Molecular Phylogeny of the *Pseudallescheria boydii* Species Complex: Proposal of Two New Species[†]

Felix Gilgado, Josep Cano, Josepa Gené, and Josep Guarro*

Unitat de Microbiologia, Facultat de Medicina i Cièncias de la Salut, Universitat Rovira i Virgili, Reus, Tarragona, Spain

Received 21 March 2005/Returned for modification 5 May 2005/Accepted 20 July 2005

Pseudallescheria boydii (anamorph *Scedosporium apiospermum*) is the species responsible for human scedosporiosis, a fungal infection with a high mortality rate and which is difficult to treat. Recently, it has been demonstrated that high genetic variation exists within this species. We have performed a morphological and molecular study involving numerous strains of clinical or environmental origins and from different countries. The analysis of partial sequences of the β -tubulin (two loci) and calmodulin genes and the internal transcribed spacer region of the rRNA gene has demonstrated that *P. boydii* is a species complex. The combined analysis of the sequences of the four loci of 60 strains has showed the presence of 44 haplotypes in the ingroup. Three species morphologically related to *P. boydii* sensu stricto, i.e., *Pseudallescheria angusta, Pseudallescheria ellipsoidea*, and *Pseudallescheria fusoidea*, which had previously been considered synonyms, could be differentiated genetically from *P. boydii* in our study. It is relevant that two of the three strains now included in *P. ellipsoidea* have caused invasive infections. The species *Pseudallescheria minutispora* and *Scedosporium aurantiacum* are clearly phylogenetically separated from the other species studied and are here proposed as new. Morphological features support this proposal. All the strains included in *S. aurantiacum* species have a clinical origin, while those included in the *P. boydii* complex have different clinical spectra and antifungal susceptibility.

Pseudallescheria boydii (anamorph *Scedosporium apiospermum*) is a ubiquitous ascomycetous fungus that causes a wide array of human infections that can affect practically all the organs of the body (8). These infections have been known for a long time, but in recent years, a marked increase in severe invasive infections has been noticed, mainly in immunocompromised hosts. The treatment of these infections has not yet been resolved, and the mortality rate is very high (3, 17). One of the most typical features of this species, which is very rare in other pathogenic fungi, is its ability to develop sexual structures on routine culture media. The presence of spherical ascomata (cleistothecia) and fusiform or ellipsoidal ascospores allows easy identification of this species and its differentiation from the other species of *Scedosporium, Scedosporium prolificans*, whose sexual state still remains unknown.

On the basis of nuclear DNA-DNA reassociation, some studies have proved that important genetic variation exists in *P. boydii*. Gueho and de Hoog (10) found three infraspecific ecological and clinical groups. Rainer et al. (16) reported the existence of five different small-subunit rRNA gene sequence lengths. Random amplified polymorphic DNA studies also demonstrated that numerous and very different genotypes can be found (7). Other authors have reported considerable differences with respect to growth and sporulation (4, 5, 9). In addition, a high variability in antifungal susceptibility of the different isolates and in their clinical response has been observed (1, 2). All these data seem to suggest that *P. boydii* is

probably a species complex. In recent years, application of the phylogenetic species concept in different biological species of pathogenic fungi has revealed phylogenetic lineages that reflected species divergence (12, 13) and the existence of cryptic species. These putative cryptic species in *P. boydii* can show different pathological behavior and different antifungal susceptibility, so their delimitation and characterization are key in order to choose the appropriate treatment of the severe infections caused by these fungi.

This paper reports the results of a combined phenotypic and phylogenetic study of numerous clinical and environmental strains, including several fresh isolates, of the *P. boydii* species complex and the description of two new species.

MATERIALS AND METHODS

Fungal isolates. Sixty isolates of *Pseudallescheria boydii* and relatives from environmental or clinical sources were included in the study (Table 1). Clinical isolates were provided by different reference culture collections (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands [CBS], Facultat de Medicina i Ciències de la Salut, Reus, Spain, [FMR], The BCCM/IHEM Biomedical Fungi and Yeasts Collection, Brussels, Belgium [IHEM], Mycotheque de l'Universite Catholique de Louvain, Belgium [MUCL], Collection of the Institute for Tropical Medicine, Antwerp, Belgium [RV], and Robert Koch Institute, Berlin, Germany [RKI]) or different physicians. Environmental isolates were generally fresh isolates recovered by the authors from soil samples from different geographical regions, and others were also provided by different culture collections. In addition, reference strains of each of the species *Pseudallescheria angusta, Pseudallescheria ellipsoidea*, and *Pseudallescheria fusoidea* were included in the study. Isolates were stored at 4 to 7°C until morphological or molecular studies were performed.

