

This article is from the  
March 2005 issue of

# *Phytopathology*

published by  
The American Phytopathological Society

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# Pathogenicity, Internal Transcribed Spacer-rDNA Variation, and Human Dispersal of *Ceratocystis fimbriata* on the Family Araceae

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Accepted for publication 22 November 2004.

## ABSTRACT

Thorpe, D. J., Harrington, T. C., and Uchida, J. Y. 2005. Pathogenicity, internal transcribed spacer-rDNA variation, and human dispersal of *Ceratocystis fimbriata* on the family Araceae. *Phytopathology* 95:316-323.

*Ceratocystis fimbriata* is a complex of many cryptic, host-specialized species that causes wilt and canker of woody species and rot diseases of storage roots and corms of many economically important plants worldwide. With the exception of the family Araceae, all confirmed hosts of *C. fimbriata* are dicotyledonous plants. We hypothesized that the isolates from members of the family Araceae would form a monophyletic lineage specialized to infect these unique hosts. Analyses of sequences of the internal transcribed spacer (ITS) region of nuclear rDNA indicate that isolates and herbarium specimens of *C. fimbriata* from the family Araceae represent three different groups: an *Xanthosoma/Syngonium* group on corms of *Xanthosoma* spp. from the Caribbean region and on ornamental *S. podophyllum* from greenhouses in Florida, Hawaii, Australia, and

Brazil; an inhome group on corms of *Colocasia esculenta* in Brazil; and a distantly related taro group on *Colocasia esculenta* in Hawaii and China and on *X. sagittifolium* in Fiji. Inoculations of three species of Araceae (*Caladium bicolor*, *S. podophyllum*, and *Colocasia esculenta*) showed that isolates from all three groups are pathogenic to these three hosts. Brazilian isolates from *Mangifera indica* and *Ficus carica* were only weakly pathogenic to *Caladium* and *Syngonium* sp. and were not pathogenic to *Colocasia* sp. *Syngonium* plants appeared to be most susceptible to isolates of the *Xanthosoma/Syngonium* group, and *Colocasia* plants were least susceptible to isolates from *Syngonium* spp. Thus, it appears that adaptations to the family Araceae have evolved more than once in the *C. fimbriata* complex. It is hypothesized that the three groups of *C. fimbriata* on the family Araceae are native to the Caribbean, Brazil, and Asia, respectively, but they have been spread elsewhere by humans.

*Additional keyword:* speciation.

*Ceratocystis fimbriata* Ellis & Halsted is a fungal pathogen with a large and diverse host range on dicotyledonous plants. Some of the many susceptible plant species include *Platanus* spp., *Theobroma cacao*, *Mangifera indica*, *Coffea arabica*, *Eucalyptus* spp., *Populus* spp., *Ficus carica*, and *Ipomoea batatas* (3,6,13, 26,41). Symptoms include discoloration of the xylem, wilting, cankers, and rot of storage roots. More than 16 dicotyledonous families are hosts to *C. fimbriata* (6,17), but only one monocotyledonous family, Araceae, contains confirmed hosts. Diseases on the family Araceae caused by *C. fimbriata* include basal rot of ornamental house and landscape plants in the genus *Syngonium* and black rot of corms of the edible aroid *Colocasia esculenta* (L.) Schott, which is known as taro in Asia and the Pacific, and as inhome in Brazil (6,20,39). *Colocasia esculenta* is the most important of the aroid hosts, with worldwide production on 1.57 million ha of 9.22 million tons annually (15). We have seen similar black rot on the edible corms of *Xanthosoma* spp. (known as yautia, tannia, or malanga in parts of the Caribbean) and have consistently isolated *C. fimbriata* from them. The edible species of *Xanthosoma* are collectively known as *X. sagittifolium* and are grown on 34,725 ha with an annual production of 286,108 tons (15).

The earliest report of *C. fimbriata* causing disease on the family Araceae was in 1939 on *Colocasia esculenta* in Japan (33). Black rot disease has also been noted on the corms of *Colocasia esculenta* or *Xanthosoma* spp. in Florida, Cuba, Brazil, Fiji, and West-

ern Samoa (1,20,37,39). Alfieri et al. (1) reported *Alocasia* sp. as a susceptible host in Florida. Davis (11) found *C. fimbriata* causing darkened, water-soaked lesions on the pseudopetioles of the ornamental houseplant *S. auritum* in California greenhouses. The disease has been reported on other *Syngonium* species, including *S. podophyllum* Schott, in greenhouses in Florida, Hawaii, and Australia (1,38–40). Inoculation studies have shown that *Alocasia* sp., *Colocasia esculenta*, *X. sagittifolium*, *Syngonium* spp., and *Caladium* sp. are susceptible to isolates of *C. fimbriata* from the family Araceae, but Araceae isolates generally do not infect dicotyledonous hosts (3,11,24,25,27,29,38,39). However, no comparisons of pathogenicity have been made among Araceae isolates.

There is substantial genetic variation within the *C. fimbriata* complex (3,4,26,34), and much of this variation is associated with unique hosts or geographic regions. Three geographic clades are believed to be centered in North America, Asia, and Latin America (17). Within each geographic clade there are numerous lineages associated with certain hosts (3,5,26), and inoculation studies have shown isolates of some of these lineages to be highly host specialized (3,13,23,35). Although there is limited morphological variation within the complex (41), several new species have been recognized based on minor morphological variation and unique host ranges (5,13,42). Many cryptic species await description using the phylogenetic species concept proposed by Harrington and Rizzo (19), in which species are recognized as monophyletic lineages with unique phenotypic characters, such as host specialization.

