

# Transcontinental Dispersal of Nonendemic Fungal Pathogens through Wooden Handicraft Imports

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ABSTRACT This study examined the viability and diversity of fungi harbored in imported wooden handicraft products sold in six retail stores in Florida, United States. Despite being subjected to trade regulations that require various sterilization/fumigation protocols, our study demonstrates high survival and diversity of fungi in wood products originating from at least seven countries on three continents. Among these fungi were nonendemic plant and human pathogens, as well as mycotoxin producers. Several products that are sold for use in food preparation and consumption harbored a novel (to North America) plant and human pathogen, Paecilomyces formosus. In addition, a high number of species isolated were thermophilic and included halophilic species, suggesting adaptability and selection through current wood treatment protocols that utilize heat and/or fumigation with methyl-bromide. This research suggests that current federal guidelines for imports of wooden goods are not sufficient to avoid the transit of potential live pathogens and demonstrates the need to increase safeguards at both points of origin and entry for biosecurity against introduction from invasive fungal species in wood products. Future import regulations should consider living fungi, their tolerance to extreme conditions, and their potential survival in solid substrates. Mitigation efforts may require additional steps such as more stringent fumigation and/or sterilization strategies and limiting use of wood that has not been processed to remove bark and decay.

**IMPORTANCE** This study, the first of its kind, demonstrates the risk of importation of nonendemic foreign fungi on wooden handicrafts into the United States despite the application of sanitation protocols. Previous risk assessments of imported wood products have focused on potential for introduction of invasive arthropods (and their fungal symbionts) or have focused on other classes of wood products (timber, wooden furniture, garden products, etc.). Little to no attention has been paid to wooden handicrafts and the fungal pathogens (of plants and humans) they may carry. Due to the large size and diversity of this market, the risk for introduction of potentially dangerous pathogens is significant as illustrated by the results of this study.

**KEYWORDS** environmental microbiology, fungal pathogens, invasive microorganisms, pathogens, plant pathogens

nternational movement of microorganisms has garnered broad attention in recent years, primarily due to the threat from pathogens to the health of naive hosts (1–6). Fungal pathogens are increasingly recognized as a major risk and threaten agricultural and ecological systems worldwide (1, 3, 4, 6–9). The recent global emergence of several mycotic diseases is leading many public health experts to raise alarms about the need to address this new threat with more resources and research (1, 3–5, 8, 10–13). One important issue is uncertainty about sources of new strains that become established in vulnerable populations or in clinical settings from presumably natural/environmental inoculum reservoirs (1, 10–21). For example, fungicide-resistant strains are emerging in several species, including *Candida auris* (8), and *Aspergillus fumigatus* (22, 23), presumably

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Exotic fungal pathogens present a particular risk due to their rapid emergence, low resistance in host populations, and limited surveillance infrastructure for detection. Invasive fungal pathogens are linked to major ecological disasters, including near extinctions of forest tree species, such as the American chestnut due to *Cryphonectria parasitica* (24) and Florida torreya due to *Fusarium torreyae* (25). They have also caused a global decline of amphibian populations due to chytridiomycosis caused by *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* (2, 26). Furthermore, numerous fungi present serious threats to food security, such as wheat stem rust caused by *Puccinia graminis* race UG 99 (4, 5).

Increasing international trade, higher capacity of shipping vessels, greater reliance on nonlocal agriculture, and the movement of horticultural plants have all been identified as major drivers of this worldwide phenomenon (3, 4, 6). Although safeguards and regulations exist to prevent inadvertent introduction of microbes and insects in wood products (6), these precautions are only as good as the enforcement mechanisms in place at the origin of export and biosecurity measures at ports of entry. Analyses of the system have revealed novel pathways recently (6) with significant focus on exchange of numerous wood and plant-based products (6). Wood products have long been a risk for importation of new invasive pests and pathogens in relation to agriculture and forestry. Most risk assessments (and regulations) related to wood products concern timber and wood-boring insect pests that they may harbor (6). Indeed, the impact from these pests has been staggering and has led to widespread attention (1, 4, 6, 24). With the exception of fungi carried by bark and ambrosia beetles harbored in wood, little attention has been given to the broader risk from fungi associated with the diverse array of wooden products that are imported into the United States through trade.

