Genetic analyses trace the Yunnan, China population of *Ceratocystis fimbriata* on pomegranate and taro to populations on *Eucalyptus* in Brazil

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**Abstract**

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Genotypes of the Latin American wilt pathogen *Ceratocystis fimbriata* have been moved around the world in vegetatively propagated material of various crop plants, including *Ipomoea batatas* (sweet potato), *Colocasia esculenta* (taro), and *Eucalyptus* spp. When compared to a worldwide collection of isolates of *C. fimbriata*, isolates from taro, *Punica granatum* (pomegranate), and *Eriobotrya japonica* (loquat) from Yunnan Province, China were found to have sequences of ITS rDNA and mating type genes that were identical to isolates from *Eucalyptus* in Brazil. Analyses of 35 isolates with 14 microsatellite markers revealed that the Yunnan population was nearly uniform, consisting of only 19 alleles and
seven closely-related genotypes, suggesting that the population is not natural and is the
result of an introduction. As in comparisons of sequences of ITS rDNA and mating type
genes, the microsatellite alleles of the Yunnan isolates were most similar to those of
_Eucalyptus_ isolates from Minas Gerais and Bahia, Brazil, where _C. fimbriata_ is native,
soilborne, and commonly infects cuttings of _Eucalyptus_ spp. used for rooting in nurseries.
Thus, the Yunnan population, which is causing severe losses on pomegranate, may have
been indirectly derived from introductions of _C. fimbriata_ in contaminated _Eucalyptus_
cuttings from Brazil.

**Keywords:** _Acacia_ spp., _Colocasia esculenta_, _Eriobotrya japonica_, _Ipomoea batatas_,
_Punica granatum_, Ceratocystis wilt

**Introduction**

The soilborne ascomycete _Ceratocystis fimbriata_ Ellis & Halsted causes lethal,
wilt-type diseases in many woody hosts and postharvest decay of root crops around the
world (5), but the species is believed to be native to Latin America (16,18). Movement of
genotypes of _C. fimbriata_ and the closely related species _C. platani_ and _C. cacaofunesta_
in vegetatively propagated plant material has resulted in losses to root crops, fruit trees,
and forest trees (8,12,14,17-19,26). Analyses of microsatellite (simple sequence repeats)
data suggest that genotypes of _C. fimbriata_ have been moved around Brazil in nursery
stock of fig (_Ficus carica_) and mango (_Mangifera indica_) and in cuttings of _Eucalyptus_
spp. and their hybrids (12,13). Several genotypes of _C. fimbriata_ and other species in the
_C. fimbriata_ complex have been moved in corms or cuttings of the family Araceae,
especially in taro (_Colocasia esculenta_) (34). A strain on sweet potato (_Ipomoea batatas_)
has been distributed to Asia, Oceania and the USA in propagation material (7,29).
The sweet potato strain of *C. fimbriata* has been recognized in China for decades (31). More recently, *C. fimbriata* has been reported to cause a serious wilt on pomegranate (*Punica granatum*) in the South China provinces of Yunnan (23) and Sichuan (38), and it also causes a postharvest rot on taro in Yunnan (22). Genetic analyses suggested that the pomegranate and taro strains from Yunnan were the same, perhaps from a single introduction (40). In addition, *C. acaciivora* Tarigan & M. van Wyk, considered a synonym of *C. fimbriata* by Harrington et al. (18), was reported on stumps of *Eucalyptus* spp. in another South China province, Guangdong (6).

The objective of this study was to genetically analyze the population of *C. fimbriata* in Yunnan Province and compare it with previously studied populations of *C. fimbriata* (12,18) in order to determine if the Yunnan population was native or introduced. Sequences of ITS rDNA and mating type genes were used to place the Chinese isolates among a worldwide collection of *C. fimbriata* isolates, and microsatellite markers were used to further identify potential sources and pathways of spread of the Chinese population in Yunnan.

