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Aspergillus alabamensis, a New Clinically Relevant Species in the Section *Terrei*▽

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Phylogenetic analyses of sequences generated from portions of three genes coding for the proteins enolase (*enoA*), β-tubulin (*benA*), and calmodulin (*calM*) of a large number of isolates within the section *Terrei*, genus *Aspergillus*, revealed the presence of a new cryptic species within this section, *Aspergillus alabamensis*. Most members of this new cryptic species were recovered as colonizing isolates from immunocompetent patient populations, had decreased in vitro susceptibilities to the antifungal drug amphotericin B, and were morphologically similar to but genetically distinct from *Aspergillus terreus* isolates.

Invasive infections caused by *Aspergillus terreus* are often disseminated with increased lethality compared with infections caused by other *Aspergillus* species and tend to be resistant to treatment with the antifungal drug amphotericin B (6, 14, 17). Despite the clinical significance of this organism, little is known about the epidemiology, genetic diversity, and population structure of *A. terreus*.

Historically, *A. terreus* has been identified in the laboratory by conventional methods such as colony morphology and microscopic characteristics. Such morphological studies have placed *A. terreus* as a single homogenous species within the section *Terrei* along with two other varieties, *A. terreus* var. *africanus* and *A. terreus* var. *aureus* (11). Recent studies have shown that morphological characteristics may not be reliable for distinguishing *Aspergillus* species, as inferred from the demonstration of multiple cryptic species within the section *Fumigati* by molecular phylogenetic methods (3–5, 13, 18).

In the past, molecular methods largely based on randomly amplified polymorphic DNA-PCR-based assays have shown that *A. terreus* isolates can have great strain diversity (1, 8, 16). One recent genotyping study of several *A. terreus* clinical isolates recovered from two different medical centers using this method concluded that nosocomial acquisition of *A. terreus* infections was highly unlikely given the great genetic diversity observed (7). Another study demonstrated that comparative sequence analyses of the D1 and D2 regions had limited utility to study relationships within the section *Terrei*, while the internal transcribed spacer regions were useful since there was more nucleotide diversity in this region (16). However, the authors of this study could not resolve species within the section *Terrei* using these molecular approaches.

In the present study, we have developed a multilocus sequence approach employing three protein-coding regions to study species diversity of the section *Terrei* using a large panel of isolates from both clinical and environmental origins recovered from various parts of the world. The studies outlined below demonstrate the presence of a new, clinically relevant species, *Aspergillus alabamensis*, and clarify the taxonomic position of the *A. terreus* variant *A. terreus* var. *aureus*.

MATERIALS AND METHODS

Fungal isolates. A total of 94 clinical and environmental *A. terreus* isolates were analyzed in this study, including 30 isolates from University of Alabama at Birmingham (UAB); 23 isolates from the Department of Hygiene, Microbiology and Social Medicine, Medical University of Innsbruck, Innsbruck, Austria; 23 isolates from the CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; 17 isolates from the Center for Microbial Biotechnology, Biocentrum-DTU, Technical University of Denmark, Lyngby; and one isolate from the National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, IL. Apart from this, two isolates of *Aspergillus allahabadii*, one isolate each of *Aspergillus niveus* var. *indicus* and *Fennellia flavipes*, and three isolates of *A. niveus* were also received from the National Center for Agricultural Utilization Research and included in this study. All fungal isolates were well separated in time and location of origin, with the years of recovery ranging from 1926 to 2006, and included isolates from Europe, South America, Asia, and North America (Table 1). Isolates were stored frozen and were subcultured on Sabouraud's dextrose agar plates before DNA isolation.

Genomic DNA isolation, PCR amplification, and sequencing. For genomic DNA isolation, fungi were grown in liquid broth for 48 h, after which the fungal material was disrupted using an Omni-Mixer (Omni International, Warrenton, VA) in the presence of buffer ATL (Qiagen, Valencia, CA) and 55 μl proteinase K; the sonicated material was incubated at 55°C for an hour in a water bath with frequent vortexing. Fungal DNA was isolated using the DNeasy blood and tissue kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations.

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TABLE 1. Source and origin of *A. terreus* isolates used in the study