Isolation from soil. Soil samples were collected mainly from the superficial layer of soil (mainly garden soils) by using sterilized polyethylene bags. These bags were closed by rubber bands and labeled. Suspensions of this material were cultured on the selective medium Dichloran Rose-Bengal chloramphenicol agar (Oxoid, United Kingdom) with benomyl added at a final concentration of 10 μ g/ml and incubated at room temperature. When typical colonies of *P. boydii* were observed, we tried to isolate them in pure culture.

^{*} Corresponding author. Mailing address: Unitat de Microbiologia Facultat de Medicina i Ciencias de la Salut, Universitat Rovira i Virgili C/Sant Llorenç 21, 43201 Reus, Tarragona, Spain. Phone: 34 977 759359. Fax: 34 977 759322. E-mail: josep.guarro@urv.net.

[†] A publication of the ECMM Working Group on Pseudallescheriasis.

biolog BT2 TUB CAL ITS FMR 4072 River sediment, Tordera River, Spain Al889592 Al880216 Al880216 Al88021	T 1 .		GenBank accession no.				
FMR 4072 River sediment, Tordera River, Spain AJ889792 AJ890122 AJ888344 FMR 4167 Ottits, Valladolid, Spain AJ888953 AJ889580 AJ890133 AJ888354 FMR 607 Sputum, Madrid, Spain AJ888955 AJ889552 AJ890155 AJ88857 FMR 6018 Garden soil, Barcelona, Spain AJ888576 AJ889767 AJ8890157 AJ888433 FMR 6920 Garden soil, Barcelona, Spain AJ88857 AJ889767 AJ8890157 AJ889157 FMR 6921 Garden soil, Barcelona, Spain AJ888764 AJ880157 AJ880157 AJ880157 AJ880157 AJ880168 AJ890126 AJ880159 AJ890129 AJ88843 FMR 6921 Garden soil, Barcelona, Spain AJ889630 AJ890126 AJ880129 A	Isolate	Source	BT2	TUB	CAL	ITS	
FMR 4167 Ottisis, Valladolid, Spain AI888952 AI8890152 AI888355 FMR 6047 Carebral abseess, Barcelona, Spain AI888954 AI889554 AI889154 AI889154 AI888355 FMR 6018 Garden soil, Barcelona, Spain AI888955 AI889574 AI88956 AI889156 AI889156 AI889156 AI889156 AI889156 AI889156 AI889156 AI889157 AI889158 AI889157 AI889356 AI889158 AI889159 AI889159 AI889159 AI889159 AI889159 AI889159 AI889159 AI889159 AI889159 AI889116 AI889359 AI890116 AI898159 AI890116 AI898159 AI8901179 AI888435 FVRR 8522 Curlivated soil, Montsia, Spain AI889567 AI890178 AI889174 AI890178 AI889457 AI890179 AI889435 AI890178 AI890178 AI889457 AI890178 AI890178 AI890178 AI898118 AI890117 AI89	FMR 4072	River sediment, Tordera River, Spain	AJ889592	AJ890122	AJ890216	AJ888384	
FNR 6604 Cerchnal abscess, Enarcelona, Spain AIS88951 AJS89551 AJS89512 AJS89151 AJS88355 FNR 6018 Garden soil, Barcelona, Spain AJS88955 AJS89552 AJS89155 AJS88157 FNR 6018 Garden soil, Barcelona, Spain AJS88576 AJS89956 AJS89175 AJS88154 FNR 6020 Garden soil, Barcelona, Spain AJS88571 AJS89756 AJS89175 AJS89175 AJS89176 AJS89177 AJS89178 AJS89177 AJS89178 AJS8	FMR 4167	Otitis, Valladolid, Spain	AJ888952	AJ889580	AJ890152	AJ888385	