Because *C. fimbriata* is an insect-dispersed pathogen (21,22, 30), natural populations of the pathogen appear to show substantial genetic differentiation and have unique internal transcribed

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DOI: 10.1094/PHYTO-95-0316

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spacer (ITS) sequences (3,14), and natural long-distance dispersal of populations to new continents and islands might be very rare events. However, there appears to have been movement by humans of certain host-specialized genotypes of *C. fimbriata* to new regions on propagative material and solid wood packing materials (3,6,13,14,17). For instance, isolates of the sweet potato pathogen (*C. fimbriata sensu stricto*) from China, Japan, Papua New Guinea, New Zealand, St. Vincent, and the United States show no genetic variation in ITS sequence and very limited variation in microsatellite markers (3,13,34), probably because a single strain of the fungus has been moved around the world on storage roots used for propagation (13). Strains of *C. fimbriata* may also have been moved around the world on partially rotted corms of edible aroids or infected slips of ornamental aroids.

We hypothesize that there is a cryptic species in the *C. fimbriata* complex that is specifically adapted to infect the family Araceae, and this strain has been moved around the world in propagative material. This hypothesis was tested by examining the ITS sequences of isolates and herbarium specimens of *C. fimbriata* from aroids in Asia, the Pacific, the Caribbean, and Latin America and testing the pathogenicity of the isolates on three species of Araceae: *Colocasia esculenta*, *S. podophyllum*, and *Caladium bicolor* (Aiton) Vent.

## MATERIALS AND METHODS

**Fungal isolates.** Most of the isolates of *C. fimbriata* were obtained from corms of *Colocasia esculenta* or *X. sagittifolium* with black rot symptoms purchased in markets and grocery stores (Table 1). Corms of these two species are similar, and in some cases the host may have been misidentified; the isolate from Cuba, in particular, may have been from either host. Isolates were also

made from diseased *Syngonium* plants in Florida and Hawaii. Isolates were obtained by transferring ascospore masses on top of perithecia formed on the corms or by placing pieces of diseased tissue between two disks of carrot (30). Ascospore masses were transferred to malt yeast extract agar (MYEA; 2% malt extract, 2% agar, and 0.2% yeast extract), and the pure cultures were stored at -80°C. Other isolates were obtained from referenced culture collections (Table 1).

Herbarium specimens from the U.S. National Fungus Collection (BPI) were also examined, and DNA was extracted from perithecia and ascospore masses of specimens on *Xanthosoma*, *Colocasia esculenta*, and *Syngonium* (Table 1). All of the herbarium specimens were of imported material intercepted by quarantine officials of the U.S. Department of Agriculture.

**DNA extraction and ITS sequencing.** Two methods were used to obtain genomic DNA from cultures for use as template in polymerase chain reactions (PCR). In one method, mycelium was grown in 25 ml of liquid medium (2% malt extract and 0.2% yeast extract) at room temperature for 2 weeks; DNA extraction followed the method of DeScenzo and Harrington (12). Another method used the Prepman Ultra Kit (Applied Biosystems, Foster City, CA) to extract DNA from mycelium scraped from the surface of plates of MYEA after 1 week of growth at room temperature.

Sequences of the ITS region and 5.8S gene of rDNA were obtained by PCR amplification of genomic DNA of isolates using the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') following the protocol of Harrington et al. (18), with slightly different cycling conditions: initial denaturation at 94°C for 95 s followed by 35 cycles of denaturation (94°C) for 35 s, annealing at 52°C for 60 s, and extension at 72°C for 60 s. Final extension was at 72°C for

TABLE 1. Host, country of origin, and date of collection of *Ceratocystis fimbriata* isolates and herbarium specimens used in phylogenetic analysis and representative GenBank accession numbers for internal transcribed spacer sequences

Isolate and specimen numbers <sup>a</sup>	GenBank accession no.	Host plant	Location	Years of collection
C1859-1861, C1863-1864, 1865 (= CBS 114713), C1866, C1873-1874, C1877, C2001	AY526286	<i>Colocasia esculenta</i>	Rio de Janeiro State, Brazil	2001-2002
C1875-1876, C1904, 1905 (= CBS 115171), C1907, C1914-1918, C1920-1922, C1925-1926, C1929	AY526288	<i>Colocasia esculenta</i>	São Paulo State, Brazil	2002
C1900 (= CBS 115170), C1902-1903, C1911-1912	AY526287	<i>Colocasia esculenta</i>	Distrito Federal, Brazil	2002
C2005-2006, C2008-2009	AY526290	<i>Colocasia esculenta</i>	Bahia, Brazil	2002
C2030, C2032	AY526289	<i>Colocasia esculenta</i>	Rondônia, Brazil	2003
C1714 (= CBS 115164, BPI 843732), C1715 (= CBS 114720, BPI 843733)	AY526306, AY526307	<i>Colocasia esculenta</i>	Hawaii, USA	1991, 1998
BPI 596161, BPI 596162	AY526304, AY526305	<i>Colocasia esculenta</i>	Imported from China	1949
C1817 (= CBS 114718)	AY526298	<i>Xanthosoma</i> sp. or <i>Colocasia esculenta</i>	Cuba	2001
C1641	AY157963	<i>Xanthosoma</i> sp.	Costa Rica	2000
C1780 (= CBS 115165)	AY526297	<i>X. sagittifolium</i>	Costa Rica	2001
C2043-2048	AY526296	<i>Xanthosoma</i> sp.	Puerto Rico	2003
BPI 595881, BPI 596216, BPI 596217, BPI 596222	AY526301, AY526300, AY526303, AY526302	<i>Xanthosoma</i> sp.	Dominican Republic	1935, 1963, 1963, 1970
BPI 595396	AY526299	<i>X. bataviense</i>	Cuba	1930
C1931 (= DAR 58902; BPI 843739)	AY526308	<i>X. sagittifolium</i>	Fiji	Unknown
BPI 595880	AY526293	<i>Syngonium</i> sp.	Brazil	1985
C1717 (= CBS 114719)	AY526294	<i>Syngonium</i> sp.	Hawaii, USA	Unknown
C2119 (= DAR 59740a)		<i>Syngonium</i> sp.	Australia	1987
C1774, C1781, C1809 (= CBS 115167)	AY526295	<i>S. podophyllum</i>	Florida, USA	2001
C1558 (= CBS 115175)	AY157965	<i>Mangifera indica</i>	Rio de Janeiro State, Brazil	1999
C1688 (= CBS 114721)	AY526291	<i>M. indica</i>	São Paulo State, Brazil	2000
C1782 (= CBS 115166)	AY526292	<i>Ficus carica</i>	São Paulo State, Brazil	2001
C925 (= CBS 115173)	AY157967	<i>Gmelina arborea</i>	Pará, Brazil	1996
C1345	AY157966	<i>Eucalyptus</i> sp.	Bahia, Brazil	1998
C996 (= C948, CBS 146.53, ATCC 14503)	AY157959	<i>Coffea arabica</i>	Suriname	1953
C1317 (= CBS 115162)	AY157958	<i>Platanus occidentalis</i>	North Carolina, USA	1998
C1548 (= CBS 114722, BPI 843730)	AY157952	<i>Theobroma cacao</i>	Costa Rica	1999