Isolate no.	Species	Geographic origin	Source	GenBank identification no. (*calM*, *enoA*, *benA*)	Yr of isolation
IBT26915	*A. terreus*	Panama	Capybara	EU147503, EU147596, EU147689	Unknown
IBT24859	*A. terreus*	Slovenia	Saltern	EU147504, EU147597, EU147690	Unknown
IBT13089	*A. terreus*	Unknown	Unknown	EU147505, EU147598, EU147691	Unknown
IBT26385	*A. terreus*	Unknown	Unknown	EU147506, EU147599, EU147692	Unknown
IBT21125	*A. terreus*	Unknown	Unknown	EU147507, EU147600, EU147693	Unknown
IBT12713	*A. terreus*	New Mexico	Kangaroo rat	EU147508, EU147601, EU147694	1989
IBT6450	*A. terreus*	Unknown	Unknown	EU147509, EU147602, EU147695	Unknown
IBT20944	*A. terreus*	Unknown	Unknown	EU147510, EU147603, EU147696	Unknown
IBT23544	*A. terreus*	Unknown	Hay	EU147511, EU147604, EU147697	Unknown
IBT14590	*A. terreus*	Merck strain	Unknown	EU147512, EU147605, EU147698	Unknown
IBT6271	*A. terreus*	Unknown	Unknown	EU147513, EU147606, EU147699	Unknown
IBT22563	*A. alabamensis*[a]	Unknown	Unknown	EU147514, EU147607, EU147700	Unknown
IBT26384 (NRRL274)	*A. terreus*	Brazil	Human ear	EU147515, EU147608, EU147701	Unknown
IBT16744	*A. terreus*	Galapagos Islands	Environment	EU147516, EU147609, EU147702	Unknown
IBT16745	*A. terreus*	Galapagos Islands	Environment	EU147517, EU147610, EU147703	Unknown
IBT15722	*A. terreus*	Unknown	Unknown	EU147518, EU147611, EU147704	Unknown
IBT13121	*A. terreus*	Japan	Soil	EU147519, EU147612, EU147705	Unknown
CBS383.75	*A. terreus*	India	Soil	EU147520, EU147613, EU147706	1975
CBS118.27	*A. terreus*	Unknown	Unknown	EU147521, EU147614, EU147707	1927
CBS601.65 (NRRL255)	*A. terreus* (type)	Connecticut	Soil	EU147522, EU147615, EU147708	1965
CBS594.65 (NRRL680)	*A. terreus*	Unknown	Soil	EU147523, EU147616, EU147709	1965
CBS106.25	*A. terreus*	Unknown	Unknown	EU147524, EU147617, EU147710	1920
CBS134.60	*A. terreus*	Unknown	Cotton (*Gossypium*)	EU147525, EU147618, EU147711	1926
CBS125.38	*A. terreus*	New Zealand	Unknown	EU147526, EU147619, EU147712	1938
CBS8G5	*A. terreus*	The Netherlands	White soy beans	EU147527, EU147620, EU147713	2005
CBS17A1	*A. terreus*	The Netherlands	Laboratory medium	EU147528, EU147621, EU147714	2006
CBS15F8	*A. alabamensis*[a]	Argentina	Soil	EU147529, EU147622, EU147715	2006
CBS15F9	*A. alabamensis*[a]	Argentina	Soil	EU147530, EU147623, EU147716	2006
CBS503.65 (NRRL1923)	*A. aureoterreus*[b]	Texas	Soil	EU147531, EU147624, EU147717	Unknown
CBS8G3	*A. terreus*	The Netherlands	Blended almond pits	EU147532, EU147625, EU147718	2005
CBS130.55 (NRRL2399)	*A. terreus* var. *africanus* (type)	Tafo, Ghana	Soil	EU147533, EU147626, EU147719	1955
1769-05	*A. terreus*	India	Clinical isolate	EU147534, EU147627, EU147720	2005
1686-05	*A. terreus*	India	Keratitis	EU147535, EU147628, EU147721	2005
1796-05	*A. terreus*	India	Keratitis	EU147536, EU147629, EU147722	2005
CBS19F3	*A. terreus*	India	Clinical isolate	EU147537, EU147630, EU147723	2005
CBS24A3	*A. terreus*	The Netherlands	Sucrose tank	EU147538, EU147631, EU147724	2006
CBS24A4	*A. terreus*	The Netherlands	Sugar silo door	EU147539, EU147632, EU147725	Unknown
CBS2B7	*A. terreus*	The Netherlands	Unknown	EU147540, EU147633, EU147726	2005
CBS469.81	*A. terreus*	Thailand	Cardiac valve	EU147541, EU147634, EU147727	1981
CBS6I9	*A. terreus*	The Netherlands	Soil	EU147542, EU147635, EU147728	Unknown
P4	*A. terreus*	Austria	Tracheal secretions	EU147543, EU147636, EU147729	1996
P12	*A. terreus*	Austria	Lung	EU147544, EU147637, EU147730	1997
P13	*A. terreus*	Austria	Brain	EU147545, EU147638, EU147731	1997
P14	*A. terreus*	Austria	BAL[c]	EU147546, EU147639, EU147732	1999
P16	*A. terreus*	Austria	BAL	EU147547, EU147640, EU147733	1996
P22	*A. terreus*	Austria	BAL	EU147548, EU147641, EU147734	2001
E5	*A. terreus*	Austria	Air, hospital	EU147549, EU147642, EU147735	2004
E7	*A. terreus*	Austria	Air, hospital	EU147550, EU147643, EU147736	2004
E8	*A. terreus*	Austria	Air, hospital	EU147551, EU147644, EU147737	2005
E9	*A. terreus*	Austria	Air, hospital	EU147552, EU147645, EU147738	2005
P7	*A. terreus*	Austria	BAL	EU147553, EU147646, EU147739	2000
P9	*A. terreus*	Austria	Tracheal secretions	EU147554, EU147647, EU147740	1999
P10	*A. terreus*	Austria	Lung	EU147555, EU147648, EU147741	2001
P11	*A. terreus*	Austria	Sputum	EU147556, EU147649, EU147742	2005
P15	*A. terreus*	Austria	Lung	EU147557, EU147650, EU147743	2002
P20	*A. terreus*	Austria	Tracheal secretions	EU147558, EU147651, EU147744	2003
P23	*A. terreus*	Austria	Sputum	EU147559, EU147652, EU147745	2003
P26	*A. terreus*	Austria	Lung	EU147560, EU147653, EU147746	2001
P29	*A. terreus*	Austria	BAL	EU147561, EU147654, EU147747	2004
P30	*A. terreus*	Austria	Sputum	EU147562, EU147655, EU147748	2005
P32	*A. terreus*	Austria	Lung	EU147563, EU147656, EU147749	2006
P33	*A. terreus*	Austria	Brain	EU147564, EU147657, EU147750	2005
P38	*A. terreus*	Austria	Lung	EU147565, EU147658, EU147751	2005
UAB1	*A. alabamensis*[a]	Alabama	Tracheal aspirate	EU147566, EU147659, EU147752	1996
UAB2	*A. terreus*	Alabama	Sputum	EU147567, EU147660, EU147753	1997
UAB3	*A. terreus*	Alabama	Bronchial wash	EU147568, EU147661, EU147754	1997
UAB4	*A. terreus*	Alabama	Bronchial wash	EU147569, EU147662, EU147755	1998
UAB5	*A. terreus*	Alabama	Bronchial wash	EU147570, EU147663, EU147756	1998
UAB6	*A. terreus*	Alabama	Sputum	EU147571, EU147664, EU147757	1999
UAB7	*A. terreus*	Alabama	Bronchial wash	EU147572, EU147665, EU147758	1999
UAB8	*A. terreus*	Alabama	Thyroid	EU147573, EU147666, EU147759	1999
UAB9	*A. terreus*	Alabama	Bronchial wash	EU147574, EU147667, EU147760	1999
UAB10	*A. terreus*	Alabama	BAL	EU147575, EU147668, EU147761	1999
UAB11	*A. terreus*	Alabama	Sputum	EU147576, EU147669, EU147762	2000
UAB12	*A. terreus*	Alabama	Sputum	EU147577, EU147670, EU147763	2000
UAB13	*A. alabamensis*[a]	Alabama	Sputum	EU147578, EU147671, EU147764	2000
UAB15	*A. alabamensis*[a]	Alabama	Sputum	EU147579, EU147672, EU147765	2000
UAB17	*A. terreus*	Alabama	Catheter tip	EU147580, EU147673, EU147766	2000