FNR 6097 Sputtum, Madrid, Spain AJ88955 AJ88955 AJ88955 AJ88955 AJ88955 AJ88955 AJ88955 AJ88956 AJ88956 AJ88956 AJ88957 AJ88956 AJ88957 AJ88657 AJ88657 AJ889017 AJ88857 AJ880178 AJ88857 AJ890017 AJ88857 AJ890017 AJ88857 AJ8900178 AJ88857 <t< td=""><td>FMR 6694</td><td>Cerebral abscess, Barcelona, Spain</td><td>AJ888953</td><td>AJ889581</td><td>AJ890153</td><td>AJ888386</td></t<>	FMR 6694	Cerebral abscess, Barcelona, Spain	AJ888953	AJ889581	AJ890153	AJ888386	
FMR 6018 Garden soil, Barcelona, Spain AJ88955 AJ889055 AJ889055 AJ889055 AJ889056 AJ889056 AJ889056 AJ889056 AJ889056 AJ889056 AJ889056 AJ889056 AJ889057 AJ890126 AJ88057 AJ890017 AJ88857 AJ890017 AJ88557 AJ890017 AJ88557 AJ890017 AJ88557 AJ890118 AJ88557 AJ890118 AJ88557 AJ890118 AJ88557 AJ890118 AJ88557 <t< td=""><td>FMR 6697</td><td>Sputum, Madrid, Spain</td><td>AJ888954</td><td>AJ889582</td><td>AJ890154</td><td>AJ888387</td></t<>	FMR 6697	Sputum, Madrid, Spain	AJ888954	AJ889582	AJ890154	AJ888387	
FMR 6919 Garden soil, Barcelona, Špain AJ88956 AJ889956 AJ889956 AJ889956 AJ889956 AJ889956 AJ889956 AJ889966 AJ889956 AJ889958 AJ889958 AJ889958 AJ889958 AJ889958 AJ889958 AJ889958 AJ889958 AJ889958 AJ8890159 AJ888359 FMR 7884 Transplant, Madrid, Spain AJ889561 AJ890016 AJ888357 AJ890016 AJ888357 FMR 7885 Forrest soil, Montsia, Spain AJ88955 AJ890018 AJ888431 FMR 8530 Cultivated soil, Montsia, Spain AJ88055 AJ890011 AJ888417 FMR 8531 Cultivated soil, Montsia, Spain AJ880561 AJ890011 AJ890183 AJ888407 FMR 8535 Soid, Buencos Aires, Argentina AJ889861 AJ890110 AJ890186 AJ890110 AJ890187 AJ888417 FMR 8535 Soid, Buencos Aires, Argentina AJ889861 AJ890114 AJ888417 AJ890189 AJ888411 FMR 8540 Soid, Buencos Aires, Argentina AJ8890565 AJ890112 AJ890190 AJ888411 <tr< td=""><td>FMR 6918</td><td>Garden soil, Barcelona, Spain</td><td>AJ888955</td><td>AJ889583</td><td>AJ890155</td><td>AJ888433</td></tr<>	FMR 6918	Garden soil, Barcelona, Spain	AJ888955	AJ889583	AJ890155	AJ888433	
FNR 6020 Garden soil, Barcelona, Spain AJS88957 AJS89967 AJS89978 AJS89917 AJS88358 FNR 6022 Garden soil, Barcelona, Spain AJS88958 AJS89907 AJS89126 AJS88359 FNR 7864 Fransplant, Madrid, Spain AJS88954 AJS900126 AJS80120 AJS88359 FNR 7855 Pleural liquid, Madrid, Spain AJS8855 AJS90017 AJS88440 FNR 8521 Forrest soil, Dellebre, Spain AJS8855 AJS90011 AJS88440 FNR 8535 Cultivated soil, Dellebre, Spain AJS8857 AJS90014 AJS8016 AJS88450 FNR 8535 Soil, Buenos Aires, Argentina AJS89860 AJS90014 AJS8016 AJS88407 FNR 8535 Soil, Buenos Aires, Argentina AJS89862 AJS90113 AJS84108 FNR 8535 Soil, Buenos Aires, Argentina AJS89864 AJS90113 AJS80109 AJS8445 FNR 8536 Soil, Buenos Aires, Argentina AJS89056 AJS90115 AJS80116 AJS80116 AJS80116 AJS80116 AJS80116 AJS80117 AJS80117 AJS80117 </td <td>FMR 6919</td> <td>Garden soil, Barcelona, Spain</td> <td>AJ888956</td> <td>AJ889985</td> <td>AJ890156</td> <td>AJ888388</td>	FMR 6919	Garden soil, Barcelona, Spain	AJ888956	AJ889985	AJ890156	AJ888388	