It was observed by the authors that many of the wooden home goods products (regulated by U.S. Department of Agriculture/Animal and Plant Health Inspection Service [USDA/APHIS] as "wooden handicrafts") commonly sold at retail stores have foreign export origins, including many Asian countries, Europe, Mexico, and Central America. Furthermore, it was also observed that these products are not being produced from milled/processed wood but often made from weathered, decayed wood that contains bark (Fig. 1) or evidence of discoloration. For example, in the arts and crafts market, raw "driftwood," pinecones and straw from foreign destinations is being sold (Fig. 2) and marketed in U.S. retail stores specifically due to their rustic, "straight from nature" appeal (Fig. 3). In some cases, evidence of microbial activity was observed on products, indicating potential reservoirs for organisms during importation (Fig. 3).

The current regulations from USDA/APHIS (27), encourage importers to engage with one of the 1,112 export firms in China (as of August 2021) that have been deemed to have met the APHIS standards. In short, this includes approved biosecurity facility, manufacturing process, and shipping requirements. If an importer desires to purchase products from an exporter not on the approved list, they will need to meet certain requirements to be added to the list, which include proper treatment of all wood craft materials larger than 1 cm in diameter, semifinished or finished with any wooden components that have not been machined. The treatments include heat (wood core temperature minimum of 60°C for 60 min), methyl bromide "for components with a diameter less than 15.24 cm/6 in. and larger than 1 cm in order to ensure the fumigant gets proper penetration," or pressure treatment (varies by weight) (27). Apparently, products containing bark or rough portions are still subject to inspection specifically for "wood-boring insects," but no mention is made of microorganisms.

For wooden handicrafts from destinations outside China, importers are directed to the general e-permits website (https://www.aphis.usda.gov/aphis/resources/permits), but no specific information is provided. Handicrafts from countries other than China do not have a specific regulatory control. According to APHIS (27), the material can be inspected for pests and then released as outlined in 7 CFR 319.40-9. If pests are mBio



**FIG 1** Three examples of wooden handicrafts tested in this study. (A) Wooden cutting board used in food preparation from China, with extensive white-pocket rot decay evident. (B) Bowl from Philippines used with bark present. (C) Bowl from Indonesia displaying decay and zone lines indicative of advanced fungal decay and colonization.

detected during inspection, then quarantine actions will be taken. Thus, these materials are not subject to the requirements outlined above.

The following research was conducted to assess the potential risk of intercontinental importation of exotic fungal pathogens on wooden handicrafts purchased from six retail stores in Gainesville, FL. Two studies were conducted, with study 1 being carried out as a pilot study and included undergraduate students enrolled in a lab-based course and study 2 was a larger repetition of study 1.



**FIG 2** Samples following incubation in moisture chambers at 22°C. (Left) Wooden bowl from Philippines with immature fruiting bodies of *Schizophyllum commune*. (Right) Pinecone from Italy with multiple fungal fruiting bodies present.





**FIG 3** Decorative, mixed-materials bowl made of driftwood and coconut fiber sold in a retail store in Gainesville, FL (Origin: Philippines). Note the label in the upper right corner marketing "inviting nature into your home" using "reclaimed wood from the East." Note also the green stain associated with recent fungal growth on wood in lower left panel and the zone lines and discoloration associated with recent decay fungi activity in lower right panel.

### RESULTS

A total of 47 pure fungal colonies were recovered from 14 out of 16 household and wooden handicraft products tested in this study (Table 1). From Study 1, a total of nine separate fungal taxa were recovered from four of the five products tested (Tables 1 and 2). The largest number of taxa, five (*Aspergillus versicolor, Paecilomyces formosus, Penicillium alfredii, Peniophora albobadia,* and *Schizophyllum commune*), were recovered from a wooden bowl that originated in Indonesia. A second bowl tested, from the Philippines, as well as *Larix* sp. cones from China, yielded one fungal taxon each, *S. commune* and *Alternaria alternata*, respectively.

