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Molecular and Phenotypic Data Supporting Distinct Species Statuses for *Scedosporium apiospermum* and *Pseudallescheria boydii* and the Proposed New Species *Scedosporium dehoogii*▽†‡

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Based on the morphological, physiologic, and molecular (β-tubulin gene) study of 141 isolates of the *Pseudallescheria boydii* species complex (including several synonyms) and relatives, the new species *Scedosporium dehoogii* is proposed. *Scedosporium apiospermum* and *P. boydii* are considered two different species and the new name *Scedosporium boydii* is proposed for the anamorph of the latter species. A summary of the key morphological and physiological features for distinguishing the species of *Pseudallescheria*/*Scedosporium* is provided.

In recent years molecular phylogenetic analyses based on DNA sequences have promoted a great change in the taxonomy of clinical fungi (4). It is now accepted that numerous common pathogenic species, traditionally considered homogeneous, are indeed polyphyletic (1, 6, 9–12). *Pseudallescheria boydii*, one of the most common clinical molds, after *Aspergillus fumigatus*, is another example. Based on a multilocus study, we recently delineated eight phylogenetic species among isolates identified as this species, grouped in five different clades (2). Clades 1 and 2 were described as new species; since only the anamorphic state was observed in isolates of the first clade, it was assigned to *Scedosporium* (the anamorph genus of *Pseudallescheria*) as *S. aurantiacum*, and clade 2 was named *Pseudallescheria minutispora* because the teleomorph was present. Clade 5 consisted of four subgroups incorporating the type strains of *P. boydii*, *Pseudallescheria angusta*, *Pseudallescheria ellipsoidea*, and *Pseudallescheria fusoidea*. Clades 3 and 4 remained unnamed. We have phenotypically characterized here these eight phylogenetic species. In order to increase the robustness of the different clades, we have included numerous fresh isolates in the present study identified by sequencing the TUB region of the β-tubulin gene, the most informative molecular marker of the four evaluated previously (2).

A total of 141 isolates was studied, including the available reference or type strains of synonymous species of *P. boydii* and *Scedosporium apiospermum* (see the supplemental material). The procedures for DNA extraction, amplification, sequencing, and phylogeny were described previously (2). Morphology was assessed by features observed on potato dextrose agar (PDA) and on oatmeal agar after incubation at 25°C for 2 months.

Fifty-nine physiological tests were performed in duplicate. Inocula, adjusted to 10^5 conidia/ml by hemacytometer counts, were prepared from 7-day-old PDA plates. Growth, including growth on cycloheximide (0.05 to 0.1%), and assimilation abilities were tested in liquid medium according to the method of Yarrow (15). All of the tests, with the exception of urease, gelatin liquefaction, acid production, and starch formation, were performed in sterile, disposable, multiwell microplates. The medium was dispensed into the wells in 150-μl volumes with a multichannel pipette, and each well was inoculated with 50 μl of the conidial suspension. The microplates were incubated at 25°C in darkness for 14 days. For thermotolerance studies, the isolates were subcultured onto PDA and incubated in darkness for 14 days.

A heuristic search of the partial sequence of the TUB region produced 18 "most-parsimonious trees"; one of them is shown in Fig. 1. The topology of the tree was similar to that obtained in our previous study (2). Most of the new isolates were included in clade 4, which makes it the most common phylogenetic species of the complex. The polymorphic nucleotides for all of the species of the *P. boydii* complex are shown in Table 1.

A relatively large number of carbon and nitrogen sources were assimilated by all of the species studied (see the supplemental material). The assimilation patterns for *P. boydii*, *P. angusta*, *P. ellipsoidea*, and *P. fusoidea* were similar, with no significant differences. Assimilation of sucrose, maltose, D-ribose, L-arabinitol, and ribitol and growth at 40 and 45°C were the most useful characteristics for discriminating the species included in the study (Table 2).