FMR 6921 Garden soil, Barcelona, Spain AJ88955 AJ889958 AJ890158 AJ883505 FMR 7884 Transplant, Madrid, Spain AJ889559 AJ890129 AJ88359 FMR 7885 Transplant, Madrid, Spain AJ889564 AJ8900160 AJ88357 FMR 8521 Forest soil, Montsia, Spain AJ889554 AJ890016 AJ888431 FMR 8522 Cultivated soil, Detlebre, Spain AJ889554 AJ890012 AJ888431 FMR 8532 Cultivated soil, Montsia, Spain AJ88955 AJ890011 AJ888401 FMR 8535 Soil, Buenos Aires, Argentina AJ889661 AJ890114 AJ880118 AJ888401 FMR 8535 Soil, Buenos Aires, Argentina AJ889654 AJ890114 AJ890118 AJ888419 FMR 8535 Soil, Buenos Aires, Argentina AJ8896564 AJ890114 AJ890118 AJ888419 FMR 8541 Soil, Buenos Aires, Argentina AJ889656 AJ890112 AJ890118 AJ888419 FMR 8540 Soil, Buenos Aires, Argentina AJ8989565 AJ890116 AJ890191 AJ890191 AJ890191	FMR 6920	Garden soil, Barcelona, Spain	AJ888957	AJ889986	AJ890157	AJ888434	
FMR 6922 Garden soil, Barcelona, Spain AJ88959 AJ889026 AJ890206 AJ88839 FMR 7884 Pieural liquid, Madrid, Spain AJ889504 AJ890176 AJ888430 FMR 8521 Forest soil, Montsia, Spain AJ889853 AJ890107 AJ888430 FMR 8522 Forest soil, Detlebre, Spain AJ889857 AJ890118 AJ889012 AJ889107 AJ88840 FMR 8532 Cultivated soil, Montsia, Spain AJ889857 AJ890113 AJ889133 AJ889113 AJ889118 AJ888418 FMR 8535 Soil, Buenos Aires, Argentina AJ889860 AJ890111 AJ890187 AJ889111 AJ889118 AJ889114 AJ889118 AJ889118 AJ889114 AJ889118 AJ889114 AJ889118 AJ889114 AJ889114 <td>FMR 6921</td> <td>Garden soil, Barcelona, Spain</td> <td>AJ888958</td> <td>AJ889987</td> <td>AJ890158</td> <td>AJ888389</td>	FMR 6921	Garden soil, Barcelona, Spain	AJ888958	AJ889987	AJ890158	AJ888389	
FMR 7884 Transplant, Madrid, Spain AJ889594 AJ880160 AJ888302 FMR 7885 Picural liquid, Madrid, Spain AJ889054 AJ88007 AJ880178 AJ88832 FMR 8521 Forest soil, Detlebre, Spain AJ889854 AJ890107 AJ88917 AJ880178 AJ889178 AJ880178 AJ880178 AJ889178 AJ880178 AJ889178 AJ880112 AJ880183 AJ888407 FMR 8535 Soil, Buenos Aires, Argentina AJ889867 AJ890118 AJ889187 AJ880118 AJ889187 AJ880111 AJ880183 AJ888443 FMR 8535 Soil, Buenos Aires, Argentina AJ889861 AJ890118 AJ889183 AJ889111 AJ889183 AJ880113 AJ880193 AJ888143 FMR 8530 Soil, Buenos Aires, Argentina AJ889864 AJ890113 AJ880112 AJ880193 AJ880115 AJ880193 AJ880115 AJ880193 AJ880115	FMR 6922	Garden soil, Barcelona, Spain	AJ888959	AJ889988	AJ890159	AJ888390	