**Materials and Methods**

**Fungal isolates.** A total of 41 isolates of *C. fimbriata* from Yunnan were used for ITS rDNA sequencing, and 35 of those isolates were used for microsatellite analyses (Table 1): 27 isolates from 27 pomegranate trees in five plantations spread over a distance of 50 km in five townships in Mengzi County (Honghe Prefecture), two isolates from loquat trees (*Eriobotrya japonica*) at two separate locations in Mengzi County, and six isolates from different taro corms collected in a storage facility in Chenggong County (Kunming City), which is about 250 km from the pomegranate plantations. Isolates from pomegranate were obtained by placing chips of discolored xylem into PDA (20% potato
infusion, 2% dextrose, and 1.5% agar). Ascospore masses from perithecia produced on
pruning cuts of loquat trees were streaked onto PDA. Pieces of black-rotted taro corms
were placed between fresh slices of carrot, and ascospore masses on top of perithecia that
formed on the carrot slices were streaked onto PDA.

**Sequencing and microsatellite markers.** Isolates were grown on MYEA (2% Difco malt extract, 0.2% Difco yeast extract, and 2% agar) for 4-10 days at room
temperature and lighting for DNA extraction using PrepMan™ Ultra (Applied
Biosystems, Foster City, CA). Sequences of the ITS rDNA region (532 bp, including
ITS1, 5.8S and ITS2) and portions of the MAT2 gene (*MAT1-2*, 1102 bp product) and
one of the MAT1 genes (*MAT1-1-2*, 1022 bp product) were generated as described in
Harrington et al. (18).

Isolates were genotyped at 14 microsatellite loci as described in Ferreira et al. (12)
using the primers developed by Steimel et al. (29). For each locus, one of the primers was
fluorescently labeled so that the size of the amplified product could be determined using a
four-capillary ABI Prism 3730 DNA Analyzer and Peak Scanner™ Software v.1.0
(Applied Biosystems Inc., Foster City, CA). Each product length (within 1 bp) was
considered a different allele, though most alleles differed by increments of 3 bp (most
markers were tri-nucleotide repeats).

**Genetic analyses of microsatellite data.** Nei’s gene diversity (*H*) for Yunnan and
representative Brazilian populations of *C. fimbriata* were calculated without and with
clone-corrected data using PopGene 1.32 software (39). 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. A matrix of Nei’s genetic distance
between populations and a UPGMA (unweighted pair group method with arithmetic
mean) dendrogram were constructed using PopGene 1.32. Bootstrap values for branches
of the population trees were calculated from 1000 replicates using SEQBOOT,

GENDIST, NEIGHBOR and CONSENSE in PHYLIP v. 3.6 (11).

To compare genotypic diversity values for populations with different sample
sizes, Stoddart and Taylor's G was scaled by the expected number of genotypes for the
smallest sample size (n = 6) being compared (15) based on rarefaction curves using the R
package v. 2.6.1 (R Development CoreTeam, 2007). Relationships among genotypes
were examined using genetic distance (Nei's) matrices, UPGMA, and 1000 bootstrap
replications generated with PAUP* (30).

Results

DNA sequences. Thirty Chinese isolates from pomegranate, nine isolates from
taro and two isolates from loquat were sequenced for the ITS rDNA region, and the 41
sequences were identical. GenBank BLAST searches (3) showed that this sequence
matched previously deposited ITS rDNA sequence of C. fimbriata isolates from
pomegranate (AM292204-5, AM293381, AM690767), taro (AM293382-3, AM712445-8)
and loquat (AM711555) in Yunnan and an isolate from pomegranate in Sichuan
(HQ529711). Another GenBank accession from a pomegranate isolate in Yunnan
(AM696283) differed from by one base pair; it is possible that this discrepancy was due
to a sequencing error, but the isolate was not available for study.