The morphology of the conidiophores and the sessile conidia (conidia borne individually along the sides of the vegetative hyphae), and the appearance of the colonies on PDA were the most useful phenotypic features for separating the different clades (Table 2). None of the isolates of clades 3 and 4 devel-

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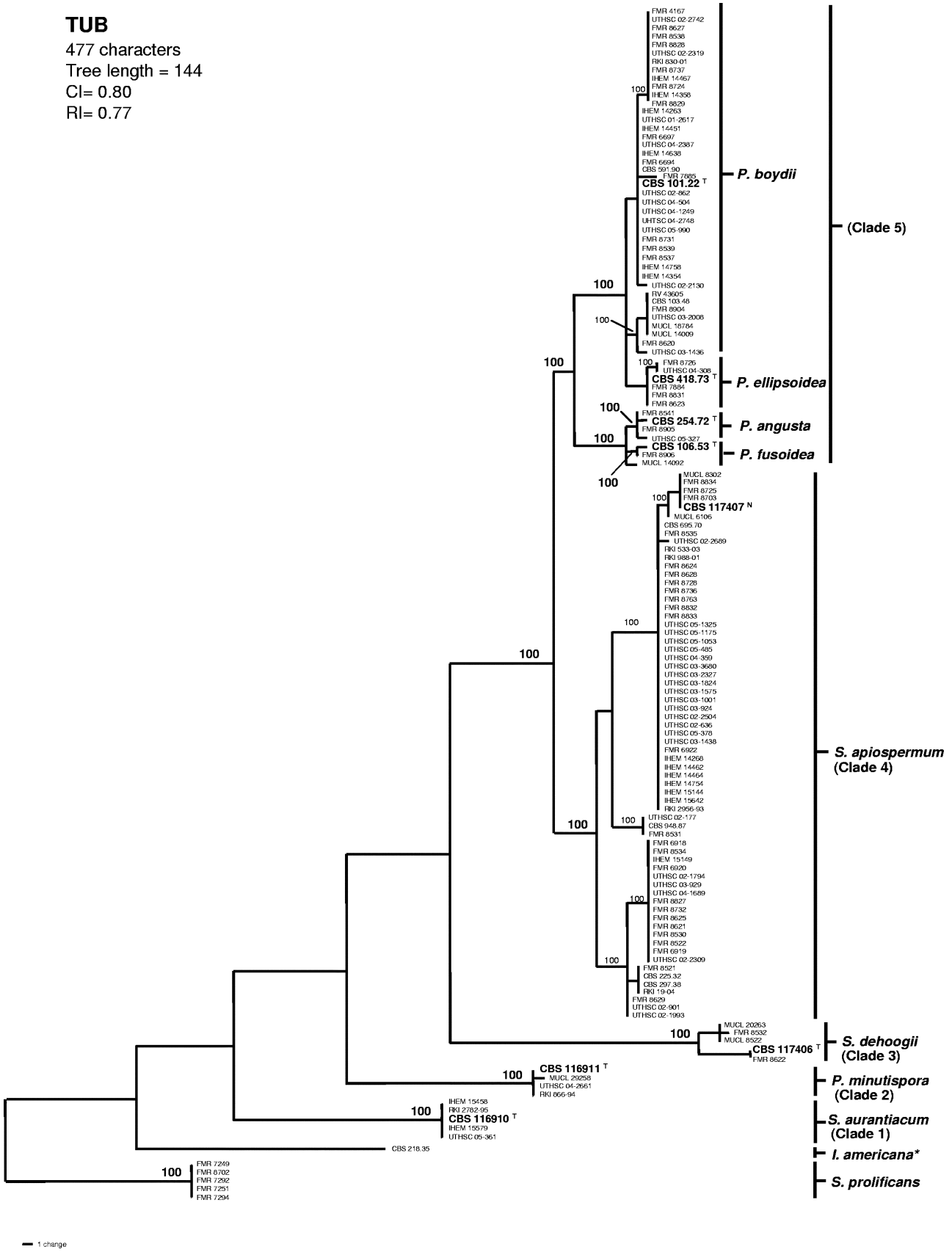

FIG. 1. One of the 18 most-parsimonious trees obtained from heuristic searches based on TUB sequences. Bootstrap support values of 100% are indicated at the nodes. Type strains are indicated with boldface type and with a superscript "T". Neotype is indicated by boldface type and with a superscript "N". Strains of *S. prolificans* were used as outgroups. CI, consistency index; RI, retention index; *I*, *Indiella*; *P*., *Pseudallescheria*; *S.*, *Scedosporium*. ∗, The reference strain of *Indiella americana*.