A total of 24 taxa were further recovered from 11 products purchased from four retail stores in study 2 (Tables 1 and 2). Eleven taxa, the largest number recovered from a single product, were obtained from *Pinus* sp. cones imported from Italy (*Aporospora terricola, Chaetomium cochlioides, Coprinellus radians, Dictyoarthrinium sacchari, Fimetariella rabenhorstii, Hormococcus conorum, Periconia macrospinosa, Rhizopus oryzae, Rhizosphaera kal-khoffii, Sarea resinae, and Trichoderma citrinoverde), followed by four taxa from a wooden food serving tray from Thailand (<i>Aspergillus flavus, C radians, R. delemar, and Xylaria badia*). The remainder of the taxa were isolated from grape vines from China sample 1 (*T. longibrachiatum*), grape vines from China sample 2 (*Bipolaris austrostipae, Curvularia lunata, and T. longibrachiatum*), a bread cutting board/serving tray from China (*Paecilomyces variotii*), straw from Mexico (*S. commune*), wooden sticks/driftwood from the Philippines (*Talaromyces rotundus*), and wooden coasters from China (*A. pseudoglaucous, Cladosporium cladosporioides, C. xanthothrix, and Humicola grisea*).

**Functional group assignment.** Of the 43 fungal taxa recovered from the two studies (Tables 1 and 2), the following 14 were determined to be plant pathogens (Fig. 4) (*A. alternata* (28), *A. flavus* (postharvest) (29, 30), *B. austrostipae* (31), *Chaetomium globosum* 

# TABLE 1 Top BLASTn results for ITS rDNA region for fungal isolates recovered from studies 1 and 2<sup>a</sup>

			BLAST statistics		
lsolate code	Top BLASTn result	% identity	Query coverage	E value	Length (bp)
RS1-S1-01 (MN547364)*	Schizophyllum commune (MH307932)	100	100	0	600
RS1-S1-02 (MN547365)*	Schizophyllum commune (MT103532)	100	100	0	600
RS1-S1-03 (MN547366)*	Paecilomyces formosus (LC317710)	100	94	0	590
RS1-S1-04 (MN547367)*	Schizophyllum commune (MF476007)	100	100	0	650
RS1-S1-05 (MN547368)*	Peniophora albobadia (KU530154)	99.77	100	0	661
RS1-S1-06 (MN547369)*	Aspergillus versicolor (MH625700)	100	99	0	595
RS1-S1-07 (MN547370)*	Penicillium alfredii (MK450726)	98	97	0	776
RS1-S1-08 (MN547371)*	Schizophyllum commune (MH307932)	99.85	100	0	674
RS2-S1-01 (MN547372)*	Alternaria alternata (MT453271)	100	100	0	608
RS2-S2-01 (MN547383)	Talaromyces rotundus (EU497950)	99.52	99	0	623
RS3-S2-01 (MN547375)	Aporospora terricola (DQ865098)	96.28	91	0	554
RS3-S2-02 (MN547376)	Rhizosphaera kalkhoffii (JX981459)	100	99.83	0	621
RS3-S2-03 (MN547377)	Rhizosphaera kalkhoffii (JX981459)	100	99.84	0	621
RS3-S2-04 (MN547378)	Periconia macrospinosa (JX981482)	99.83	100	0	587
RS3-S2-05 (MN547380)	Coprinellus radians (MH855978)	100	100	0	631
RS3-S2-06 (MN547385)	Rhizosphaera kalkhoffii (JX981459)	100	100	0	605
RS3-S2-07 (MN547386)	Dictyoarthrinium sacchari (MT482325)	98.83	87	0	515
RS3-S2-08 (MN547387)	Rhizosphaera kalkhoffii (JX981459)	100	100	0	606
RS3-S2-09 (MN547388)	Fimetariella rabenhorstii (KX869958)	99.49	100	0	585
RS3-S2-10 (MN547389)	Chaetomium cochlioides (MT279444)	100	100	0	592
RS3-S2-11 (MN547391)	Sarea resinae (MT809245)	100	100	0	588
RS3-S2-12 (MN547392)	Hormococcus conorum (KF993412)	100	92	0	564
RS3-S2-13 (MN547393)	Hormococcus conorum (KF993412)	100	93	0	564
RS3-S2-14 (MN547394)	Sarea difformis (MH857896)	99.15	100	0	587
RS3-S2-15 (MN547395)	Sarea difformis (MH857896)	99.18	99	0	608
RS3-S2-16 (MN547396)	Sarea difformis (MH857896)	99	100	0	588
RS3-S2-17 (MN547397)	Sarea difformis (MH857896)	99	100	0	587
RS3-S2-18 (MN547398)	Chaetomium globosum (KX674657)	100	99	0	793
RS3-S2-19 (MN547400)	Trichoderma longibrachiatum (MT634694)	100	100	0	450
RS3-S2-20 (MN547401)	Curvularia sp. (MT066189)	99.83	99	0	583
RS3-S2-21 (MN547402)	Curvularia lunata (MT683262)	100	100	0	587
RS3-S2-22 (MN547403)	Trichoderma longibrachiatum (KY225659)	100	99.81	0	533
RS3-S2-23 (MN547404)	Bipolaris austrostipae (NR147491)	99.68	99	0	624
RS3-S2-24 (MN547406)	Trichoderma citrinoverde (MG972800)	100	100	0	630
RS3-S2-25 (MN547407)	Rhizopus oryzae (MT316366)	100	100	0	655
RS3-S2-26 (OM262198)	Schizophyllum commune (MH307932)	99.84	100	0	645
RS3-S2-27 (MN547409)	Paecilomyces variotii (FJ345354)	100	100	0	622
RS3-S2-28 (MN534797)	Paecilomyces variotii (FJ345354)	100	100	0	447
RS4-S2-01 (MN547373)	Aspergillus flavus (CP051065)	100	100	0	636
RS4-S2-02 (MN547374)	Xylaria badia (GU322446)	98.44	95	0	586
RS4-S2-03 (MN547379)	Coprinellus radians (LC612525)	100	100	0	697
RS4-S2-04 (MN547384)	Rhizopus delemar (LC514308)	99.69	100	0	645
RS4-S2-05 (MN547405)	Rhizopus delemar (LC514308)	99.84	99	0	646
RS5-S2-01 (MN547381)	Coprinellus xanthothrix (MK573918)	100	100	0	706
RS5-S2-02 (MN547382)	Humicola grisea (KU705826)	100	99	0	578
RS5-S2-03 (MN547390)	Aspergillus pseudoglaucus (MT316341)	100	100	0	569
RS5-S2-04 (MN547399)	Cladosporium cladosporioides (MT598826)	100	100	0	539