In comparisons to an extensive database of 435 ITS rDNA sequences from 435
isolates representing the worldwide diversity of the C. fimbriata complex (T. Harrington,
unpublished), the sequence of the 41 Yunnan isolates matched precisely that of 15
isolates of C. fimbriata in the Latin American Clade (LAC). This sequence corresponded
to the ITS5 haplotype (AY157966, FJ236715) designated by Harrington et al. (18,19),
which was found to be common in isolates of C. fimbriata in Bahia, Brazil from diseased
Eucalyptus trees (10 isolates, including C1345 and C1985) and was also found in two isolates from soil taken from a healthy Eucalyptus plantation (isolates C2114 and C2116). The ITS5 haplotype was also found in an isolate (C2059) from mango in Rio de Janeiro, Brazil and isolates from Acacia mearnsii in Paraná, Brazil (C2042) and in South Africa (C1181). Harrington et al. (18) also reported the ITS5 haplotype in accessions deposited as C. fimbriata, C. eucalypticola M. van Wyk & M. J. Wingfield, or C. acaciivora from Eucalyptus spp. collected in China (Guandong Province), Indonesia, Thailand and Uruguay (AF453438-40, FJ236716-20, FJ236728-30, FJ236735-6, FJ236740-1, FJ236743-4, JQ862733-6) and accessions deposited as C. acaciivora from A. mangium in Indonesia (EU588655-6).

The MAT1-2 and MAT1-1-2 sequences of five Yunnan isolates (C2845, C2847, C2849, and C2867 from pomegranate and C2841 from taro) were identical to each other. In comparisons with the databases of MAT1-2 sequences (123 isolates) and MAT1-1-2 sequences (136 isolates) representing the worldwide diversity of the C. fimbriata complex (T. Harrington, unpublished), the sequences of the Yunnan isolates matched precisely the MAT1-2 and MAT1-1-2 sequences (HQ157550 and KF482985, respectively) of mating type haplotype 3a in the LAC of the C. fimbriata complex (18). Consistent with the results of the ITS rDNA sequences, the Yunnan combined MAT1-2 and MAT1-1-2 sequence was the most common haplotype among Eucalyptus isolates from plantations in Bahia (six isolates, including C1345 and C1985), as well as that of mango isolates from Rio de Janeiro (C2059) and Distrito Federal, Brazil (C2176) and the A. mearnsii isolates from Paraná (C2042) and South Africa (C1181). A sixth Yunnan isolate (C2840, from taro) had a unique, single-base substitution (an A for a G) near the end of the MAT1-1-2 sequence that was not found in other C. fimbriata isolates (18), but the MAT1-2 and ITS sequences of C2840 were identical to that of the other Yunnan isolates.
Microsatellite diversity. Of the 14 microsatellite loci, 11 were monomorphic in the Yunnan population (estimated allele size in bp: AAG8 = 180, AAG9 = 397, CAA9 = 172, CAA10 = 134, CAA15 = 324, CAT1 = 261, CAT12X = 377, CAGDL5 = 320, CAG900 = 194, GACA60 = 187, GACA6K = 215). For the three polymorphic loci, the respective allele sizes (number of isolates) were: CAA38 = 201 (1), 238 (32), and 247 (2); CAA80 = 311 (9), 317 (25), and 323 (1); and CAG15 = 262 (34) and 271 (1).

The Yunnan population had gene and genotypic diversity values similar to those of introduced populations of *C. fimbriata* and other related species in the LAC (8,9,12,26). Nei’s gene diversity ($H$) for the Yunnan population was only 0.0456, or $H = 0.0899$ when clone corrected (Table 1). The clone corrected values for $H$ were 0.1905 and 0.1486, respectively, for a mango population in eastern Rio de Janeiro (ManRJ2) and a taro population from São Paulo (ColSP3), but the other Brazilian populations, including the five populations from individual *Eucalyptus* plantations, had much higher clone-corrected values of $H$, ranging from 0.2286 to 0.3661 (Table 1).