TABLE 1. Polymorphic sites in the TUB locus (exons 5 and 6) identified by using DnaSP v.4.10.3

Species	Nucleotide at polymorphic site at indicated position[a]																																							
	47	74	113	116	119	125	128	131	146	155	182	209	230	250	254	257	258	261	262	263	268	273	275	282	283	284	285	286	290	291	296	297	298	300	302						
P. boydii	C	T	G	T	T	T	T	T	T	G	T	C	C	A	T	A	T	C	T	A	—	—	—	—	—	—	—	C	A	A	C	T	A	G	T						
S. apiospermum	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	C/·	·	—	—	—	—	—	—	—	·	·	G	·	·	·	·	·						
S. aurantiacum	G	·	·	C	·	·	·	·	·	·	·	·	T	G	·	T	C	—	—	—	G	C	A	C	C	C	C	·	C	C/—	·	C	C	A	A						
S. dehoogii	·	C	T	·	G	·	C	·	·	·	C	·	·	·	·	T	·	T/·	·	T/—	—	—	—	—	T	T	T	·	T	·	·	·	·	A	A						
P. angusta	·	·	·	·	·	C	·	·	C	·	·	·	·	·	·	·	·	T	·	·	—	—	—	—	—	—	—	A	·	G	T	·	·	A	·						
P. ellipsoidea	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	—	—	—	—	—	—	—	·	·	·	·	·	·	·	·						
P. fusoidea	G	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	T	·	·	—	—	—	—	—	—	—	A	·	G	T	·	·	A	·						
P. minutispora	G	·	·	·	·	·	·	C	·	T	·	·	·	·	G	T	·	—	—	—	G	G	T	T	T	T	T	·	—	—	—	—	—	—	—						

oped the teleomorph (sexual state) after 2 months, while all of them developed the *Scedosporium* anamorph. Synnematous conidiophores (*Graphium* anamorph) were usually absent in isolates from clade 3, whereas they were present in more than 90% of the isolates in clade 4. Clade 3 was characterized by solitary and usually unbranched conidiophores, subhyaline to pale gray and thin-walled, sessile conidia, and pale gray colonies (Fig. 2), whereas members of clade 4 showed branched conidiophores, brownish and thick-walled sessile conidia, and brownish colonies (Fig. 3).

Given the genotypic and phenotypic uniqueness of clade 3, we therefore propose the new species *Scedosporium dehoogii*.

Scedosporium dehoogii Gilgado, Cano, Gené et Guarro, sp. nov. = *Clade 3* sensu Gilgado et al. (2).

Coloniae dilute griseae coloratae. Conidiophora solitaria plerumque non-ramosa. Conidia sessilis subhyalina vel dilute grisea, tenuitunicata, plerumque obovata, 5 vel 8 per 5 vel 6 μm. Teleomorphosis ignota. Assimilantur ribitolum, L-arabinitolum, sucrosum et maltosum. Non assimilantur D-ribosum. Augmentum fit in temperatura 37°C.

The colonies on PDA attained a diameter of 45 to 60 mm at 25°C after 14 days. They were cottony and white to pale gray with a colorless reverse. Solitary conidiophores were usually reduced to conidiogenous cells, which were subhyaline, smooth-walled, usually cylindrical, 6 to 50 μm long by 1 to 1.5 μm wide, and produced pale brown, obovoid or ellipsoidal conidia measuring 6 to 11 μm long by 4 to 5 μm wide. Synnematous conidiophores were erect, 80 to 450 μm long, and terminated in a slimy head of conidia. Those conidia were cylindrical or claviform, 6 to 11 μm long by 3 to 4 μm wide, with a wide truncate base. Sessile conidia were subhyaline to pale gray, thin-walled, mostly obovate, 5 to 8 μm long by 5 to 6 μm wide. A teleomorph was not observed for any isolate after 2 months. Maximum growth temperature was at 37°C (5 to 10 mm in diameter after 14 days). The fungus was able to assimilate ribitol, L-arabinitol, sucrose and maltose, but not D-ribose.

Etymology. Derived from the name of the mycologist G. Sybren de Hoog.

A dry culture of the strain isolated from garden soil (Barcelona, Spain) has been deposited in the International Mycological Institute-CABI Bioscience (Egham, England) as IMI 394089 (holotype). A living culture of the isolate has been deposited in the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) as CBS 117406.

The members of clade 4 are morphologically indistinguishable from the anamorph of *Pseudallescheria boydii* (clade 5), but they can be separated by the response to D-ribose test and by the absence of a teleomorph (Table 2). Clade 4 also included the

TABLE 2. Morphological and physiological key characters for differentiating among species of the *Pseudallescheria*/*Scedosporium* complex and *S. prolificans*[a]