<sup>a</sup> Isolate numbers are from the collection of T. C. Harrington, Iowa State University, and equivalent numbers in other collections are given in parentheses (ATCC = American Type Culture Collection, CBS = Centraalbureau voor Schimmelcultures; DAR = Agricultural Institute, New South Wales, Australia). Specimens with BPI numbers are in the U.S. National Fungus Collections.

15 min. Complementary sequences of the PCR product were obtained with the same primers at the Iowa State University DNA Sequencing and Synthesis Facility.

Template DNA was extracted from herbarium specimens using mycelium or three to four perithecia with ascospores and the Prepman Ultra Kit (Applied Biosystems). Because these specimens frequently included mold spores, primers specific to *C. fimbriata* (Cerat1F = 5'-GCGGAGGGATCATTACTGAG-3' and 1CFL-R = 5'-TGATCCGAGGTCAACCTTGTG-3') were designed and used to amplify the ITS region in 100- $\mu$ l reactions. Cycling conditions were the same as described previously. The PCR products from three to four reactions of each specimen were concentrated and combined using MicroCon filters (Millipore, Billerica, MA). The products were run on a 2% agarose gel, and bands of the appropriate size (600 kb) were cut out, purified with a Gene Clean Kit (Qbiogene, Carlsbad, CA), and inserted into PGEMT-Easy Vector (Promega, Madison, WI). Transformed plasmids were inserted into competent cells, clones were grown, and plasmids were purified using the Qiagen Kit (Valencia, CA). Sizes of the insertions were checked after restriction with *Eco*RI (Invitrogen, Carlsbad, CA), and those of the correct size were sequenced using primers of the vector sequence.

**Phylogenetic analysis.** In addition to the sequences of isolates and herbarium specimens from the family Araceae, representative sequences of isolates of *C. fimbriata* from the Latin American clade (3) were used in the analyses. Among more than 280 ITS sequences currently available, we selected sequences most similar to those of Araceae isolates. Isolates from Hawaii and Fiji and dried specimens from China had unique ITS sequences that matched most closely those of the recently described species *C. pirilliformis* (5), and these sequences (accession nos. AF427104, AF427105, and AF427106) were added to the data set. *C. albofundus* isolate C1048 from *Acacia mearnsii* in South Africa was used as the outgroup taxon. There were 75 ITS sequences in the data set. Sequences were aligned manually by adding gaps, and the data set was analyzed with phylogenetic analysis using parsimony (PAUP) version 4.0b10 (35).

As has been found earlier in ITS analyses of the *C. fimbriata* complex (3), there were numerous large insertion-deletions (indel) in the aligned data set, and these indel regions could not be unambiguously aligned. Of the 712 total characters in the ITS/5.8S data set, including gaps, 215 were ambiguously aligned and excluded from the analysis, 97 remaining sites were variable, and of these, 59 were parsimony-informative. After deletion of ambiguously aligned characters, all gaps were only a single base pair (bp). Thus, gaps were treated as a fifth character without fear of biasing the data analysis. A maximum parsimony heuristic search was performed with all characters having equal weight. Stepwise addition was used to obtain starting trees, and tree-bisection-reconnection was used. Bootstrap analysis with 1,000 replications of heuristic searches was used to assess support for the internal branches.

**Pathogenicity tests. Host plants.** Representative isolates of the *C. fimbriata* lineages from the family Araceae (identified by ITS sequences) were inoculated into *Caladium bicolor* cv. Pink Beauty, *S. podophyllum* (believed to be cv. White Butterfly), and *Colocasia esculenta*. Brazilian isolates of *C. fimbriata* from the dicot hosts *M. indica* (mango) and *F. carica* (edible fig) were also inoculated into the three hosts.

The *Caladium bicolor* plants were grown from bulbs that were purchased from Caladium Bulb Company (Lakeland, FL), and the *Colocasia esculenta* plants were grown from corms that were purchased from various grocery stores in Ames and Des Moines, IA. The bulbs and corms were placed directly into 4- to 6-in. pots, and the plants were allowed to grow for 3 to 4 months and 1 to 2 months, respectively, before inoculation. The *S. podophyllum* plants were vegetatively propagated from a single houseplant, placed into 4-in. pots, and grown for 7 months before inoculation.