Continued on following page

TABLE 1—*Continued*

Isolate no.	Species	Geographic origin	Source	GenBank identification no. (*calM*, *enoA*, *benA*)	Yr of isolation
UAB18	*A. alabamensis*[a]	Alabama	Sputum	EU147581, EU147674, EU147767	2000
UAB19	*A. terreus*	Alabama	BAL	EU147582, EU147675, EU147768	2000
UAB20	*A. alabamensis*[a]	Alabama	Wound	EU147583, EU147676, EU147769	2000
UAB21	*A. terreus*	Alabama	Sputum	EU147584, EU147677, EU147770	2000
UAB22	*A. alabamensis*[a]	Alabama	Tracheal aspirate	EU147585, EU147678, EU147771	2000
UAB23	*A. alabamensis*[a]	Alabama	Sputum	EU147586, EU147679, EU147772	2000
UAB26	*A. terreus*	Alabama	Sputum	EU147587, EU147680, EU147773	2000
UAB28	*A. alabamensis*[a]	Alabama	BAL	EU147588, EU147681, EU147774	2000
UAB30	*A. alabamensis*[a]	Alabama	Sputum	EU147589, EU147682, EU147775	2001
UAB31	*A. terreus*	Alabama	BAL	EU147590, EU147683, EU147776	2001
UAB32	*A. terreus*	Alabama	Fingernail	EU147591, EU147684, EU147777	2001
UAB33	*A. alabamensis*[a]	Alabama	Foot tissue	EU147592, EU147685, EU147778	2001
UAB34	*A. terreus*	Alabama	Sputum	EU147593, EU147686, EU147779	2001
UAB37	*A. terreus*	Alabama	Tracheal aspirate	EU147594, EU147687, EU147780	2001
UAB38	*A. alabamensis*[a]	Alabama	Left ear	EU147595, EU147688, EU147781	2001
NRRL4609	*A. terreus* var. *africanus*	Panama	Soil	NA[d]	Unknown
NRRL515 (CBS114.33)	*A. niveus*	Unknown	Unknown	NA	1933
NRRL4101	*A. allahabadii*	San Salvador	Soil	NA	Unknown
NRRL4539	*A. allahabadii*	India	Soil	NA	Unknown
NRRL4751	*A. niveus*	Unknown	Unknown	NA	Unknown
NRRL5505	*A. niveus*	Unknown	Unknown	NA	Unknown
NRRL6134 (CBS444.75)	*A. niveus* var. *indicus*	Maharashtra, India	Soil	NA	1975
NRRL5504	*Fennellia flavipes*	Unknown	Unknown	NA	Unknown

[a] Isolates originally identified by morphology as *A. terreus*, currently assigned to the new species *A. alabamensis* based on phylogenetic analyses described in the text.
[b] Originally identified as *A. terreus* var. *aureus*.
[c] BAL, bronchoalveolar lavage.
[d] NA, not applicable.

For the multilocus analysis, genes encoding enolase (*enoA*) and β-tubulin (*benA*) were identified using the publicly available *A. terreus* genome database (*A. terreus* Sequencing Project, Broad Institute of Harvard and MIT; http://www.broad.mit.edu). Primers were designed using the Genefisher program to yield a product size of about 300 bp for *enoA* (*enoA* F, 5′ CCGTCTACGACTCTCGCGGTA; and *enoA* R, 5′ TGAGGAACTCGTCAACCTTGGA) and a product size of 400 bp for *benA* (*benA* F, 5′ GGGGATAGGATGTTTTGTGACA; and *benA* R, 5′ GGTCAACGAGGACGGCACGA). For calmodulin (*calM*), previously described degenerate primers CF1 F (5′ GCCGACTCTTTGACYGARGAR) and CF4 R (5′ TTTYTGCATCATRAGYTGGAC), predicted to yield a 700-bp product, were used (9). PCR amplification was performed with 1 μl of genomic DNA as the template in a final reaction volume of 25 μl consisting of PCR buffer (20 mM Tris-HCl [pH 8.4], 50 mM KCl); 0.2 mM each of dATP, dGTP, dCTP, and dTTP; 1.2 mM $MgSO_4$; 0.2 pmol of primers; 1 U of *Pfx* DNA polymerase (Invitrogen-BRL, Life Technologies, Carlsbad, CA); and 1× PCR enhancer (Invitrogen). Amplification was performed in a GeneAmp PCR system 9700 thermocycler (PE-Applied Biosystems) after initial denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 15 s, 55°C for 30 s, and 68°C for 30 s, and the last cycle was followed by a final extension at 68°C for 2 min.

PCR products were visualized in a 1.2% agarose gel using ethidium bromide and purified using the ExoSAP-IT enzyme system (USB, Cleveland, OH). For sequencing, 1 μl of the purified PCR amplicon was added to the sequencing mixture containing 4 μl Big Dye, 1.6 μl of 1 μM primer (same as the respective PCR primers), and 3.4 μl water. The sequence cycle was 96°C for 5 s, followed by 30 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Both strands were sequenced in an ABI 3730 DNA sequencer, and the resultant nucleotide sequences were edited with the Sequencher version 4.7 software (Genecodes, Inc., Ann Arbor, MI).