A UPGMA tree was constructed using allele frequencies of microsatellite markers in order to compare the Yunnan population (35 isolates) with previously studied (12) *C. fimbriata* populations from Brazil and the worldwide sweet potato population, as well as representative populations of the closely related *C. cacaofunesta* Engelbr. & TC Harr. and *C. platani* (Walter) Engelbr. & TC Harr. (Fig. 1). The *C. cacaofunesta* and *C. platani* populations were distinct, as were the *C. fimbriata* population from sweet potato and Brazilian populations ManRJ2 and ColSP3, which were represented by only a few genotypes. The Yunnan population grouped with the other Brazilian populations from mango and *Eucalyptus* with weak bootstrap support (40%) (Fig. 1).

Among a database of the microsatellite alleles for 817 isolates of *C. fimbriata* representing the diversity of the LAC (8,9,12,13,26), the alleles of 13 loci of the Yunnan
isolates were most similar to (most commonly found in) *C. fimbriata* isolates from 
*Eucalyptus* spp. in Bahia. All the alleles identified among the Yunnan isolates were also 
identified in Bahia in earlier studies (12,13), with the exception of the 320 bp allele for 
locus CAGDL5, which has previously been found only in some mango isolates from 
northeastern Brazil (Pernambuco) and *A. mearnsii* isolate C2042 from Paraná (19).

A dendrogram based on UPGMA and Nei’s genetic distance matrix showed that 
the Yunnan genotypes grouped together (Fig. 2). Although there was no bootstrap support 
for most of the branches in the tree, the seven Yunnan genotypes were most similar to 
Brazilian genotypes from *Eucalyptus*. The *A. mearnsii* isolates from Paraná (C2042) and 
South Africa (C1181) and a mango isolate (C2059) from Rio de Janeiro, which had ITS 
and mating type sequences identical to the Yunnan isolates, also had microsatellite 
genotypes that were similar to *Eucalyptus* isolates from Brazil (Fig. 2).

**Genotypic diversity.** Seven genotypes (i.e., unique combinations of microsatellite 
alleles) were found among the 35 isolates from Yunnan. The most common genotype 
(CAA38 = 238, CAA80 = 317, CAG15 = 262) was found in 19 of 27 pomegranate 
isolates, the two loquat isolates, and one of the six taro isolates. The next most common 
genotype (CAA38 = 238, CAA80 = 311, CAG15 = 262) was found in five pomegranate 
isolates and three taro isolates. Three pomegranate isolates and two taro isolates had 
unique genotypes. No isolate from Brazil had the exact same combination of 
microsatellite alleles as any of the Yunnan isolates.

Stoddart and Taylor’s genotypic diversity (G) for the Yunnan population of 35 
isolates was 2.67 (on a 1 to 6 scale; 1 = only one genotype, 6 = each isolate a unique 
genotype), a lower value of G than two populations on mango and five populations on 
*Eucalyptus* in Brazil, each of which was collected from a single *Eucalyptus* plantation 
(Table 1). Two other Brazilian populations had relatively low values of G (mango
population ManRJ2, G = 1.95; and taro population ColSP3, G = 2.96), perhaps due to local spread of certain genotypes by human activity (12). The six Yunnan isolates from taro had higher genotypic diversity than the 27 pomegranate isolates, though the genetic diversity of taro and pomegranate subpopulations were similar (Table 1).

Discussion

Sequence comparisons of mating type genes and ITS rDNA from hundreds of representatives of the *C. fimbriata* complex from around the world clearly place the Yunnan population in the Latin American Clade and specifically among populations on *Eucalyptus* spp. in Bahia, Brazil. Analyses of microsatellite alleles suggest that the *C. fimbriata* population in Yunnan is not natural and instead is the result of an introduction of one or a few closely related strains. The Yunnan microsatellite alleles were compared to those of more than 800 isolates of the LAC from native and introduced populations around the world and found to be most similar to those of *C. fimbriata* isolates on *Eucalyptus* in Brazil.