Species	Conidiogenous cells	Sessile conidia	Colony reverse in orange shades[b]	Yellow diffusible pigment[b]	Assimilation of:					Growth at:	
					Ribitol	L-Arabinitol	Sucrose	Maltose	D-Ribose	40°C	45°C
P. boydii and relatives[c] (clade 5)	Cylindrical	Globose to subglobose, thick-walled	−	V	+	+	+	+	+	+	−
P. minutispora (clade 2)	Cylindrical	Ellipsoidal to obovoid, thin-walled	−	−	+	+	+	−	−	+	−
S. apiospermum (clade 4)	Cylindrical	Globose to subglobose, thick-walled	−	V	+	+	+	+	−	+	−
S. aurantiacum (clade 1)	Cylindrical or slightly flask-shaped	Mostly obovoid, thick-walled	+	+	+	+	−	+	+	+	+
S. dehoogii (clade 3)	Cylindrical or slightly flask-shaped	Mostly obovoid, thin-walled	−	−	+	+	+	+	−	−	−
S. prolificans	Flask-shaped	Globose to subglobose, thick-walled	−	−	−	−	−	+	−	+	V

[a] Abbreviations and symbols: −, all strains of the species displayed a negative response; +, all strains of the species displayed a positive response; V, variable (i.e., some strains were positive and others were negative).
[b] That is, on PDA at 25°C.
[c] That is, *P. angusta*, *P. ellipsoidea,* and *P. fusoidea*. These species can be identified by other molecular and morphological characteristics.

TABLE 1—*Continued*

Nucleotide at polymorphic site at indicated position[a]

303	305	306	307	320	321	322	329	332	333	335	336	337	358	359	360	361	363	364	368	369	370	379	380	382	383	384	385	389	392	400	415	439	496	499	508	511	532	535
C	C	T	G	C	G	A	C	T	C	C	C	—	G	T	G	A	G	A	C	T	C	A	G	A	G	T	T	C	T	C	T	G	C	T	T	T	C	T
·	·	·	·	A/G	·	·	·	·	·	T	—	—	·	C	·	G	·	·	·	·	·	·	A	G	·	G	G	T	·	·	·	·	·	·	A	·	·	·
·	A	C	A	·	·	G	·	·	G	T	G	T	—	—	A	G	·	G	—	C	·	—	·	G	·	C	·	T	C	T	·	·	T	C	C	C	·	C
·	·	G	A	T	A	G	G	C	G	T	—	—	A	·	·	·	A	C	—	·	T	G	T	G/C	T	·	·	—	·	T	·	·	·	·	C	·	·	·
·	·	·	·	·	·	·	·	·	·	·	T	—	·	·	·	·	·	·	T	·	·	·	·	G	·	G	·	T	·	·	·	·	·	·	·	·	·	·
T	·	·	·	·	·	·	·	·	·	·	T	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
·	·	·	·	·	·	·	·	·	·	·	T	—	·	·	·	·	·	·	T	·	·	·	·	G	·	G	·	T	·	·	·	·	·	·	·	·	·	·
—	—	—	—	—	—	—	G	—	—	—	—	—	—	—	·	·	A	G	—	·	·	C	·	G	T	G	·	·	·	T	C	A	·	C	C	C	T	·

[a] Sites with alignment gaps were included if there was a polymorphism. The sequence of type strain of *P. boydii* CBS 101.22 (GenBank accession no. AJ890121) was used as the master sequence. Nucleotides identical to the corresponding nucleotides in the *P. boydii* type strain sequence are shown as dots. A dash (—) denotes a gap. The data were adapted from a study by Rozas et al. (12a).

type strains of *Acremonium suis*, *Sporocybe borzinii*, and *Polycytella hominis* and the only reference strain of *Scedosporium apiospermum*. Therefore, the clade 4 must be identified as *S. apiospermum*, since this name has priority from a nomenclatural point of view over the other species included in the clade. A previous name is *Monosporium apiospermum*, which was proposed by Saccardo (13) in 1911 based on a mycetoma isolate, but later *Monosporium* was considered as a nomen illegitimum (5). One of the most important aspects of this study has been to precisely demonstrate that *S. apiospermum* (clade 4) and *P. boydii* (clade 5) are really two different species. Up to now, the former had been considered as the anamorph of the latter (7, 8).