Phylogenetic analysis. The number of polymorphic sites, number of genotypes, and parsimony informative sites generated by each of the three loci and all loci combined were determined using PAUP* v4B10 (15). The aligned sequences were used to estimate phylogenetic trees under the maximum likelihood criterion (ML) as employed by PAUP* v4B10. An HKY + I + G model of evolution was used to correct for multiple hits. Specifically, we used a neighbor-joining tree to generate the first tree (neighbor-joining start) and then optimized the start tree with the subtree pruning and regrafting swapping algorithm. Trees were rooted with the midpoint rooting algorithm, as implemented in PAUP* v4B10. Support for nodes was generated by nonparametric bootstrapping of the data with the same ML model of evolution estimated from the complete data set. Sequences of the loci *enoA*, *benA*, and *calM* of *A. terreus* NIH2624, whose genome has been sequenced, were downloaded from the *A. terreus* Sequencing Project (http://www.broad.mit.edu/annotation/genome/aspergillus_terreus/Home.html) and included for reference.

Morphological studies. For macromorphological observations, isolates were grown on Czapek yeast autolysate (CYA), malt extract agar (MEA), and Czapek agar and incubated at 25°C in the dark for 7 days and at 37°C and 42°C on CYA. For micromorphological observations such as size of conidia, phialides, vesicles and conidiophores, and stipe wall morphology, microscopic mounts from MEA colonies were made in lactic acid and a drop of alcohol was added to remove air bubbles and excess conidia.

Nucleotide sequence accession numbers. Sequences of the *enoA*, *benA*, and *calM* of the section *Terrei* isolates have been submitted to GenBank and assigned the accession numbers listed in Table 1.

RESULTS

Genomic DNA from all isolates was amenable to PCR amplification and sequencing in the selected gene regions. ML trees generated from the three genes (Fig. 1, 2, and 3) and the combine ML tree generated from the sequences from all the threeloci (Fig. 4) revealed two distinct, well-supported clades within section *Terrei*. Clade 1 included 85.1% of isolates (80/94) previously identified as *A. terreus*, including the type isolate (CBS601.65) and the genome sequence isolate (NIH2624), indicating that this clade represents, at least in part, that taxon (Fig. 1 to 3). There was no clustering of genotypes from environmental or clinical origin within clade 1 (Fig. 1 to 3). The two *A. terreus* var. *africanus* isolates (CBS130.55 = NRRL2399; NRRL4609) clustered within clade 1 in all three loci. Another isolate, IBT13121, clustered with *A. terreus* var. *africanus* in the *benA* and *calM* loci.

The type strain of *A. terreus* var. *aureus* (CBS503.65 = NRRL1923) was distinct from all other isolates in all three loci as well as in the combined-locus tree (Fig. 1 to 4).

Clade 2 was distinct from the *A. terreus* clade (clade 1) and included 14 isolates, of which 11 were clinical specimens recovered from 11 different patients attending UAB (Fig. 1 to 3). This clade had high bootstrap support in all three loci studied. The combined-gene tree also gave strong support for the reciprocal monophyly of clades 1 and 2 (Fig. 4).