FMR 7885 Pieural liquid, Madrid, Spain AJ889050 AJ890077 AJ880178 AJ888453 FMR 8521 Forest soil, Detlebre, Spain AJ889853 AJ890101 AJ889178 AJ889457 FMR 8532 Cultivated soil, Montsia, Spain AJ889857 AJ890113 AJ889183 AJ889101 AJ889183 AJ889103 AJ889103 AJ889103 AJ889103 AJ889103 AJ889108 AJ889104 AJ889104 AJ889105 <	FMR 7884	Transplant, Madrid, Spain	AJ889594	AJ890126	AJ890209	AJ888391	
FMR 8521 Forest soil, Montsia, Spain AJ889853 AJ890078 AJ890179 AJ888451 FMR 8530 Cultivated soil, Detlebre, Spain AJ889854 AJ890012 AJ890173 AJ888451 FMR 8530 Cultivated soil, Montsia, Spain AJ889858 AJ890113 AJ890183 AJ888407 FMR 8535 Soil, Buenos Aires, Argentina AJ889860 AJ890114 AJ890186 AJ888409 FMR 8535 Soil, Buenos Aires, Argentina AJ889861 AJ890113 AJ890188 AJ888445 FMR 8530 Soil, Buenos Aires, Argentina AJ889864 AJ890113 AJ880199 AJ888445 FMR 8530 Soil, Buenos Aires, Argentina AJ889054 AJ890113 AJ880199 AJ88841 FMR 8541 Soil, Buenos Aires, Argentina AJ889554 AJ890115 AJ880193 AJ88841 FMR 862 Cystic Brooks, Barcelona, Spain AJ889557 AJ890114 AJ890194 AJ888419 FMR 862 Leukernic patient, Barcelona, Spain AJ88957 AJ890118 AJ890194 AJ889149 FMR 8625 Leukernic patient, Spain	FMR 7885	Pleural liquid, Madrid, Spain	AJ888960	AJ889989	AJ890160	AJ888392	
FMR 852 Forest soil, Deltebre, Spain AJ889854 AJ890018 AJ890179 AJ888431 FMR 853 Cultivated soil, Montsia, Spain AJ889858 AJ890012 AJ890183 AJ888407 FMR 8535 Soil, Buenos Aires, Argentina AJ889460 AJ890114 AJ890186 AJ889160 FMR 8535 Soil, Buenos Aires, Argentina AJ889460 AJ890111 AJ890186 AJ889145 FMR 8535 Soil, Buenos Aires, Argentina AJ889461 AJ890113 AJ890188 AJ888414 FMR 8540 Soil, Buenos Aires, Argentina AJ889464 AJ890113 AJ890190 AJ888415 FMR 8541 Soil, Buenos Aires, Argentina AJ889654 AJ890115 AJ890192 AJ888415 FMR 8612 Cystic fibrosis, Barcelona, Spain AJ889586 AJ890115 AJ890194 AJ888417 FMR 8623 Leukemic patient, Barcelona, Spain AJ889596 AJ8901125 AJ890149 AJ888427 FMR 8624 Leukemic patient, Zaregaze, Spain AJ889577 AJ890113 AJ890149 AJ888420 FMR 8625 Leukemic patient, Zarega	FMR 8521	Forest soil, Montsia, Spain	AJ889853	AJ890007	AJ890178	AJ888430	
FMR 8530 Cultivated soil, Dottsin, Spain AJ89857 AJ890011 AJ890182 AJ888406 FMR 8534 Cultivated soil, Montsia, Spain AJ889859 AJ890013 AJ890185 AJ888406 FMR 8535 Soil, Buenos Aires, Argentina AJ889861 AJ890110 AJ890185 AJ888410 FMR 8535 Soil, Buenos Aires, Argentina AJ889863 AJ890111 AJ890186 AJ889101 FVR 8530 Soil, Buenos Aires, Argentina AJ889863 AJ890112 AJ890189 AJ888411 FVR 8541 Soil, Buenos Aires, Argentina AJ889605 AJ890112 AJ890125 AJ888412 FVR 8610 Keratitis, Brazil AJ889558 AJ890116 AJ890193 AJ888417 FVR 8622 Foot skin, Barcelona, Spain AJ890557 AJ890116 AJ890196 AJ888419 FVR 8625 Leukemic patient, Zaragoza, Spain AJ890588 AJ890119 AJ890196 AJ888420 FVR 8626 Leukemic patient, Zaragoza, Spain AJ889576 AJ890196 AJ888420 FVR 8626 Leukemic patient, Zaragoza, Spain AJ889577	FMR 8522	Forest soil, Deltebre, Spain	AJ889854	AJ890008	AJ890179	AJ888431	