All plants were grown in pasteurized potting mix (25% peat, 25% soil, and 50% perlite) in a greenhouse in full sun, with a maximum daily temperature of approximately 25°C. The plants were adequately watered and fertilized (25% Peter's Excel 21-5-20, 75% Peter's Cal-Mag Special 15-5-15) weekly.

**Inoculations.** Seven days prior to inoculation, plants were placed in a growth chamber set at 25°C with a 16/8 h light/dark cycle with illumination of approximately 95  $\mu$ mol cm<sup>-2</sup> s<sup>-1</sup> of photosynthetically active radiation. Plants were well-watered in the growth chamber prior to and during inoculation experiments.

Self-fertile, single-ascospore progeny of each isolate were transferred onto MYEA and grown at room temperature for 7 days. Inoculum was prepared by flooding each culture with 10 ml of sterile deionized water (SDW), scraping the mycelium with a sterile spatula, and filtering the suspension through four layers of sterile cheesecloth, which was rinsed with an additional 5 ml of SDW. Spore concentration was estimated with a hemacytometer, diluted to 2.0  $\times$  10<sup>5</sup> spores per ml, and loaded into sterile syringes (needle gauge 21). Spore suspensions consisted almost entirely of cylindrical endoconidia, although there were some ascospores and hyphal fragments. Controls were prepared following the same technique using a sterile MYEA plate.

For each of the three host species, pseudopetioles of the youngest, fully expanded leaf were wounded by punching a hole through the pseudopetiole about 3 cm above the soil line with the syringe, and approximately 0.2 ml of spore suspension was injected. The inoculation site was wrapped with Parafilm. The inoculated leaves were monitored daily for death or wilting. After 25 to 31 days, each pseudopetiole was sliced open vertically above and below the point of inoculation, and the length of xylem discoloration was measured. To reisolate the fungus, discolored tissue was placed between two carrot disks and incubated under humid conditions at room temperature. After 7 to 14 days, the carrot disks were observed for growth of *C. fimbriata*.

**Statistical analyses.** Two experiments with each host were performed with four replications (for the *Caladium* experiments, the first *Colocasia* experiment, and the control treatment in the second experiment) or five replications of each treatment in a completely randomized design. Only one host was in the growth chamber in each experiment. Data on the length of xylem discoloration and days until death (dead pseudopetioles per leaves) were analyzed using a multifactorial analysis of variance (ANOVA) including isolate, source (host) of inoculum, and experiment. Data from the two experiments on each host were combined if there was homogeneity of variances, otherwise the data from the two experiments were analyzed separately. When ANOVA indicated significant variation among isolates ( $P \leq 0.05$ ), Fisher's protected least significant difference test was used to make pairwise mean comparisons among the treatments. Percent mortality and reisolation success were compared between each isolate and the control using Fisher's exact test adjusted for multiple comparisons by the Bonferroni method. All statistics were performed using SAS statistical software (SAS Institute, Cary, NC).