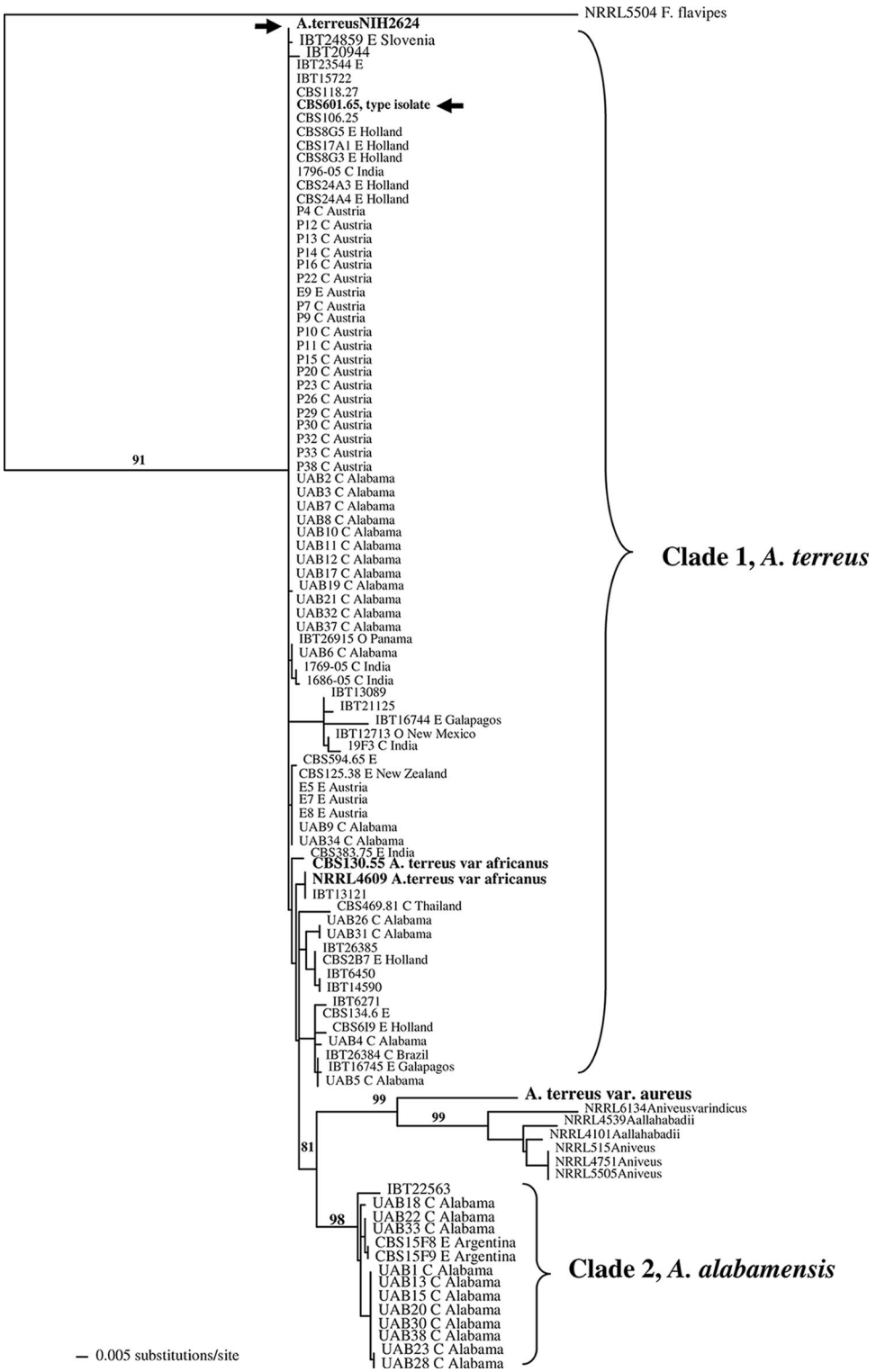

FIG. 1. ML tree generated from partial sequences from the calmodulin (*calM*) gene region. *Fennellia flavipes* is used as the outgroup. Bootstrap values are shown above the branches. Isolate NIH2624, whose genome has been sequenced, is indicated with an arrow. Environmental isolates are denoted by E, and clinical isolates are denoted by C.

Eight of 11 clinical isolates were colonizing isolates as they were recovered from sputum or tracheal aspirates of immunocompetent hosts and not from a sterile body fluid or an invasive site. One isolate each was recovered from a broncheoalveolar lavage sample of a kidney/pancreas transplant patient, a wound from a burn patient, and the ear of a patient with external otitis. This clade also included three soil isolates—one from Florida and two from Argentina.

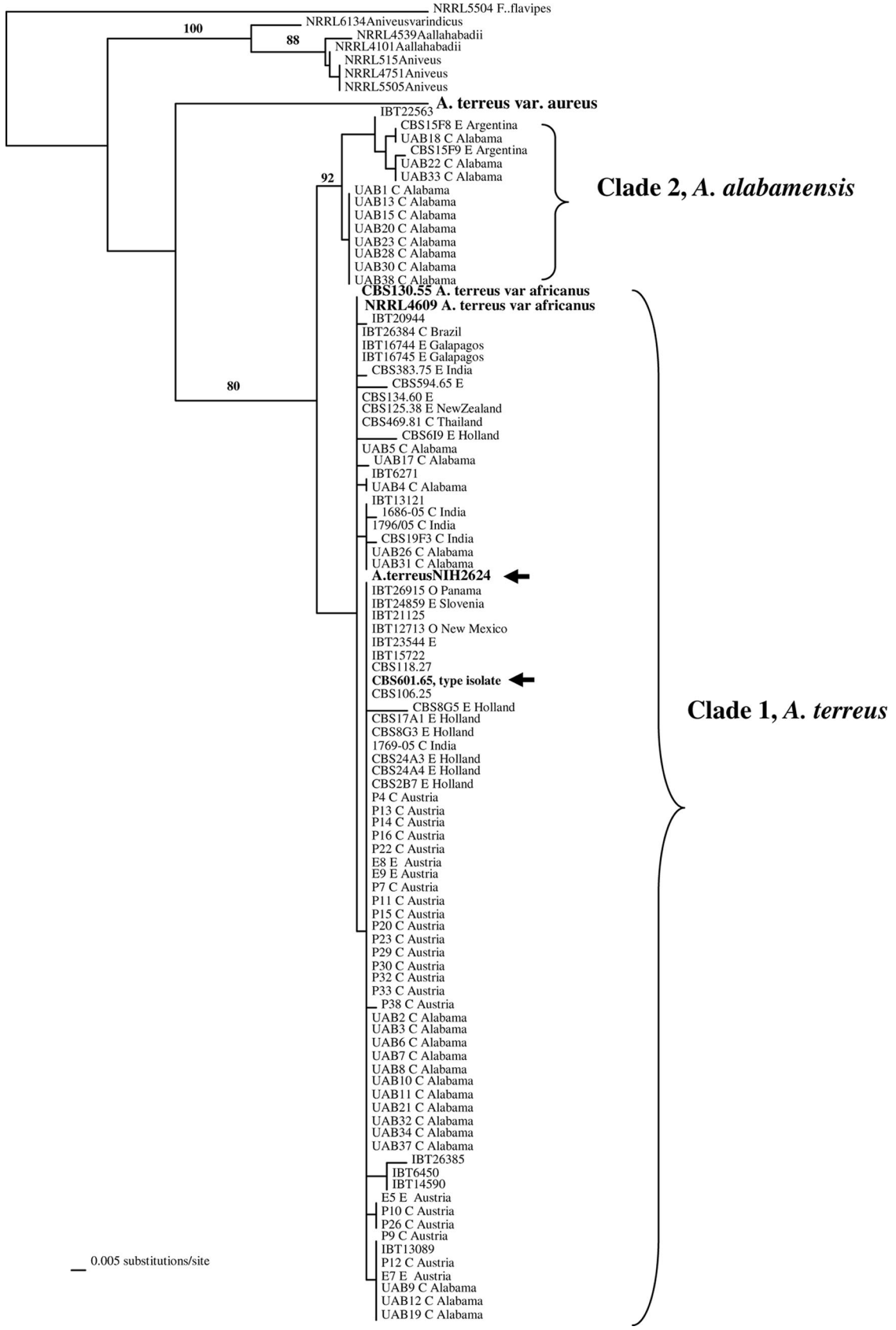

FIG. 2. ML tree generated from partial sequences of the enolase (*enoA*) gene region. Arrows indicate *A. terreus* isolate NIH2624, whose genome has been sequenced. Bootstrap values are shown above the branches. Environmental isolates are denoted by E, and clinical isolates are denoted by C.