FMR 8532 Cultivated soil, Montsia, Spain AJ889858 AJ89012 AJ891135 AJ888407 FMR 8535 Soil, Buenos Aires, Argentina AJ889866 AJ890114 AJ890186 AJ889101 FMR 8535 Soil, Buenos Aires, Argentina AJ889862 AJ890111 AJ890188 AJ888443 FMR 8535 Soil, Buenos Aires, Argentina AJ889862 AJ890111 AJ890188 AJ888412 FMR 8540 Soil, Buenos Aires, Argentina AJ889864 AJ890113 AJ890190 AJ888412 FMR 8540 Soil, Buenos Aires, Argentina AJ889584 AJ890115 AJ890190 AJ888415 FMR 8619 Keratitis, Brazil AJ889586 AJ890115 AJ890190 AJ888417 FMR 8621 Cycit fibrosis, Barcelona, Spain AJ889586 AJ890117 AJ890190 AJ888420 FMR 8622 Leukemic patient, Zaragoza, Spain AJ88957 AJ890116 AJ888420 FMR 8625 Leukemic patient, Zaragoza, Spain AJ88957 AJ890164 AJ888420 FMR 8626 Cysit fibrosis, patient 4, Giens, France AJ88957 AJ890164	FMR 8530	Cultivated soil, Deltebre, Spain	AJ889857	AJ890011	AJ890182	AJ888406	
FMR 8534 Cultivated soil, Montsia, Spain AJ889855 AJ890013 AJ889055 AJ889061 AJ890110 AJ889156 AJ889166 AJ889166 AJ889166 AJ889166 AJ889166 AJ889166 AJ889165 AJ889165 AJ889165 AJ889165 AJ889165 AJ880112 AJ889168 AJ888443 FNR 8539 Soil, Buenos Aires, Argentina AJ889665 AJ890112 AJ889100 AJ888412 FNR 8541 Soil, Buenos Aires, Argentina AJ889565 AJ890116 AJ890122 AJ888416 FNR 8610 Keratitis, Brazil AJ889585 AJ890116 AJ890192 AJ888418 FNR 8620 Keratitis, Brazil AJ889576 AJ890118 AJ890194 AJ888418 FNR 8621 Costs (Brossis, Barcelona, Spain AJ889578 AJ890119 AJ890104 AJ888420 FNR 8625 Leukemic patient, Zaragoza, Spain AJ889579 AJ890119 AJ890120 AJ888420 FNR 8625 Leukemic patient, Zaragoza, Spain AJ889579 AJ890113 AJ890120 AJ88844 RV 34005 Human, Zaire	FMR 8532	Cultivated soil, Montsia, Spain	AJ889858	AJ890012	AJ890183	AJ888407	
FMR 8535 Soil, Buenos Aires, Argentina AJ889860 AJ89014 AJ89016 AJ889167 FMR 8537 Soil, Buenos Aires, Argentina AJ889862 AJ890111 AJ890187 AJ88841 FMR 8539 Soil, Buenos Aires, Argentina AJ889866 AJ890113 AJ890199 AJ88841 FMR 8540 Soil, Buenos Aires, Argentina AJ889584 AJ890115 AJ890125 AJ888413 FMR 8619 Keratitis, Brazil AJ889585 AJ890116 AJ890192 AJ888417 FMR 8612 Cystic fibrosis, Barcelona, Spain AJ889586 AJ890117 AJ890193 AJ888417 FMR 8623 Leukemic patient, Barcelona, Spain AJ889596 AJ890112 AJ890106 AJ888410 FMR 8625 Leukemic patient, Zaragoza, Spain AJ889597 AJ890113 AJ890106 AJ888420 RV 43063 Human, Zaire AJ888956 AJ890113 AJ890164 AJ889461 RV 43064 Cystic fibrosis, patient 1, Angers, France AJ888957 AJ890113 AJ890164 AJ889451 HIEM 14256 Cystic fibrosis, patient 1, Giens, France<	FMR 8534	Cultivated soil, Montsia, Spain	AJ889859	AJ890013	AJ890185	AJ888408	