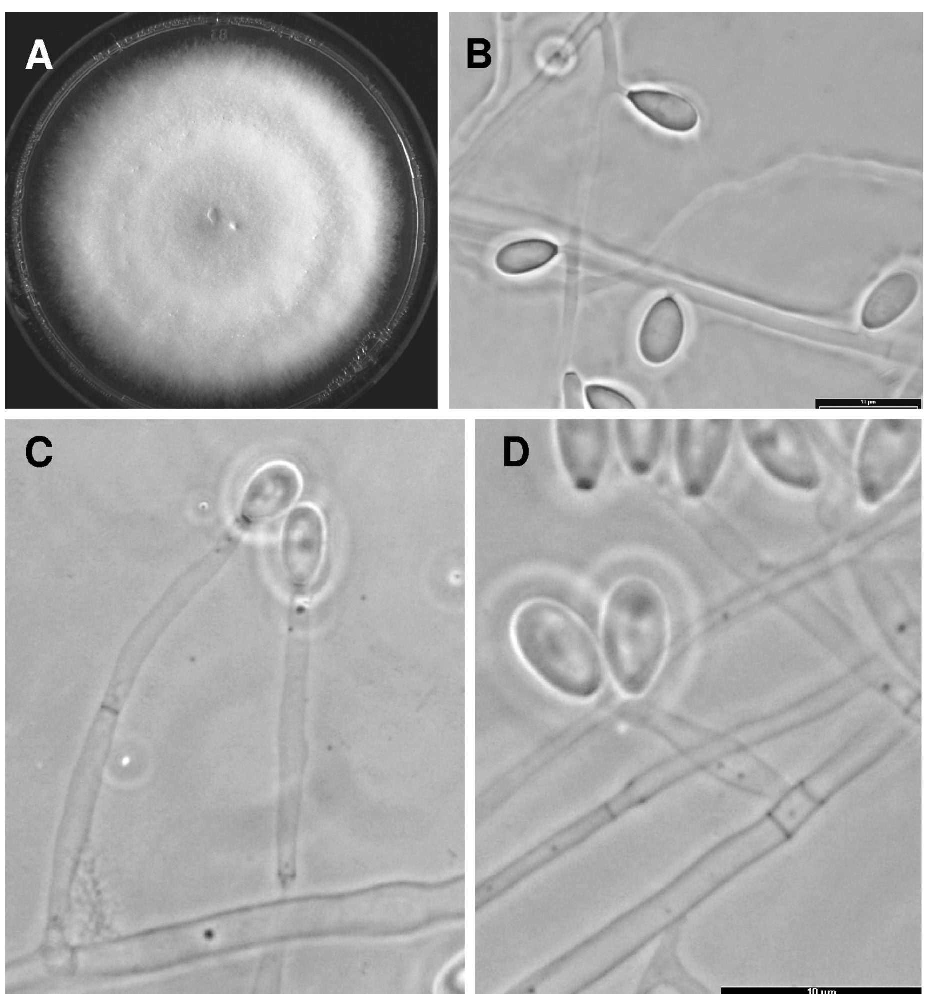

FIG. 2. *Sedosporium dehoogii* (CBS 117406). (A) Colony growing on PDA after 14 days at 25°C. (B) Sessile conidia. (C and D) Conidiogenous cells and conidia.