a\*, Samples from study 1. GenBank accession numbers are in parentheses. Isolate codes indicated in boldface were recovered from samples incubated in moist chambers.

(32), *C. cladosporioides* (33), *C. lunata* (34), *Curvularia* sp. (34), *P. formosus* (35, 36), *P. albobadia* (37), *Periconia macrospinosa* (38), *R. oryzae* (39), *R. kalkhoffii* (40), *S. commune* (40), and *X. badia* (decay fungus) (41, 42), with three of these not previously reported in the United States: *B. austrostipae*, *P. formosus*, and *X. badia*. These pathogens represent disease agents of cereal crops, fruits/vegetables, trees, and postharvest pathogens, as well as decayers (29, 33, 40, 42). They also include several major mycotoxin producers, including *A. alternata* (28, 43), *A. flavus* (29, 30), *A. versicolor* (15), and *P. macrospinosa* (44). One saprophytic fungus, *D. sacchari*, has never been reported in the United States before (45; https://www.gbif.org), being found in a wide range of African, Asian, and Caribbean locations.

Retail store	Product description	Country of origin	No. of fungal taxa recovered	No. of samples	Plating method
Study 1					
1	Wooden bowl <sup>a</sup>	Indonesia	5	9	Streaking
	Wooden coaster	India	0	1	Streaking
	Wooden bowl <sup>b</sup>	Philippines	2	9	Streaking
2	Larix sp. cones for crafts	China	1	5	Streaking
	Wooden eggs	China	1	1	Streaking
Study 2					
1	Dried grape vines 1	China	2	5	Streaking
	Dried grape vines 2	China	3	1	Direct plating
	Bread board platform <sup>c</sup>	China	1	1	Direct plating
	Pinus sp. cones <sup>d</sup>	Italy	11	1	Streaking
	Straw	Mexico	1	5	Streaking
	Wooden letter "S"	China	0	1	Direct plating
2	Wooden rice bowls/spoons	Thailand	2	1	Streaking
	Wooden spatula	China	0	7	Streaking
	Wooden serving tray <sup>e</sup>	Thailand	7	1	Streaking
3	Wooden "cookie" disks <sup>f</sup>	China	5	3	Streaking
4	Wooden sticks/driftwood	Philippines	2	9	Streaking