Corroborating the results of Yu et al. (40), the isolates from pomegranate, taro and loquat showed very low levels of gene diversity, suggesting a genetic bottleneck. Members of the *C. fimbriata* complex are homothallic due to unidirectional mating type switching, and insect dispersal of ascospores is generally of minor importance in the epidemiology of Ceratocystis wilt (17). Thus, limited outcrossing generally leads to low gene and genotypic diversity in introduced populations (1,8,9,12,37). In spite of the very limited number of microsatellite alleles found among the six taro isolates in Yunnan (17 alleles at 14 loci), four different combinations of alleles were found among isolates collected from a single storage facility, perhaps due to some recombination of microsatellite alleles. Nonetheless, using the same microsatellite markers, the gene
diversity value of the Yunnan population was very low and comparable to that of
introduced populations of *C. cacaofunesta* in Bahia and Costa Rica (8), *C. platani* in
Europe and California, USA (9,26), *C. fimbriata* in various plantings of infected nursery
stock in Brazil (12,13), and the worldwide population of *C. fimbriata* on sweet potato
(12,29). Much greater gene diversity has been found in populations recovered from
*Eucalyptus* planted in naturally infested soil in Brazil (12,13).

Although the ITS sequences of *C. fimbriata* isolates from Yunnan and Sichuan are
similar to those of taro isolates in Brazil (22,38), and *C. fimbriata* strains are easily spread
in infected corms of taro and other Araceae (20,34), the ITS5 haplotype, the mating type
haplotype 3a, and the microsatellite alleles found in Yunnan are distinct from those of
taro isolates in Brazil, and from sweet potato isolates in Asia and elsewhere (18). Instead,
the genetic markers for the Yunnan isolates are typical for *Eucalyptus* isolates in Minas
Gerais and Bahia, where the pathogen is native and diverse, but some genotypes are
common in *Eucalyptus* cuttings used for rooting and establishment of clonal plantations
(12-14,18,19).

Isolates recovered from cut stumps of *Eucalyptus* spp. in Guandong (6) have the
same ITS5 haplotype as the Yunnan isolates (18), and the same ITS rDNA sequence was
reported by Xu et al. (38) for pomegranate isolates in Sichuan, where the pathogen also is
aggressive on pomegranate and is causing serious losses. Outside of China, the only other
report of Ceratocystis wilt on pomegranate is from India (28), but a pomegranate isolate
from India had a different genetic profile (40), and it had the mating type haplotype of
introduced strains of *C. fimbriata* in Oman and Pakistan (18), where Ceratocystis wilt is
causing important losses on mango, *Dalbergia sissoo* and other hosts (1,2,10,27). Based
solely on distinct ITS sequences (ITS7B and ITS6, respectively), the causal agent of the
Oman and Pakistan epidemics on mango could be referred to as *C. manginecans* M. van
Wyk, Al Adawi & M. J. Wingfield and/or *C. acaciivora* (2,18,35). However, multiple ITS rDNA sequences were recovered from single-ascospore strains of *C. fimbriata* from Oman, Pakistan and India (2,18). Thus, ITS rDNA sequences are hypervariable and helpful in genotyping strains, but they are not suitable for delineating species in the *C. fimbriata* complex. *C. acaciivora* and *C. manginecans*, as well as *C. eucalypticola*, are considered synonyms of *C. fimbriata* and apparently represent introduced strains from Brazil (18). Regardless, the India/Oman/Pakistan population is genetically distinct from the Yunnan population.

Besides Brazil and China, the ITS5 haplotype has been identified on *Eucalyptus* in Indonesia, Thailand and Uruguay and *Acacia* spp. in Indonesia (18), but it is not clear that *C. fimbriata* is causing serious disease on *Eucalyptus* in Asia (6,17,36). Isolates from *Acacia* spp. with the ITS5, ITS6 and ITS7b haplotypes (18) were found to be associated with a serious disease in *A. mangium* plantations in Indonesia, and isolates of these three ITS haplotypes were aggressive on *Acacia* spp. in inoculation studies (32). However, more detailed genetic analyses are needed to compare the Southeast Asian and Chinese populations of *C. fimbriata*.