FMR 8537 Soil, Buenos Aires, Argentina AJ889861 AJ890110 AJ890187 AJ88917 FMR 8538 Soil, Buenos Aires, Argentina AJ8898663 AJ890112 AJ890190 AJ888443 FMR 8539 Soil, Buenos Aires, Argentina AJ8898663 AJ890128 AJ890120 AJ888412 FMR 8541 Soil, Buenos Aires, Argentina AJ889565 AJ890116 AJ890122 AJ888416 FMR 8610 Keratitis, Brazil AJ889586 AJ890116 AJ890192 AJ888418 FMR 8621 Cystic fibrosis, Barcelona, Spain AJ889586 AJ890117 AJ890195 AJ888418 FMR 8622 Foot skin, Barcelona, Spain AJ889586 AJ890133 AJ880210 AJ888418 FMR 8623 Leukemic patient, Barcelona, Spain AJ889597 AJ890133 AJ880210 AJ888420 FMR 8630 Ulcer of ankle, Santiago de Compostela, Spain AJ889597 AJ890133 AJ880216 AJ889999 AJ890161 AJ888438 HEM 14263 Cystic fibrosis, patient 1, Angers, France AJ888966 AJ889999 AJ890162 AJ888438 <t< td=""><td>FMR 8535</td><td>Soil, Buenos Aires, Argentina</td><td>AJ889860</td><td>AJ890014</td><td>AJ890186</td><td>AJ888409</td></t<>	FMR 8535	Soil, Buenos Aires, Argentina	AJ889860	AJ890014	AJ890186	AJ888409	
FMR 8538 Soil, Buenos Aires, Argentina AJ88962 AJ890111 AJ890189 AJ888411 FMR 8530 Soil, Buenos Aires, Argentina AJ889643 AJ890112 AJ890189 AJ888411 FMR 8540 Soil, Buenos Aires, Argentina AJ889665 AJ890112 AJ8890125 AJ889012 AJ88912 FMR 8619 Keratitis, Brazil AJ889585 AJ890116 AJ890192 AJ88841 FMR 8621 Cystic fibrosis, Barcelona, Spain AJ889586 AJ890116 AJ889193 AJ888419 FMR 8622 Foot skin, Barcelona, Spain AJ889587 AJ890118 AJ890196 AJ889109 AJ8891016 AJ88910161 AJ888420 AJ880161 AJ888420 AJ880161 AJ888430 AJ8890161 AJ888430 AJ889106 AJ889164 AJ889164 AJ889164 AJ889164 AJ889164 AJ889164 AJ889166 AJ889166 AJ8891066 AJ889166 AJ889166	FMR 8537	Soil, Buenos Aires, Argentina	AJ889861	AJ890110	AJ890187	AJ888410	
FMR 8539 Soil, Buenos Aires, Argentina AJ88963 AJ890112 AJ890190 AJ888412 FMR 8514 Soil, Buenos Aires, Argentina AJ889664 AJ890128 AJ890125 AJ88912 AJ888412 FMR 8619 Kerattits, Brazil AJ889584 AJ890115 AJ890192 AJ888416 FMR 8610 Cystic fibrosis, Barcelona, Spain AJ889586 AJ890117 AJ890194 AJ888427 FMR 8622 Foot skin, Barcelona, Spain AJ889586 AJ890118 AJ890195 AJ888427 FMR 8623 Leukemic patient, Barcelona, Spain AJ889586 AJ890113 AJ8890196 AJ888427 FMR 8630 Ulcer of ankle, Santiago de Compostela, Spain AJ889597 AJ890153 AJ889219 AJ8890164 JA888438 HEM 14263 Cystic fibrosis, patient 1, Angers, France AJ888962 AJ889991 AJ890164 JA888438 HEM 14254 Cystic fibrosis, patient 7, Giens, France AJ888966 AJ889993 AJ890165 AJ888438 HEM 14451 Cystic fibrosis, patient 8, Tours, France AJ888966 AJ889996 AJ890166 AJ888	FMR 8538	Soil, Buenos Aires, Argentina	AJ889862	AJ890111	AJ890188	AJ888443	