#### TABLE 2 Isolation of fungi in studies 1 and 2

<sup>a</sup>Evidence of decay was present.

<sup>b</sup>Evidence of both decay and fungal zone lines were present.

<sup>c</sup>White pocket rot evident.

<sup>d</sup>Pycnidia present on cone scales.

<sup>e</sup>Bark and some sapstain present.

Bark present. The number of samples refers to the number of times a tissue was collected for plating. Each sample was replicated twice per media type.

In addition, we recovered 17 taxa that are considered human pathogens (Fig. 4); these 17 represent over a third of all taxa recovered. Of these, *P. formosus* (18, 46), which is known from the Middle East and Asia, has not yet been reported in the United States. The others—*A. alternata* (28, 47–49), *A. flavus* (23, 50), *A. pseudoglaucous* (23, 51), *A. versicolor* (14, 15), *C. cochlioides* and *C. globosum* (49, 52, 53), *C. cladosporioides* (49, 54), *C. radians* (55), *C. lunata* (49, 56), *P. variotii* (9, 16, 21), *P. macrospinosa* (49, 57), *R. delemar* (58–60) and *R. oryzae* (61–64), *S. commune* (65), *T. citrinoverde* (66, 67), and *T. longibrachiatum* (66, 67)—are associated with clinical infection records at various global locations, including the United States.

Three livestock/domestic animal pathogens were also recovered (Fig. 4): A. versicolor (14), C. globosum (68), and C. cladosporioides (54, 69). These are also considered human pathogens.

Thirteen thermophilic/thermotolerant species were found and included A. alternata (28, 70, 71), A. flavus (70-73), A. versicolor (15, 51, 74), Chaetomium spp. (74, 75), C. cladosporioides (71, 74, 76, 77), H. grisea (74, 78), P. variotii (21), P. macrospinosa (44, 57), R. oryzae (79, 80), T. rotundus (81), T. citrinoverde (78), and T. longibrachiatum (78, 80). Many of these species are also halophilic/halotolerant, such as A. alternata (18, 70), A. flavus (29, 73), A. versicolor (73), C. globosum (70, 75), C. cladosporioides (76), Humicola grisea (78), P. variotii (21, 82), P. macrospinosa (44), R. oryzae (70, 79), T. citrinoverde (70, 83, 84), and T. longibrachiatum (70, 83, 84). Four xerophylic/xerolerant species were also found: A. flavus (29, 50, 51), A. pseudoglaucous (51), A. versicolor (15), and P. formosus (85). Five species were determined to be resinicolous based on the literature (A. pseudoglaucous [51], H. conorum [86-88], R. kalkhoffii [40, 86-88], and S. difformis [87, 88]), and nine species were identified as xylophytic (A. alternata [28], A. flavus [29], C. globosum [75, 89], F. rabenhorstii [90, 91], P. formosus [35, 36], P. albobadia [37], S. commune [40], and X. badia [decay fungus] [41, 42]). Other species also possess heavy metal tolerance; these species include A. alternata (92), A. flavus (50, 93), C. globosum (75), C. cladosporioides (76), P. formosus (85), P. variotii (21), and T. longibrachiatum (49, 93) (Fig. 5).