In addition to Brazilian *Eucalyptus* isolates, the Yunnan mating type and ITS rDNA sequences were found in a mango isolate from Rio de Janeiro and *A. mearnsii* isolates from Paraná and South Africa, but *A. mearnsii* is not a likely source for introduction of genotypes of *C. fimbriata* because it is propagated from seed, and *C. fimbriata* is not likely seedborne (5). The South African *A. mearnsii* isolate is believed to be part of an introduced population of *C. fimbriata* on *Eucalyptus* in South Africa (18,37), which was recently named *C. eucalypticola* (36). It was suggested that the name *C. eucalypticola* may represent diverse strains of *C. fimbriata* distributed in *Eucalyptus* throughout the world, including those in Guandong (36), but there is no evidence of host
specialization of these *Eucalyptus* strains, and with no other genetic or phenotypic
distinction, *C. eucalypticola* is considered a synonym of *C. fimbriata* (18).

Strains of *C. fimbriata* generally have a broad host range, and Brazilian isolates
from *Eucalyptus* vary greatly in aggressiveness to *Eucalyptus* and other hosts (4,19,41). A
*Eucalyptus* isolate from Bahia (C1345) with the same ITS5 haplotype and mating type
haplotype 3a found in Yunnan was inconsistent in pathogenicity when inoculated into *E.
grandis* × *E. urophylla* clones and did not appear to be pathogenic to *A. mearnsii*, but it
was pathogenic to taro (4,19,33). When 18 Brazilian clones of *E. grandis* × *E. urophylla*
were screened for resistance against *Eucalyptus* isolates C1345 and C1422 (with the ITS4
haplotype and mating type haplotype 3a), C1422 was generally more aggressive, and
some clones were highly susceptible and others resistant to either or both isolates (41).

Although Ceratocystis wilt can be serious on certain *Eucalyptus* clones in Brazil (14,17),
some relatively resistant clones may prove to be symptomless carriers of *C. fimbriata.

*Ceratocystis fimbriata* and other members of the LAC are most easily dispersed to
new continents in vegetative cuttings taken from asymptomatic hosts (8,12,17), especially
in *Eucalyptus* cuttings from a region of Brazil where decades ago Aracruz Cellulose S.A.
developed highly productive clones of *Eucalyptus* spp. and hybrids. Seeds from some of
these clones were introduced to China as early as 1984 (21), but seeds would not have
likely carried *C. fimbriata* (5,17). However, it is possible that some cuttings were also
taken from this region of Brazil and brought to China. Among clones of *Eucalyptus* spp.
that were widely planted in South China in the 1990s (24), two were *E. urophylla* × *E.
grandis* hybrids from Brazil and two were *E. urophylla* clones from Indonesia, where the
ITS5 haplotype of *C. fimbriata* was identified in *Eucalyptus* and *A. mangium* (18,32,36).

*Acacia mangium* can be vegetatively propagated, so *C. fimbriata* may be moved in *A.*
mangium cuttings, as it can be in taro (20,34) and probably pomegranate, which also is propagated from hardwood cuttings or air layering. However, international movement of C. fimbriata would more likely be in cuttings of Eucalyptus (13,17).

The broad and unpredictable host range of C. fimbriata strains complicates management of Ceratocystis wilt. Clearly, much greater care should be taken in moving propagative material (8,12,13,17,18). Also, the pathogen is mechanically transmitted, and sanitation practices are needed in nursery production (12-14,17,19). Once introduced, C. fimbriata and close relatives in the Latin American clade of the C. fimbriata complex may become waterborne or soilborne and infect roots of various susceptible crops (17). It appears that C. fimbriata is established in some soils in Yunnan, so Ceratocystis wilt may continue to be a problem, even if clean planting material is utilized. A clearer understanding of the host range of the Yunnan population of C. fimbriata may be useful in identifying alternative crops or crop rotations, and selection for pomegranate clones resistant to Ceratocystis wilt may be feasible.