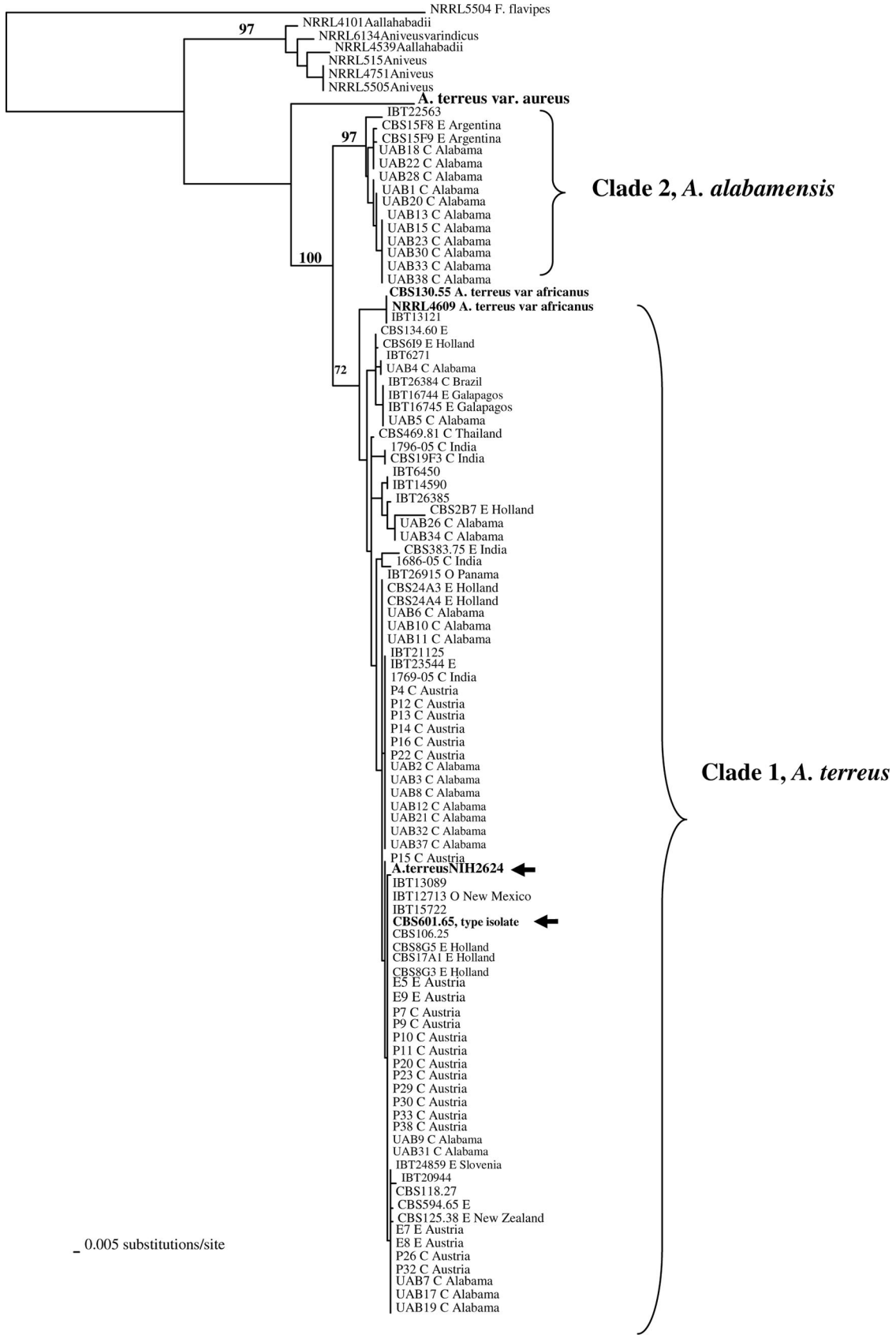

FIG. 3. ML tree generated from partial sequences from the β-tubulin (*benA*) gene region. Isolate NIH2624, whose genome has been sequenced, is indicated by arrows. Bootstrap values are shown above the branches. Environmental isolates are denoted by E, and clinical isolates are denoted by C.

