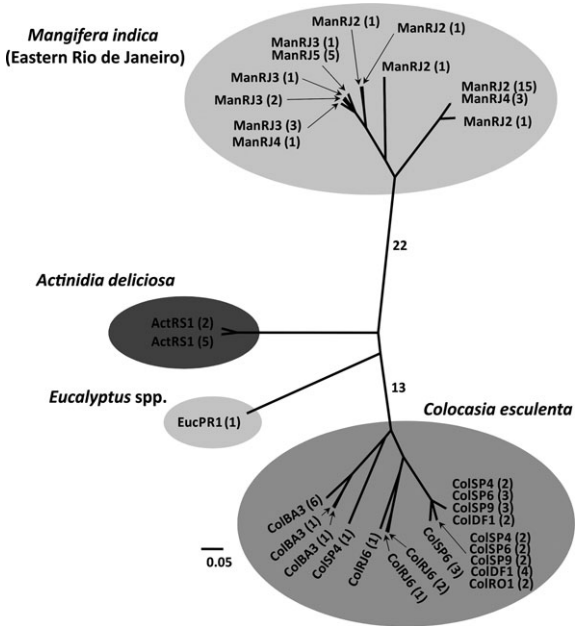

Figure 3 A UPGMA (unweighted pair group method, arithmetic mean) dendrogram of genotypes of *Ceratocystis fimbriata* from the Mata Atlântica region in Brazil based on alleles of 14 microsatellite loci. The first three letters of each population name indicate the host (*Colocasia*, *Eucalyptus*, *Actinidia*, *Mangifera*), the last two letters indicate the state of origin (Bahia, Rondônia, São Paulo, Rio de Janeiro, Distrito Federal, Paraná and Rio Grande do Sul), and each population within a state was given a separate number. The number of isolates from each population with that genotype is given in parentheses. Bootstrap values are shown alongside the branches. Scale bar indicates genetic distance.

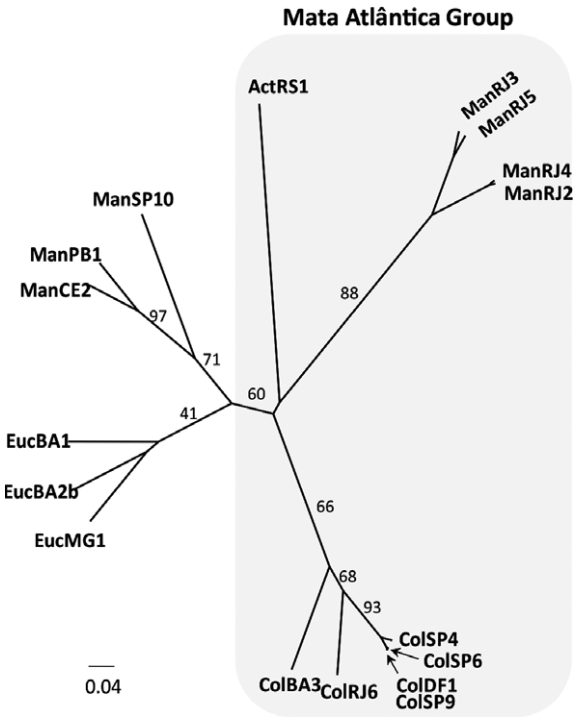

Figure 4 Dendrogram of populations of *Ceratocystis fimbriata* generated by UPGMA (unweighted pair group method, arithmetic mean) based on allele frequencies of 14 microsatellite loci. The first three letters of each population name indicate the host (*Colocasia*, *Eucalyptus*, *Actinidia*, *Mangifera*), the last two letters indicate the state of origin (Bahia, São Paulo, Rio de Janeiro, Distrito Federal and Rio Grande do Sul) and the last number distinguishes the populations within a state. Bootstrap values are shown alongside the branches. Scale bar indicates genetic distance.

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

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*

Host	Population[a]	No. of isolates	Locus												
			AAG8	AAG9	CAA9	CAA10	CAA15	CAA38	CAA80	CAG5	CAG15	CAG900	CAT1	CAT12	GACA60	GACA6K
Colocasia	ColSP4	5	180	400, 403	209	128	321, 324	223	314	326	265, 274	194	248, 261	373	187, 214	213
	ColSP6	6	180	400	209	128	324	223	302, 314	326	274	194	261	373	187, 214	213
	ColSP9	5	180	400	209	128	324	223	314	326	274	194	261	373	187, 214	213
	ColRJ6	4	180	400	209	128	321, 324	241, 250	314	326	265	194	261	373	187, 214	213
	ColDF1	6	180	400	209	128	324	223	314	326	274	194	261	373	187, 214	213
	ColBA3	8	180	403	191	128	324	223	314	326	265, 280	194	261	373	187	215
Mangifera	ManRJ2	19	186, 198	391	223	128	330	168	311	323, 326	277, 286	194	248, 261	371, 377, 380	187, 221	219
	ManRJ3	7	186	391	197, 223	128	321, 330	168, 211	311	323	286	194	248	380	187	219
	ManRJ4	4	186, 198	391	223	128	321, 330	168	311	323, 326	277, 286	194	248	380	187, 221	219
	ManRJ5	5	186	391	223	128	330	168	311	323	286	194	248	380	187	219
Actinidia	ActRS1	7	183	403	266	125	324	202, 226	314	326	283	194	248	371	200	219

[a]Only populations that contain more than four isolates are shown.

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

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 (*Annona 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 genotypes (Ferreira *et al.*, 2017), were very aggressive in all kiwifruit cultivars tested, but the aggressiveness of these isolates on other hosts was not tested (Piveta *et al.*, 2016).

Movement of select genotypes by humans could explain why most of the populations from the Mata Atlântica group exhibited low gene diversity, suggesting that the populations had gone through genetic bottlenecks (Harrington *et al.*, 2015; Li *et al.*, 2016). On taro, populations of *C. fimbriata* had very low gene and genotypic diversity values, similar to those found in introduced populations of the LAC (Engelbrecht *et al.*, 2004, 2007; Ocasio-Morales *et al.*, 2007; Ferreira *et al.*, 2010, 2011; Harrington *et al.*, 2015; Li *et al.*, 2016). Strains of *C. fimbriata* are easily spread in infected corms of taro and other Araceae used for propagation (Harrington *et al.*, 2005; Thorpe *et al.*, 2005), explaining the dominance of two common genotypes in most of the taro populations in Brazil. Taro corms were taken from São Paulo for establishing plantings in a farm near Brasília, and isolates from that farm had the common São Paulo microsatellite genotype. Taro is not grown in the state of Rondônia, but a local grocery store in Ouro Preto do Oeste purchased corms from the large distribution centre in São Paulo (CEAGESP); isolates from that grocery store had the same dominant genotypes found in São Paulo populations on taro. The most distinct population based on microsatellite alleles was from a grocery store in Bahia, but the exact source of those corms could not be determined.

Dissemination of *C. fimbriata* strains in plant propagative material has been suggested frequently as the source of new disease outbreaks (Engelbrecht *et al.*, 2004, 2007; Engelbrecht & Harrington, 2005; Johnson *et al.*, 2005; Thorpe *et al.*, 2005; Ocasio-Morales *et al.*, 2007; 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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