#### DISCUSSION

This is the first known study to examine the viability of fungal pathogens in wooden handicrafts imported into the United States. These products are highly varied in their

#### Fungal Pathogens in Wooden Handicraft Imports



FIG 4 Fungal taxa organized by lifestyle/guild based on searches of multiple databases and literature searches. Several taxa are represented in more than one category. Taxa in red font are not known to have been documented in North America previously.

source materials, origin, destination, function/use, and biosecurity treatment prior to shipment. Although APHIS has rules that regulate the importation of these products, these regulations are based on potential risks to plant commodities based on products used in gardens (for example, bamboo stakes used in gardens). Little to no consideration appears to be given to products used in crafts or in food preparation (27). Furthermore, these rules were mainly written with potential wood-boring insects being prioritized as the greatest threat. Given that many of these products are destined for use in food preparation and/or utensils, handcrafting, or in bath and beauty industries, the potential threat of microbes to other hosts (nonplant; i.e., human) from this commodity appears to have been given little attention in the current regulations. Similar products exist in pet stores (toys, perches, ornaments, etc.). The studies reported here demonstrated that from a small sampling of products and retail stores, potentially dangerous and nonendemic fungal pathogens could be recovered.

The fungi recovered include 25 plant and human pathogens, including three that have never been reported in North America previously (Fig. 4): *B. austrostipae, P. formosus*, and *X. badia*. Although the exact distributions of these species are not clear, the latter two have been found previously in Asia (including China, the products' import origin location), and in particular, on wood substrates. *Bipolaris austrostipae* has only been found in Australia previously, but all three of these are plant pathogens, and *P. formosus* is both a plant and human pathogen. *Bipolaris austrostipae* was described from Queensland (31), Australia, on *Austrostipa* sp., and other species of *Bipolaris* are important plant pathogens on numerous hosts globally. Several are important emerging fungal pathogens, and some species (31) are known as human pathogens (49). The global importance of *B. oryzae* as a major pathogen of rice cannot be understated as the cause of brown spot. This disease is known to have been a major cause of the Great Bengal Famine of 1942 when rice yield losses of 50 to 90% were reported due to an epidemic of this disease (94). *Paecilomyces formosus* is particularly interesting because it has the potential to infect both plants and humans (18, 35, 36, 46). It is

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**FIG 5** Fungal taxa organized by functional trait, based on searches of multiple databases and literature searches. Several taxa are represented in more than one category. Taxa in red font are not known to have been documented in North America previously.

associated with cankers and dieback of oaks and pistachio trees in the Mediterranean region and has also caused cutaneous and lung infections in both premature infants and immunocompromised adults in the Middle East (18, 46). It is not known whether strain-level differentiation exists for host specificity within this species but, given the wide potential for threats to both tree and human hosts, this nonendemic species is of significant concern and should be a surveillance target in future sampling. Interestingly, *X. badia*, thought to function as a decay fungus of wood, was recently described (42) from the Mekong Delta region of Vietnam, where it is apparently endemic. The only other record was as an endophyte of orchids in tropical regions of China (41). This species was recovered from a product imported from China; however, the exact origin of the original raw wood material is unknown.

Many wooden handicraft commodities are made up of minimally or unprocessed wood. The widespread popularity of "rustic" home goods products—those with bark, decay, zone lines, and discoloration present—increases the likelihood of fungi being present. Likewise, products used in arts and crafts, such as pinecones, grape vines, and driftwood, are unprocessed and raw material and likely to contain a wide diversity of microbial organisms. Indeed, in this study we recovered fungi from products containing unprocessed wood and products containing bark and evidence of decay, suggesting fungi were already well established when shipped.

An evaluation of the functional traits (Fig. 5) of the fungi recovered illustrates a high proportion of the taxa (76.6%) with tolerances that would increase their likelihood for survival through potential heat and/or methyl bromide treatments (thermophilic/thermotolerant, halophilic/halotolerant, xerophylic/xerotolerant, xylophytic, resinicolous, and heavy metal tolerant). Although it is difficult to determine the exact treatments for these samples, knowledge of the fungi recovered and their tolerances can guide new strategies for policies to reduce future introductions and develop new treatment methods that can control the viability of fungi within imported wooden products.

These results suggest that the current regulations are not sufficient to prevent entry of novel fungal pathogens on wooden handicrafts. Although numerous plant and

human pathogens were recovered from this study, the significance of the results should be placed on the overall potential pathway rather than on individual species and illustrate the importance of including fungi in risk assessments associated with wood-based commodities.