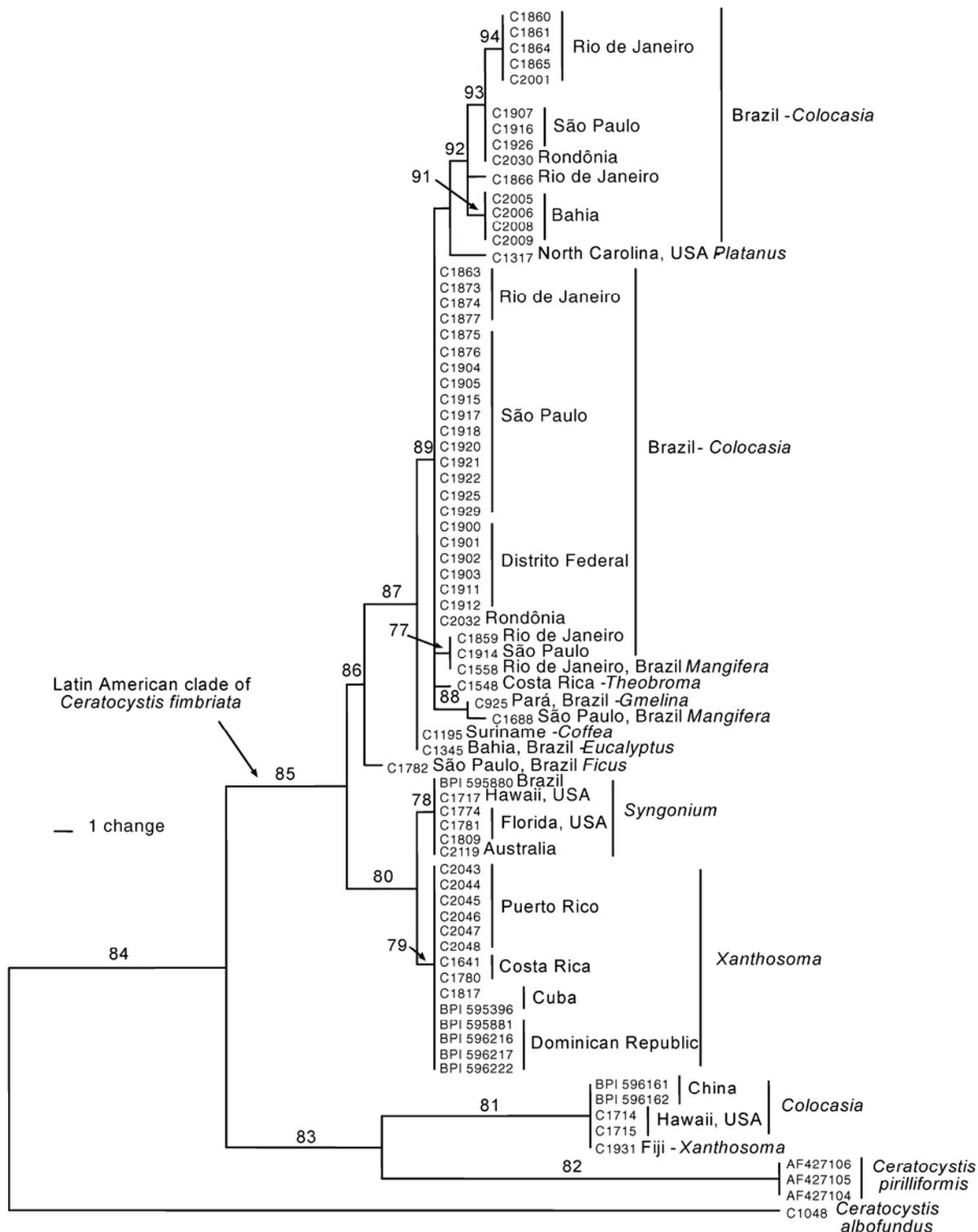
## RESULTS

**ITS sequence analysis.** There was substantial variation in ITS sequences among the isolates and herbarium specimens from the family Araceae. There were six ITS sequences (genotypes) identified among the 49 Brazilian isolates from *Colocasia* found in markets, distribution centers, and grocery stores in four states and the Distrito Federal. Three of these genotypes were found in São Paulo, the main production area for inhamé in Brazil and where most of the diseased corms are believed to have been infected (20). The *Syngonium* isolates from Florida, Hawaii, and Australia had an ITS/5.8S sequence of 528 bp that was identical to that of the *Syngonium* specimen from Brazil. The nine *Xanthosoma*

isolates from Costa Rica, Cuba, and Puerto Rico had identical ITS/5.8S sequences of 527 bp, and this sequence was identical to that of the five herbarium samples from *Xanthosoma* collected in Cuba and the Dominican Republic. *Colocasia esculenta* isolates from Hawaii, *Colocasia esculenta* specimens from China, and the *Xanthosoma* isolate from Fiji had a distinct ITS/5.8S sequence of 565 bp that differed greatly from other isolates of *C. fimbriata*.

Two most-parsimonious trees of 133 steps were derived from the 59 parsimony-informative characters from the total aligned

data set. As seen in Figure 1, the ITS sequences of *Colocasia* isolates from Brazil were found among the sequences of various South American isolates from dicot hosts representing the Latin American clade of the *C. fimbriata* complex (3,17). Included in this moderately supported clade (bootstrap value = 86) of South American isolates was a North Carolina isolate of the *Platanus* pathogen, recently recognized as *C. platani*, and the *T. cacao* pathogen *C. cacaofunesta* (13). Sister to the South American group of isolates were isolates from *Xanthosoma* and *Syngonium*.



**Fig. 1.** One of two most-parsimonious trees of 133 steps using rDNA-internal transcribed spacer sequences of *Ceratocystis fimbriata* isolates and herbarium specimens and *C. pirilliformis*. *C. albofundus* was used as the outgroup taxon. The consistency index, retention index, and rescaled consistency index were 0.827, 0.952, and 0.787, respectively. Bootstrap values greater than 50 are shown above the branch.

The sequences of the *Syngonium* and *Xanthosoma* genotypes differed by only 2 bp, and these genotypes grouped together in a clade with a moderate bootstrap support of 80. The ITS sequence of the Asian and Pacific isolates and specimens from *Colocasia* and *Xanthosoma* was most similar to that of *C. pirilliformis* (Fig. 1). In the second most-parsimonious tree, BPI 596162 differed from the other Asian *Colocasia* isolates by a single base substitution.

**Pathogenicity tests.** *Caladium bicolor*. In both experiments there was substantial, natural senescence of both inoculated and control pseudopetioles of *Caladium* during the course of the experiments. Half of the pseudopetioles inoculated with the water control died (Table 2). Counting dead pseudopetioles only, days to mortality in the two experiments varied significantly by isolate ( $F = 3.35$ ;  $P = 0.0011$ ) and experiment ( $F = 5.79$ ;  $P = 0.019$ ), and there was significant isolate–experiment interaction ( $F = 2.67$ ;  $P = 0.007$ ). There was no difference in the time (in days) to mortality (of the pseudopetiole and leaf) between the inoculated and the control plants in the first experiment, but in the second experiment, pseudopetioles inoculated with all *C. fimbriata* isolates except the *Mangifera* isolate died sooner than those inoculated with water only.

There was homogeneity of the variances in the two experiments for length of xylem discoloration, and the means shown in Table 2 are for the combined data of the two experiments. All isolates from the family Araceae caused more xylem discoloration than the controls, but the *Mangifera* and *Ficus* isolates caused no more discoloration than the controls (Table 2). With all the isolates, the pathogen was reisolated from most of the inoculated plants, but the fungus was not recovered from the control plants.

*S. podophyllum*. The two isolates from *Syngonium* and the related *Xanthosoma* isolates from Costa Rica and Cuba killed all inoculated pseudopetioles of *Syngonium* (Table 2). Two of the control pseudopetioles also died, and one of the *Colocasia* isolates from Hawaii killed 90% of the inoculated pseudopetioles. Only the pseudopetioles inoculated with the *Syngonium* and *Xanthosoma* isolates and *Colocasia* isolate C1900 died sooner than the control pseudopetioles.