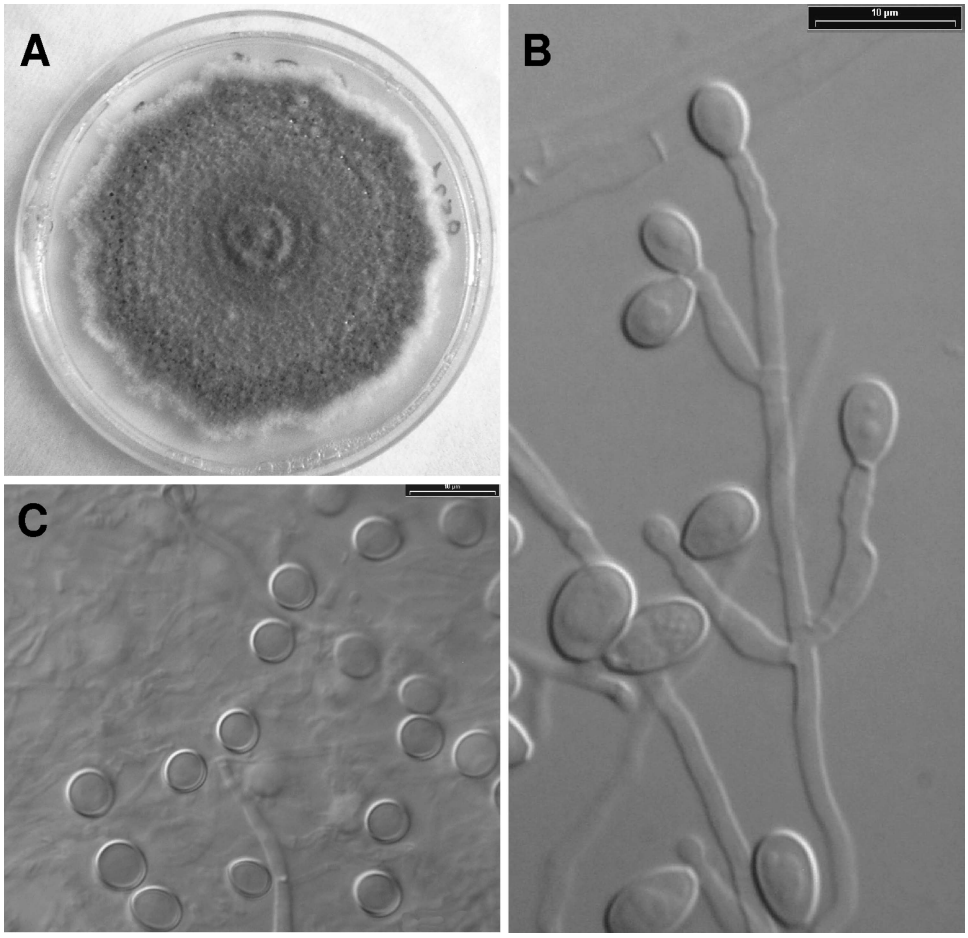

FIG. 3. *Scedosporium apiospermum* (CBS 117407). (A) Colony growing on PDA after 14 days at 25°C. (B) Conidiogenous cells and conidia. (C) Sessile conidia.

Since the type of *S. apiospermum* is apparently lost, choosing a neotype for this species is required taxonomically. It may be possible to select the reference strain CBS 225.32 since, according to the CBS curators (unpublished data), this strain is presumed to be a subculture of the original strain from which Saccardo based the description of the species. However, over the years this strain has degenerated, and it sporulates very poorly in all of the media tested. We therefore preferred to choose as a neotype an isolate of the same clade which showed a better sporulation in order to illustrate the most important morphological features of *S. apiospermum* (Fig. 3). A dry culture of the strain isolated from human keratitis (São Paulo, Brazil) has been deposited in the CABI Bioscience (Edgham, England) database as IMI 394090 (neotype). A living culture of the isolated has been deposited in the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) database as CBS 117407.

A new name is then required for the anamorph of *P. boydii*. Because it was originally described as *Cephalosporium boydii* by Shear in 1922 (14) and later defined as a *Scedosporium* species, it can be classified as *Scedosporium boydii* (Shear) Gilgado, Gené, Cano et Guarro comb. nov. (Basionym: *Cephalosporium boydii* Shear, Mycologia **14:** 242, 192).

The study of the type strains of *Acladium castellanii*, *Sporocybe chartoikoon*, and *Sporotrichum councilmanii* (see the supplemental material), also considered synonyms of *S. apiospermum*/*P. boydii*, revealed that they are morphologically incompatible with the anamorphs of the *P. boydii* complex. The internal transcribed spacer regions of these strains were sequenced and, when compared to the sequences of the GenBank database, none matched the species in the *P. boydii* complex. The only synonymous species that was morphologically compatible with anamorphs of *Pseudallescheria* was *Indiella americana*. However, the TUB sequence placed this species in a branch phylogenetically distant to the species of the *P. boydii* complex (Fig. 1). Because the taxonomy of *I. americana* is ill defined, an extensive study with more strains would be required for the proper delineation of this species.

Traditionally, the classification of the fungi of clinical interest has been based on morphology. However, molecular methods, based mainly on sequencing rRNA genes, have recently evolved as useful tools for this purpose. Combining both approaches is not yet a common practice but seems to be the best approach for obtaining a more natural classification. In recent years, different authors have used multigene analyses to demonstrate the existence of numerous cryptic species in important clinical fungi (6, 9, 11). In our study, we used a polyphasic approach that combines morphological, physiologic, and mo-

lecular data sets to characterize the species of the genus *Pseudallescheria* and its relatives.

Although it has been shown that the TUB region is a good marker for delimiting the different species of the complex, many clinical laboratories may not have the capability for molecular characterization of isolates. The fact that these species can also be identified by using simple and inexpensive phenotypic methods has made their delineation in routine laboratories possible. Now that phylogenetic species of the *P. boydii* complex can be recognized and their different responses to the antifungals drugs have been demonstrated (3), it will be of interest to see whether they also elicit different clinical manifestations.

We are grateful to G. Sybren de Hoog (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) for useful comments on the taxonomy of the fungi included in the study.

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