Future strategies for mitigating risks associated with importation of novel fungal pathogens on wooden handicrafts could be employed by increasing stringency standards for the products themselves and the treatment process. The importation of rustic, unprocessed wood with bark and/or decay and of products such as cones, straw, and driftwood would likely increase the risk of entry of microbes associated with these products. Although our study did not evaluate this variable, we did observe numerous products with evidence of fungal growth (staining, fruiting bodies, zone lines). By limiting imports to clean, processed wood only, the probability of importing established fungal growth is reduced. Likewise, by standardizing the product raw materials, fumigation and other sterilization treatments can be improved and designed to match the substrate and adequately sterilize taking into account potential thermophilic and halophilic taxa as identified in this study.

## **MATERIALS AND METHODS**

Two studies were completed during 2019 and 2020 to examine the survival and diversity of fungi in wood products sold in retail stores in Gainesville, FL.

Undergraduate students in a forest pathology course participated in the processing and culturing of samples purchased by the first author at two retail stores. This was considered a pilot study that is described as study 1. Five products (Table 2) were purchased from two retail stores, representing four countries of origin, transported to the laboratory and maintained at 4°C until processed within 3 days of purchasing. Processing involved culturing on selective media (potato dextrose agar [Gibco] acidified with 1 mL/L lactic acid [APDA], malt extract agar [MEA; Gibco], and basidiomycete select agar [BSA]), and incubation of select wood pieces in moist chambers. Fungal cultures were identified by Sanger sequencing of the internal transcribed spacer region (ITS) rDNA, followed by sequence comparisons using BLASTn searches in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 1).

In study 2, a larger experiment was completed with 11 products sampled from four additional retail stores, representing five countries of origin (Table 2). The products were handled in the same way as study 1 and were also processed within 3 days of purchasing. Processing involved culturing and sequencing cultures as described above.

Wood product sampling. The objects (Fig. 1) were surface sterilized with 70% ethanol to remove any external material and were drilled with an oscillator saw or drill. Cutting instrument parts that touched the wood, such as blades, and drill bits, were surface sterilized with 96% ethanol and flamed between each sampling. Sawdust or wood/straw pieces were obtained from the samples. Samples varied in size and some included multiple components such as several utensils packaged together. Sampling was done to ensure all components that were packaged in aggregate were sampled (Table 2). Between one to nine samples were taken from the products using the cutting instruments (Table 2). Two replicates from each sample were then plated onto one plate each for each replicate and medium type. In the case of sawdust, approximately 25 mg for bark and 100 mg for cambium and other tissue types was resuspended in 500  $\mu$ L of sterile double-deionized water. Sawdust samples were aliquoted (100  $\mu$ L), vortexed and then streaked with a sterile glass rod onto plates of three different types of media MEA, APDA, and BSA. Wood or straw pieces, approximately 50 mm imes 10 mm in size were excised from the larger sample using a sterile scalpel. These were sterilized further with half-strength bleach (5% sodium hypochlorite) diluted with sterile water for 30 s, followed by a wash in sterile autoclaved water prior to plating four times (pseudoreplicates) onto the three respective medium types in a sterile laminar flow hood. Plates were wrapped in Parafilm (Bemis, Inc.) and then incubated at room temperature in the dark and monitored daily for fungal growth. The specific culturing methods applied for each sample are available in Table 2.

**Wood tissue incubation.** To encourage the growth of existing viable fungi present inside wooden handicrafts, products cut in half (study 1) or wood pieces (study 2) were incubated in moist chambers. Two wooden bowls obtained from the Philippines and Indonesia and wooden coasters from China exhibiting evidence of fungal colonization were used for incubation in study 1. The products were surfaced disinfected with 10% sodium hypochlorite and then cut in half with a sterilized cutting tool. The wood pieces were soaked in sterile water for 1 h, and then two replicates were placed in sterile plastic boxes lined with premoistened sterile paper towels. The boxes were sealed with Parafilm and maintained at room temperature and monitored every 1 to 3 days for emergence of fungal fruiting bodies. No fungal fruiting bodies in study 1 were used for sequencing. Six products (derived from samples 01-MIC-CHI, 02-MIC-CHI, 09-ROS-THA, 04-MIC-ITA, 05-MIC-MEX, and 03-MIC-CHI, isolates highlighted in boldface in Table 1) were used for incubation in study 2. Cross-sections of wood, 2 to 4 cm in diameter (or straw for 05-MIC-MEX, 2 cm in length), were surface sterilized by washing with 5% sodium hypochlorite, allowed to dry, followed by spraying with 70% ethanol. Two (for wooden products) or six (straw products) replicates were sealed with Parafilm and maintained at room temperature and monitored every 1 to