The ANOVA indicated that there was significant variation in the length of xylem discoloration among the isolates inoculated into the *Syngonium* plants ( $F = 8.90$ ;  $P < 0.0001$ ). All isolates except C1865 from *Colocasia* in Brazil and the *Mangifera* isolate caused

more xylem discoloration than the controls. The *Syngonium* and *Xanthosoma* isolates caused significantly more discoloration in the pseudopetioles of *Syngonium* than all other isolates and the control, except for the *Ficus* isolate. The fungus was reisolated from 100% of the plants inoculated with isolates C1809, C1780, and C1688.

*Colocasia esculenta*. Two of eight control pseudopetioles died, and *C. fimbriata* was recovered from each of these pseudopetioles, though there was little discoloration in the xylem of either plant. Thus, there was cross-contamination, but *C. fimbriata* may have colonized the pseudopetiole of the control plants after the pseudopetiole died. All Araceae isolates, except C1717, caused 100% mortality of inoculated pseudopetioles of *Colocasia*, significantly greater than that of the *Ficus* and *Mangifera* isolates and the controls (Table 2). Days until death and percent reisolation from *Colocasia* (100% for all isolates) did not differ among treatments.

Xylem discoloration in inoculated *Colocasia* plants varied significantly by isolate ( $F = 7.34$ ;  $P < 0.0001$ ), experiment ( $F = 46.29$ ;  $P < 0.0001$ ), and isolate–experiment interaction ( $F = 2.5$ ;  $P = 0.009$ ). The variances of the two experiments were not homogeneous, so the two experiments were analyzed separately. In the first experiment, the three Brazilian isolates from *Colocasia*, one Hawaiian isolate from *Colocasia*, and one *Xanthosoma* isolate caused more discoloration than the controls. In the second experiment, all isolates from the corms of *Colocasia* or *Xanthosoma* caused xylem discoloration greater than that found in the control inoculations, but isolates from *Syngonium*, *Mangifera*, and *Ficus* caused no more discoloration than the controls.

## DISCUSSION

Analysis of ITS sequences from isolates and herbarium specimens up to 70 years old suggest that *C. fimbriata* genotypes from the family Araceae represent several cryptic species that have been moved around the world by humans. All isolates and specimens from *Xanthosoma* species in the Caribbean region had the identical ITS sequence (the *Xanthosoma* genotype), the *Syngonium* isolates and specimen had an identical ITS sequence (the *Syngonium* genotype) that differed by only 2 bp from the *Xanthosoma* genotype, and the *Colocasia* specimens from China had the same ITS sequence (the taro genotype) as Hawaiian

TABLE 2. Percent mortality, days from inoculation to death of pseudopetiole, and average length of xylem discoloration in family Araceae plants inoculated with isolates of *Ceratocystis fimbriata*

Source of isolate	Location	Isolate number	<i>Caladium bicolor</i>				<i>Syngonium podophyllum</i>			<i>Colocasia esculenta</i>			
			% Mortality <sup>w</sup>	Days to death <sup>xy</sup>		Xylem discoloration (cm) <sup>z</sup>	% Mortality <sup>w</sup>	Days to death <sup>xz</sup>	Xylem discoloration (cm) <sup>z</sup>	% Mortality <sup>w</sup>	Days to death <sup>xz</sup>	Xylem discoloration (cm) <sup>y</sup>	
				Exp. 1 <sup>z</sup>	Exp. 2 <sup>z</sup>							Exp. 1 <sup>z</sup>	Exp. 2 <sup>z</sup>
<i>Syngonium</i>	Hawaii	C1717	100	8.8	7.5 ab	1.8 a	100**	16.6 ab	3.4 a	55.6	21.6 c	1.7 cde	1.6 c
	Florida	C1809	100	10.0	8.0 ab	1.5 a	100**	16.2 ab	3.1 a	100*	19.1 c	2.3 bcd	3.5 bc
<i>Xanthosoma</i>	Costa Rica	C1780	100	10.0	7.5 ab	1.8 a	100**	15.0 a	3.3 a	100*	13.8 ab	2.5 bc	9.3 a
		C1817	100	9.3	9.0 ab	1.9 a	100**	15.0 a	3.0 ab	100*	12.3 ab	2.0 cde	6.1 ab
<i>Colocasia</i>	Brazil	C1865	100	13.5	9.3 ab	1.3 ab	40	19.8 bcd	1.3 ef	100*	15.0 b	2.5 bc	6.2 ab
		C1900	100	11.8	9.0 ab	1.6 a	30	17.0 abc	1.9 cde	100*	12.6 ab	4.3 a	7.8 a
		C1905	100	9.8	9.3 ab	1.4 a	50	19.8 bcd	2.1 cd	100*	12.9 ab	3.6 ab	7.3 a
	Hawaii	C1714	100	10.0	6.5 a	1.6 a	90	19.8 cd	2.1 cd	100*	10.0 a	2.3 bcd	7.0 ab
		C1715	100	10.0	8.0 ab	1.3 a	20	22.5 cd	1.8 cde	100*	11.2 ab	2.4 bc	7.4 a
<i>Mangifera</i>	Brazil	C1688	75	14.5	9.8 bc	0.5 c	10	22.0 cd	1.5 def	22.2	25.0 cd	0.7 e	2.0 c
<i>Ficus</i>	Brazil	C1782	75	15.3	6.0 a	0.6 bc	10	17.0 abcd	2.3 bc	22.2	29.5 d	1.1 cde	1.4 c
Control			50	12.0	25.0 c	0.3 c	20	25.0 d	0.7 f	25.0	18.0 bc	0.8 de	0.5 c

<sup>w</sup> Values of percent mortality (of pseudopetioles) within a column followed by one or two asterisks are significantly different ( $P < 0.05$  and  $0.01$ , respectively) from the control according to Fisher's exact test (Bonferroni adjusted  $\alpha = 0.0045$  and  $0.00091$ , respectively). No isolate caused significantly more mortality than the control in the *Caladium* experiment.