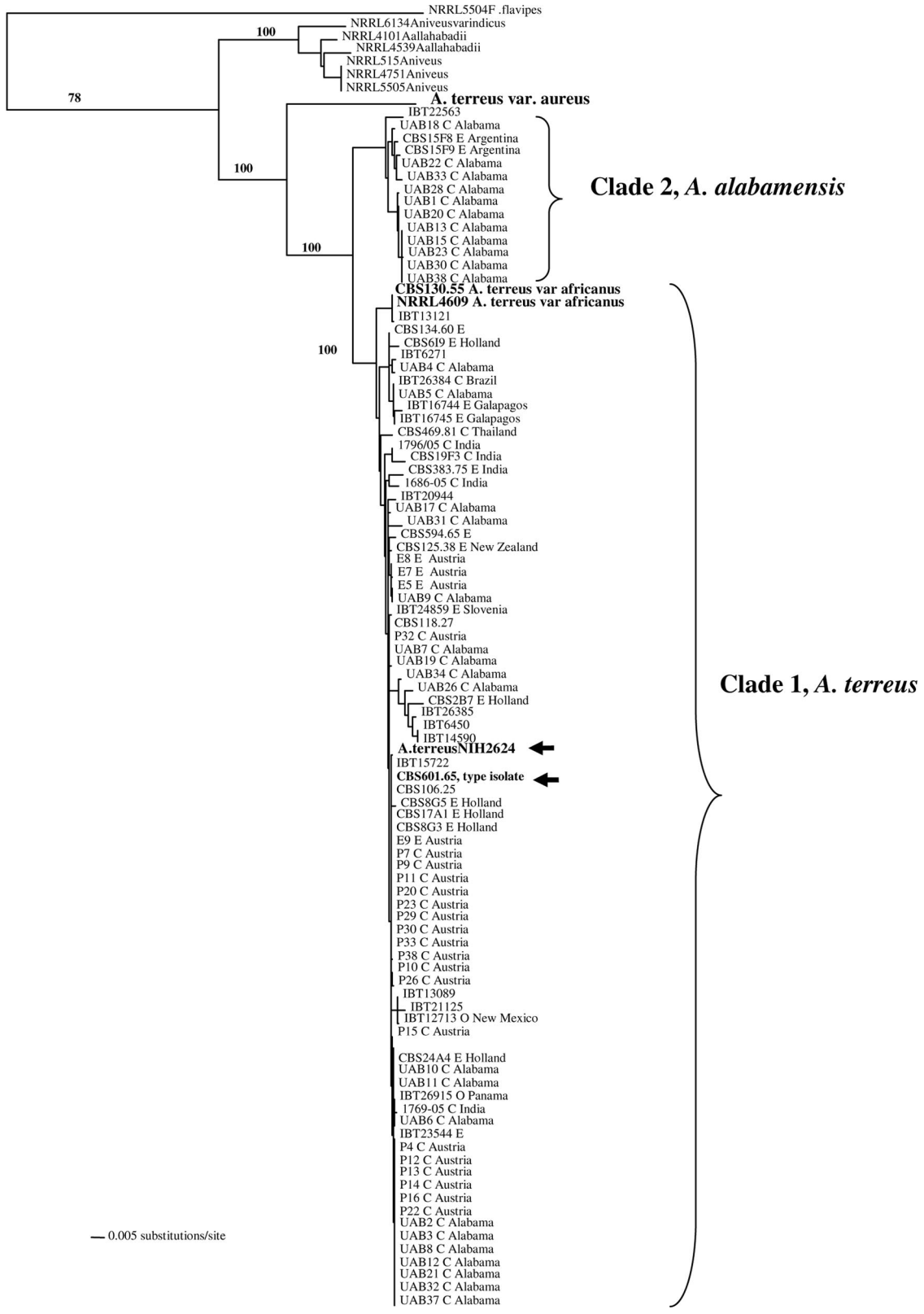

FIG. 4. Combined ML tree generated from sequences from the three protein-coding loci *calM*, *enoA*, and *benA* showing *A. alabamensis* as a separate species with high bootstrap values.

The source and origin of one isolate (IBT22563) are not known.

Members of clade 2 were recognized as a new species within the section *Terrei*, which is formally described below.

***Aspergillus alabamensis* Balajee, Baddley, Frisvad & Samson, sp. nov.**

Holotype. Isolate UAB 20, recovered from a clinical specimen received from UAB, is designated as the holotype and has been deposited in the CBS Fungal Biodiversity Centre.

Coloniae in agaris CYA et MEA luteobrunneae vel cinnamomeae, saepe ex strato coacto conidiophororum constantes, etsi auctus floccosus praebentes. Capitula conidialia longa, dense columnaria, in maturitate 30–50 μm diam, 150–500+ μm longa. Conidiophora biseriata, laevia, hyalina, 100–250 × 4.5–6.0 μm; vesiculae subglobosae, 10–16 μm diam; phialidae 5.0–7.0 × 2.0–2.5 μm; metulae arcte contiguae, 5.5–7.5 × 1.5–2.0 μm; conidia globosa vel subelliptica, laevia, 1.8–2.4 μm diam.

The basic morphological characteristics of *A. alabamensis* are illustrated in Fig. 5. Colonies on Czapek yeast extract and MEA are yellowish-brown to cinnamon-brown, often consisting of a dense felt of conidiophores but also showing floccose growth. Conidial heads are densely columnar. Conidial heads are long, columnar, 30 to 50 μm in diameter, and 150 to 500 μm or more in length at maturity; conidiophores are biseriate, smooth, colorless, and 100 to 250 μm by 4.5 to 6.0 μm. Vesicles are subglobose and 10 to 16 μm in diameter. Phialides are 5.0 to 7.0 μm by 2.0 to 2.5 μm. Metulae are closely packed and 5.5 to 7.5 μm by 1.5 to 2.0 μm. Conidia are globose to slightly elliptical, smooth, and 1.8 to 2.4 μm in diameter.

Etymology. The name *Aspergillus alabamensis* was chosen since most of the members of this new species were recovered as clinical specimens from patients at the UAB.

DISCUSSION

The present study, by means of a multilocus phylogenetic approach, characterized a large number of *A. terreus* isolates assembled from clinical and environmental origins representing different geographic locations of the world.

Results of the present study demonstrated that 33% of the clinical isolates (11/33) received from the UAB consistently clustered into a separate clade (clade 2) in the single-locus and combined-gene genealogies with strong bootstrap support; this clade was distinct from the *A. terreus* clade (Fig. 1 to 4), and thus the members of clade 2 are recognized as belonging to a new species, *A. alabamensis*. Antifungal susceptibility data were available for 7/11 isolates through a previous study (1); all 7 had higher MICs to amphotericin B (1 to 2 μg/ml) and lower MICs to voriconazole (0.25 to 0.5 μg/ml) and itraconazole (0.25 to 1 μg/ml). This susceptibility pattern was similar to the MICs of *A. terreus* to amphotericin B, voriconazole, and itraconazole (1). Interestingly, most of the clinical isolates in this new species were recovered as colonizing isolates, but given the small sample size of the study population, it is unclear at this time if these isolates have a decreased propensity to cause invasive infection. Apart from clinical isolates, this new species also included isolates recovered from soil in Florida and Argentina. Detailed morphological analyses revealed that *A. alabamensis* is macroscopically similar to *A. terreus*, with no striking macroscopical and microscopical differences. A feature common to both *A. alabamensis* and *A. terreus* is the colony pattern diversity (variation in color from yellow-brown to cinnamon-buff with or without orange tints) observed in this study as well as documented decades ago (11). Although *A. alabamensis* and *A. terreus* share many common secondary metabolites, the characteristic metabolite in *A. alabamensis* is citrinin, while *A. terreus* produces mevinolin (none of the *A. alabamensis* isolates tested produced this lovastatin derivative) and citreoviridin. This metabolite pattern appears to be a distinguishing feature between the species and warrants further detailed examination (J. Frisvad, personal communication).

A. terreus var. *aureus* has been previously recognized as a variety of *A. terreus* based on morphological characteristics, and the phenotype of this species is strikingly distinct from that of *A. terreus* (11). Specifically, this variety presents on MEA as slow-growing, floccose colonies with a bright golden yellow color due to pigmentation of the vegetative mycelium, with slower sporulation than *A. terreus*. Conidiophores are long, often becoming 500 μm or more in length, and bear rather small but definitely columnar heads that range from white to cream or light buff-colored conidia. Combined phylogenetic and morphological evidence demonstrates that this variant should be considered a new species within the section *Terrei*.