3 days for the emergence of fungal fruiting bodies. In addition, cones from an unidentified *Pinus* sp. imported from Italy were incubated the same way; however, these were maintained whole, rather than cut, with six replicates being used. Fungal growth was identified by scraping fruiting bodies that developed and identification by DNA extraction and Sanger sequencing of the ITS rDNA as described above (Table 1).

**Fungal culture identification.** Within 24 to 72 h, individual colonies were transferred to new plates when they were observed to be culturable (free of contamination by neighboring microbial growth). Colony counts were not completed, and growth was not quantified for this study because the growth was generally too heterogenous and in many cases groups of colonies were consolidated, making accurate quantification of growth impossible. Isolates were subcultured onto the same type of media they were originally isolated from by aseptically transferring a small plug of mycelia from the edge of the colonies. Isolates were grouped into unique morphotypes and given accession codes. If possible, unique morphotypes were counted for each product tested and, in several cases, multiple products produced the same morphotype. Once colony growth reached approximately 2.5 cm in diameter, a sample of mycelium was scraped off and used for DNA extraction with Extract-N-Amp procedure (Sigma). PCR amplification was performed using the primers ITS1F-ITS4 (95). PCR amplificons were visualized on a 1.2% agarose gel stained with SYBR green (Sigma-Aldrich, Inc., St. Louis, MO) to confirm amplification prior to Sanger sequence-ing. Sequences were aligned using Geneious 10.2.6 (http://www.geneious.com) software, and low-quality bases were trimmed and/or edited to produce a final consensus sequence for tentative identification. Final aligned sequences were deposited in GenBank (Table 1).

Sequences were compared against the NCBI database using BLASTn (https://blast.ncbi.nlm.nih.gov/ Blast.cgi), using default settings to determine the potential taxonomic identity of the isolates (Table 1). BLAST results were considered a "top match" based on the following criteria: (i) high identities/query coverage (query coverage of > 95% was cutoff); (ii) matching well-annotated specimens, with priority given to deposited sequences originating from living cultures, particularly vouchers; and (iii) sequences deposited from noncultured sources (i.e., metasequencing studies) were filtered and not considered in the analyses. If identifications could not be determined (identity 90 to 95%) at the species level, but there was no ambiguity at the genus level, the identification was designated as "Genus sp." No identifications were given for identities below 90%.

Fungal biogeography and functional group determination. Each fungal taxon name was checked against Index Fungorum (www.indexfungorum.org) and MycoBank (https://www.mycobank.org) for current taxonomic status and synonyms. For each of the taxa, the biogeography was assessed using multiple sources, including both Index Fungorum and Mycobank, as well as the U.S. National Fungus Collections Nomenclature Database (https://nt.ars-grin.gov), the National Center for Biotechnology Information life-map tree database (http://lifemap-ncbi.univ-lyon1.fr/), and the Global Biodiversity Information Facility (GBIF; https://www.gbif.org) using the "occurrences" filter to determine known records for the taxa identified in this study. For each of the taxa searched, their biogeography, lifestyle (pathogen [and type] versus saprophyte) and toxin production ability was determined by searching the literature using multiple databases, including PubMed (https://pubmed.ncbi.nlm.nih.gov), Google Scholar (https://scholar.google.com), Agris (https://www.fao.org/agris/), MycoPortal (https://mycoportal.org/portal/), and GBIF (https://www.gbif.org). In addition, they were grouped based on traits determined to increase likelihood of survival through the biosecurity measures: halophile, resinicolous, thermophile, xerophile, and xylophyte. Tolerance to heavy metals was also determined based on the literature. Data for each analysis was visualized (Fig. 4 and 5) using Raw Graphs (https://rawgraphs.io) and then figures were exported, and text and colors were customized using an html editor.

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