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Table 1. Number of genotypes, genotypic diversity, and gene diversity based on 14 microsatellite loci in populations of *Ceratocystis fimbriata* on *Ipomoea batatas*, *Eucalyptus* spp., *Mangifera indica*, and *Colocasia esculenta* and a population in Yunnan, China.

<table>
<thead>
<tr>
<th>Population</th>
<th>Location</th>
<th>Host(s)</th>
<th>No. of isolates</th>
<th>No of genotypes</th>
<th>Stoddart &amp; Taylor's genotypic diversity (G) with rarefaction(^a)</th>
<th>Nei’s gene diversity ((H))</th>
</tr>
</thead>
<tbody>
<tr>
<td>EucBA1(^c)</td>
<td>Plantation, Bahia, Brazil</td>
<td><em>E. grandis</em> × <em>E. urophylla</em></td>
<td>27</td>
<td>13</td>
<td>4.45</td>
<td>0.2206 0.2500</td>
</tr>
<tr>
<td>EucBA2b</td>
<td>Plantation, Bahia, Brazil</td>
<td><em>E. grandis</em> × <em>E. urophylla</em></td>
<td>6</td>
<td>4</td>
<td>4.00</td>
<td>0.2262 0.2899</td>
</tr>
<tr>
<td>EucMG1</td>
<td>Plantation, Minas Gerais, Brazil</td>
<td><em>E. grandis</em> × <em>E. urophylla</em></td>
<td>20</td>
<td>14</td>
<td>5.34</td>
<td>0.3076 0.3528</td>
</tr>
<tr>
<td>EucMG2</td>
<td>Plantation, Minas Gerais, Brazil</td>
<td><em>E. grandis</em> × <em>E. urophylla</em></td>
<td>6</td>
<td>6</td>
<td>6.00</td>
<td>0.2659 0.2659</td>
</tr>
<tr>
<td>EucMG3</td>
<td>Plantation, Minas Gerais, Brazil</td>
<td><em>E. grandis</em> × <em>E. urophylla</em></td>
<td>6</td>
<td>5</td>
<td>5.00</td>
<td>0.2381 0.2286</td>
</tr>
<tr>
<td>ManRJ1</td>
<td>Western Rio de Janeiro, Brazil</td>
<td><em>Mangifera indica</em></td>
<td>14</td>
<td>10</td>
<td>4.83</td>
<td>0.3092 0.3661</td>
</tr>
<tr>
<td>ManSP1</td>
<td>Central São Paulo, Brazil</td>
<td><em>M. indica</em></td>
<td>9</td>
<td>5</td>
<td>4.39</td>
<td>0.3192 0.3600</td>
</tr>
<tr>
<td>ManRJ2</td>
<td>Eastern Rio de Janeiro, Brazil</td>
<td><em>M. indica</em></td>
<td>19</td>
<td>4</td>
<td>1.95</td>
<td>0.0910 0.1905</td>
</tr>
<tr>
<td>ColSP3</td>
<td>Eastern São Paulo, Brazil</td>
<td><em>Colocasia esculenta</em></td>
<td>12</td>
<td>5</td>
<td>2.96</td>
<td>0.0893 0.1486</td>
</tr>
<tr>
<td>IpoWW</td>
<td>Worldwide</td>
<td><em>Ipomoea batatas</em></td>
<td>16</td>
<td>2</td>
<td>1.62</td>
<td>0.0156 0.0357</td>
</tr>
<tr>
<td>Current Study</td>
<td>Mengzi County, Yunnan, China</td>
<td><em>Punica granatum</em></td>
<td>27</td>
<td>5</td>
<td>2.41</td>
<td>0.0366 0.0857</td>
</tr>
<tr>
<td>Current Study</td>
<td>Kunming City, Yunnan, China</td>
<td><em>C. esculenta</em></td>
<td>6</td>
<td>4</td>
<td>4.00</td>
<td>0.0556 0.0714</td>
</tr>
<tr>
<td>Current Study</td>
<td>Yunnan, China</td>
<td><em>P. granatum, C. esculenta, and Eriobotrya japonica</em></td>
<td>35</td>
<td>7</td>
<td>2.67</td>
<td>0.0456 0.0899</td>
</tr>
</tbody>
</table>

\(^a\)Values of G range from 1 (only one genotype found in the population) to 6 (each isolate in the population a unique genotype).