<sup>x</sup> Days to death include only those plants that died before the end of the experiment, which was 25 days for the *Caladium* experiments, 28 days for the *Syngonium* experiments, 31 days for the first *Colocasia* experiment, and 28 days for the second *Colocasia* experiment.

<sup>y</sup> There was no homogeneity of variances for the first and second experiments, so the data for the two experiments were analyzed separately.

<sup>z</sup> Numbers within a column followed by the same letter are not significantly different ( $P < 0.05$ ) based on Fisher's protected least significant difference. Analysis of variance indicated that there was no significant variation among the isolates in the days until death in the first *Caladium* experiment.

*Colocasia* isolates and the *Xanthosoma* isolate from Fiji. In contrast, there was some limited variability in ITS sequences of *Colocasia* isolates from Brazil (the inhome genotypes).

Isolates from the family Araceae appear to be uniquely pathogenic to aroids. In our pathogenicity tests, the *Mangifera* and *Ficus* isolates, in contrast to Araceae isolates, caused no more xylem discoloration than the controls in inoculated *Caladium* or *Colocasia* pseudopetioles, and there was only slight discoloration in *Syngonium* plants inoculated with the *Ficus* isolate. Baker et al. (3) found that isolates from *M. indica*, *Eucalyptus* sp., *T. cacao*, *I. batatas*, *Platanus occidentalis*, *Coffea arabica*, and *G. arborea* from the United States and Latin America were not pathogenic to *Colocasia esculenta*, and Costa Rican isolates from *Coffea arabica* and *T. cacao* were not pathogenic to *X. sagittifolium*. In contrast, our *Xanthosoma* isolate from Costa Rica was not pathogenic to *I. batatas*, *Platanus occidentalis*, *Coffea arabica*, or *T. cacao* (3). A *C. fimbriata* isolate from *Syngonium* in Australia failed to infect *I. batatas*, *Platanus orientalis*, or *Prunus* spp. but was pathogenic to various cultivars of *Syngonium* sp., *Caladium* sp., *Alocasia* sp., and *X. lindenii* (39). Japanese isolates from *I. batatas* were pathogenic to *I. batatas* plants but not to *Colocasia esculenta*, and the reverse was true for Japanese *Colocasia esculenta* isolates (24,25,27,29).

There was no significant difference among Araceae isolates in days until death or the length of xylem discoloration in *Caladium* sp., which had been shown to be susceptible to a *Syngonium* isolate of *C. fimbriata* in an earlier study (39). However, natural infection of this common ornamental plant has not been reported. We found that *Syngonium* plants were most severely affected by the two *Syngonium* isolates and the related *Xanthosoma* isolates from Cuba and Costa Rica. The two *Syngonium* isolates appeared to be less aggressive than the other Araceae isolates on *Colocasia esculenta*. However, the *Syngonium* isolate from Hawaii that was not pathogenic to *Colocasia esculenta* is very old, and it was earlier shown to be pathogenic to this host (38). Our Costa Rican isolate from *Xanthosoma* was pathogenic to *X. sagittifolium* and *Colocasia esculenta*, and *X. sagittifolium* appeared to be more susceptible than *Colocasia esculenta* (3).

Much of the ITS sequence data had to be eliminated from analysis because of ambiguous alignment (3), and there are other limitations for using rDNA sequences for inferring evolutionary history (2). Nonetheless, ITS analyses, incompatibility testing, and pathogenicity tests suggest that the isolates and specimens from the family Araceae represent at least three cryptic species in the *C. fimbriata* complex. *Colocasia* specimens from China, the *Colocasia* isolates from Hawaii, and the *Xanthosoma* isolate from Fiji had the identical ITS sequence, which was distinct from the sequences of other aroid isolates. Analyses of isozyme electromorphs and *MAT-2* sequences place this taro genotype in the Asian clade of the *C. fimbriata* complex (17,23,32), and our ITS sequence analysis groups the taro pathogen with the recently described species *C. pirilliformis*, which was collected from wounds on *Eucalyptus nitens* in Australia (5). The taro pathogen also has minor morphological characters that distinguish it from *C. fimbriata sensu stricto*, the *Ipomoea* pathogen, which is in the Latin American clade (13,32).

The other aroid isolates and specimens fell into the Latin American clade based on ITS analysis (2,17). *Xanthosoma* isolates from the Caribbean region and the *Syngonium* specimen and isolates formed a moderately supported subclade that was sister to the rest of the Latin American clade, including isolates from dicots and Brazilian isolates from *Colocasia*. Our ongoing multiple gene phylogenies (*unpublished data*) also separate the Caribbean *Xanthosoma/Syngonium* isolates from Brazilian *Colocasia* isolates and other South American isolates. Isolates of the *Xanthosoma/Syngonium* subclade are sexually compatible with each other but not with Brazilian *Colocasia* isolates in interfertility tests (*unpublished data*). The *Colocasia* isolates from

Brazil had ITS sequences similar to each other, but they did not form a monophyletic group. The related *Ficus* and *Mangifera* isolates from Brazil were weakly pathogenic to *Colocasia* in the current study. Brazilian isolates from *M. indica*, *F. carica*, *Eucalyptus* spp., and *G. arborea* were at least weakly pathogenic to *Colocasia esculenta* in other inoculation experiments and were interfertile with Brazilian *Colocasia* isolates in laboratory pairings (36). More work will be done on the host range and relatedness of Brazilian isolates of *C. fimbriata*, but sexual compatibility and variability in ITS sequences and aggressiveness to various exotic hosts suggest that the Brazilian isolates are representatives of single species native to Brazil (36).