Aspergillus terreus var. *africanus* is morphologically distinct from *A. terreus* in that the colonies grow as bright yellow colonies on both Czapek agar and MEA, with less sporulation and the presence of globose sclerotium-like bodies in light tan shades. Despite this distinctive phenotype, phylogenetic analyses placed these isolates (CBS 130.55 = NRRL 2399; NRRL 4609) in a basal cluster within a broader, well-supported *A. terreus* clade. Thus, the taxonomic status of *A. terreus* var. *africanus* could not be clearly resolved in this study using the three-locus phylogeny. Several *A. terreus* isolates recovered from diverse geographic origins appeared to share the same multilocus haplotype, with no clear correlation between genotypes of *A. terreus* isolates, their source (environmental versus clinical), and their geographic origin. These results are similar to the findings that *A. fumigatus* persists as a single, global phylogenetic population with no evidence of endemism (10, 12). Thus, *A. terreus* appears to be a cosmopolitan fungus, with several genotypes spread worldwide. Although, in this study, sequences generated from the three loci yielded sufficient diversity to clearly delineate species within the section *Terrei*, the proposed scheme had limited utility in strain discrimination. It was recently shown that a multilocus sequence typing scheme was not useful for strain discrimination in *A. fumigatus* because of low genetic diversity (2). Similarly, data from this study indicate that a multilocus sequence typing scheme, albeit using highly conserved genes, may not be suitable to discriminate individuals in a population of *A. terreus*: other subtyping schemes such as microsatellite marker-based formats may be more useful for *A. terreus* strain typing.

In conclusion, employing multilocus phylogenetic analyses, the present study describes a new species within the section *Terrei*: *A. alabamensis*. Preliminary evidence suggests that members of the newly recognized species *A. alabamensis* are often colonizing isolates, but a larger screening study and pathogenesis studies with an in vivo model of invasive aspergillosis will be needed to validate this observation.

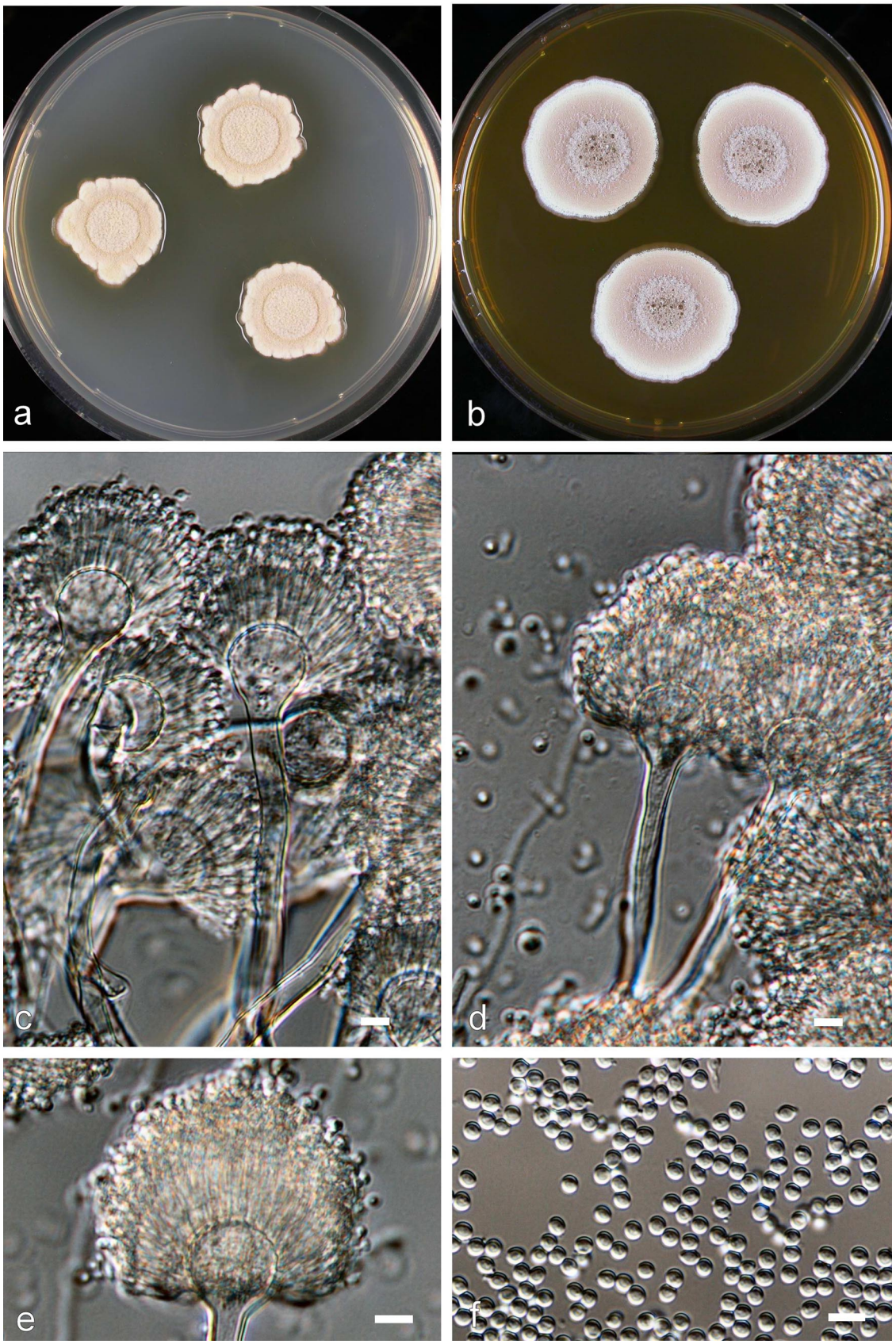

FIG. 5. *Aspergillus alabamensis* sp. nov. UAB 20T. Shown are colonies on MEA after 7 days at 25°C on CYA (a) and on MEA (b), conidial heads (c and d), a single conidial head (e), and conidia (f). Bar, 10 μm.

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