\(^b\)Clone correction removed isolates that had genotypes identical to other isolates from the same site.

\(^c\)Population designations and data from Ferreira et al. (12), based on 14 microsatellite loci.
Figure 1. Dendrogram generated by UPGMA (unweighted pair group method, arithmetic mean) based on alleles frequencies of 14 microsatellite loci of populations of *Ceratocystis cacaofunesta*, *C. platani*, and populations of *C. fimbriata* on *Ipomoea batatas* (isolates from Asia, Oceania and USA), on *Eucalyptus* spp., *Mangifera indica*, and *Colocasia esculenta* from Brazil, and *Punica granatum*, *C. esculenta* and *Eriobotrya japonica* from Yunnan, China. Designations for the Brazil populations as in Ferreira et al. (12) and in Table 1, with a two-letter designation for state of origin (RJ = Rio de Janeiro, SP = São Paulo, BA = Bahia, MG = Minas Gerais). Branch lengths are proportional to the genetic distance between a population and a node. Bootstrap values from 1000 replications are shown alongside the branches.

Figure 2. Dendrogram of the genetic relatedness of representative genotypes of *Ceratocystis cacaofunesta*, *C. platani* and *C. fimbriata* generated by UPGMA (unweighted pair group method, arithmetic mean) and Nei’s genetic distance. Isolates with numbers following a ‘C’ are stored at Iowa State University, and all other isolates are stored at the Universidade Federal de Viçosa. Host genus precedes the isolate numbers, and state of origin of Brazilian isolates are given (RJ = Rio de Janeiro, SP = São Paulo, BA = Bahia, MG = Minas Gerais, PA = Pará, PN = Paraná). Two of the genotypes found in Yunnan (represented by isolates C2836 and C2840, respectively) were found in multiple isolates as indicated in parentheses. Bootstrap values from 1000 replications are shown alongside the branches.
Figure 1. Dendrogram generated by UPGMA (unweighted pair group method, arithmetic mean) based on alleles frequencies of 14 microsatellite loci of populations of Ceratocystis cacaofunesta, C. platani, and populations of C. fimbriata on Ipomoea batatas (isolates from Asia, Oceania and USA), on Eucalyptus spp., Mangifera indica, and Colocasia esculenta from Brazil, and Punica granatum, C. esculenta and Eriobotrya japonica from Yunnan, China. Designations for the Brazil populations as in Ferreira et al. (12) and in Table 1, with a two-letter designation for state of origin (RJ = Rio de Janeiro, SP = São Paulo, BA = Bahia, MG = Minas Gerais). Branch lengths are proportional to the genetic distance between a population and a node. Bootstrap values from 1000 replications are shown alongside the branches.
Figure 2. Dendrogram of the genetic relatedness of representative genotypes of Ceratocystis cacaofunesta, C. platani and C. fimbriata generated by UPGMA (unweighted pair group method, arithmetic mean) and Nei's genetic distance. Isolates with numbers following a 'C' are stored at Iowa State University, and all other isolates are stored at the Universidade Federal de Viçosa. Host genus precedes the isolate numbers, and state of origin of Brazilian isolates are given (RJ = Rio de Janeiro, SP = São Paulo, BA = Bahia, MG = Minas Gerais, PA = Pará, PN = Paraná). Two of the genotypes found in Yunnan (represented by isolates C2836 and C2840, respectively) were found in multiple isolates as indicated in parentheses. Bootstrap values from 1000 replications are shown alongside the branches.