It has been hypothesized that natural populations of *C. fimbriata* tend to have restricted geographic ranges, populations vary in their aggressiveness to various hosts, and some isolated populations of *C. fimbriata* have become specialized to native hosts (3). Host specialization may be facilitated by propagation and cultivation of certain hosts, which may select for genotypes of *C. fimbriata* that are particularly aggressive to that host. Natural populations of *C. fimbriata* tend to be geographically restricted and have limited genetic variation (3,13,14,17) because the fungus is insect dispersed and not wind dispersed (21,22,31). The primary insect vectors (Nitidulidae: Coleoptera) are fungal feeding insects that are not associated with particular plants. Successful establishment of a host-specialized strain of *C. fimbriata* on a new island or continent would require that the fungus survive as spores on the insect during the flight, and the insect would then have to visit a fresh wound on a susceptible host, an extremely unlikely event. In contrast, host-specialized strains of *C. fimbriata* are easily dispersed by humans on diseased propagative material or in wood (3,13,14,17).

Dispersal of Araceae strains of *C. fimbriata* by humans is likely to have occurred on cuttings or corms. The edible aroids are typically propagated from corms, and the rot caused by *C. fimbriata* is often superficial and generally does not destroy the entire corm. More isolates of the Asian taro pathogen are needed, but it could be speculated that the taro ITS genotype had a limited geographic range before human cultivation of its host, which is believed to be native to eastern or southern Asia and has been cultivated in southern China for millennia (7,9). *Colocasia esculenta* was widely dispersed by humans throughout the Pacific before European exploration (28), and the taro genotype of *C. fimbriata* could have been carried on black-rotted corms to Fiji, the Samoan Islands, and Hawaii. The Hawaiian Islands are particularly remote and did not have taro until colonization by Polynesians. One of our taro isolates (C1715) was from a long-abandoned paddy of an ancient, noncommercial cultivar of taro that was used by native Hawaiians. This paddy was in a roadless area in the Nualolokai Valley on the island of Kauai, far from known cultivation of taro in recent times.

The species of *Xanthosoma* that are cultivated for their edible corms and foliage may have originated from northern South America and were carried to various Caribbean Islands and Central America by Amerindians before the arrival of Columbus (8,16). Identical ITS sequences were found for the *Xanthosoma* isolates from Costa Rica, Cuba, and Puerto Rico and for the herbarium specimens from Cuba and the Dominican Republic, suggesting that the Caribbean population on cultivated *Xanthosoma* plants has gone through a genetic bottleneck. We have twice collected the *Xanthosoma* genotype of *C. fimbriata* from corms in small markets in the northern (Caribbean) region of Costa Rica, where Amerindians were cultivating *Xanthosoma* before the arrival of Europeans (16).

The *Syngonium* isolates may represent a single genotype of the *Xanthosoma/Syngonium* pathogen that has been dispersed on vegetatively propagated *Syngonium* plants. It was earlier suggested (39,40) that the *Syngonium* pathogen was introduced to Australia on vegetatively propagated material, and the Australian

isolate we studied had the same ITS sequence as *Syngonium* isolates from Florida and Hawaii and the specimen from Brazil. Some of the commonly used cultivars of *Syngonium* are native to the Caribbean and Central America (10), and the *Syngonium* genotype of *C. fimbriata* may have originated from a naturally infected wild plant used for cultivation.

Ribosomal DNA sequences proved highly valuable in identifying genotypes of *C. fimbriata*. There are limitations in using rDNA sequences for phylogenetic analyses, and rDNA gene trees do not always reflect species trees (2), but rDNA sequences are easily generated because of the universal PCR primers for amplifying these regions and the high copy number of nearly identical tandem repeats. This high copy number allows for the amplification of rDNA regions from poor quality template DNA, such as that extracted from small quantities of herbarium material more than 70 years old. Taxa-specific primers were needed to preferentially amplify from the genomic DNA of the target organism versus the genomic DNA of molds, mycoparasites, and other eukaryotic organisms. With many of our herbarium samples, PCR had to be attempted several times, PCR products from several reactions had to be combined, and cloning of the PCR fragments was necessary to obtain quality DNA sequences. The fidelity of those sequences was evident in that the sequences generated from herbarium specimens collected from *Xanthosoma*, *Syngonium*, and *Colocasia* isolates matched precisely the sequences of isolates from these respective hosts. Herbarium specimens appear to be an underutilized resource in the phylogenetics, species delimitation, and historical tracking of genotypes of fungal plant pathogens.

The monocotyledonous genera *Colocasia*, *Syngonium*, *Xanthosoma*, and *Alocasia* are unique among the 28 genera known to be susceptible to *C. fimbriata* (6), and isolates of *C. fimbriata* from dicotyledonous hosts are not strongly pathogenic to the family Araceae. We speculate that at least three host-specialized populations of *C. fimbriata* from Asia and the Caribbean region have been moved by humans to new continents and islands on infected corms of *Colocasia* and *Xanthosoma* plants and in vegetatively propagated *Syngonium* plants. We are conducting further studies with Brazilian populations of *C. fimbriata* on *Colocasia esculenta* (20) and other hosts, but the inhame pathogen appears to be indigenous to Brazil, perhaps to the São Paulo region, and it may not be restricted to the family Araceae (36).

## ACKNOWLEDGMENTS

This research was supported by the National Science Foundation through grants DEB-987065 and DEB-0128104. We thank C. J. B. Engelbrecht and J. Steimel for generating some of the DNA sequences; T. Hurt (University of Florida) and M. Priest (Agricultural Institute, New South Wales, Australia) for assistance in obtaining isolates; and the U.S. National Fungus Collection for providing access to herbarium specimens and cooperation.

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