FMR 8540 Soil, Buenos Aires, Argentina AJ88964 AJ890113 AJ890120 AJ888413 FMR 8510 Keratitis, Brazil AJ889605 AJ890115 AJ8890125 AJ889012 AJ888413 FMR 8610 Keratitis, Brazil AJ889586 AJ890116 AJ890193 AJ888419 FMR 8621 Cystic fibrosis, Barcelona, Spain AJ889587 AJ890117 AJ889019 AJ888419 FMR 8621 Leukemic patient, Barcelona, Spain AJ889596 AJ890112 AJ889016 AJ888429 FMR 8625 Leukemic patient, Jaragoza, Spain AJ889579 AJ890113 AJ889016 AJ888440 RV 43005 Human, Zaire AJ889561 AJ889090 AJ890121 AJ888440 RV 43005 Human, Zaire AJ888561 AJ889090 AJ890161 AJ888451 HEM 1426 Cystic fibrosis, patient 1, Giens, France AJ888063 AJ8890163 AJ8890164 AJ88840 HEM 14451 Cystic fibrosis, patient 3, Giuns, France AJ888064 AJ8890164 AJ88840 HEM 14462 Cystic fibrosis, patient 8, Tours, France	FMR 8539	Soil, Buenos Aires, Argentina	AJ889863	AJ890112	AJ890189	AJ888411	
FMR 8541 Soil, Buenos Aires, Argentina Al889605 Al890128 Al89015 Al88015 Al88013 FMR 8610 Keratitis, Brazil Al889584 Al890116 Al890193 AJ888417 FMR 8621 Cystic fibrosis, Barcelona, Spain Al889586 Al800117 Al890195 AJ88419 FMR 8622 Foot skin, Barcelona, Spain Al889587 Al800128 AJ890120 AJ88427 FMR 8623 Leukemic patient, Earcelona, Spain AJ889588 AJ80013 AJ890120 AJ88427 FMR 8630 Ulcer of ankle, Santiago de Compostela, Spain AJ889571 AJ890133 AJ890219 AJ888440 RV 43605 Human, Zaire AJ8889561 AJ889901 AJ8890161 AJ888433 HEM 14263 Cystic fibrosis, patient 4, Giens, France AJ888962 AJ889993 AJ890164 AJ88843 HEM 14451 Cystic fibrosis, patient 9, Tours, France AJ889966 AJ889994 AJ890166 AJ888305 HEM 14462 Cystic fibrosis, patient 8, Tours, France AJ88967 AJ889996 AJ890166 AJ888304 HEM 14464 </td <td>FMR 8540</td> <td>Soil, Buenos Aires, Argentina</td> <td>AJ889864</td> <td>AJ890113</td> <td>AJ890190</td> <td>AJ888412</td>	FMR 8540	Soil, Buenos Aires, Argentina	AJ889864	AJ890113	AJ890190	AJ888412	
FMR 8619 Keratitis, Brazil AJ889584 AJ890115 AJ890192 AJ888416 FMR 8621 Cystic fibrosis, Barcelona, Spain AJ889585 AJ890117 AJ889144 AJ889587 AJ890117 AJ889144 AJ889587 AJ890118 AJ8890116 AJ88917 AJ88957 AJ890121 AJ888385 FMR 8601 Ulcer of ankle, Santiago de Compostela, Spain AJ88957 AJ88997 AJ890161 AJ888397 AJ890161 AJ888395 AJ890161 AJ888399 AJ890162 AJ888395 AJ889990 AJ890162 AJ888395 AJ889991 AJ890164 AJ888438 HIEM 1435 Cystic fibrosis, patient 9, Tours, France AJ888966 AJ889996 AJ890166 AJ88397 HIEM 14467 Cystic fibrosis, patient 8, Tours, France AJ889966 AJ889996 AJ890166 AJ88399 AJ890166 <td>FMR 8541</td> <td>Soil, Buenos Aires, Argentina</td> <td>AJ889605</td> <td>AJ890128</td> <td>AJ890215</td> <td>AJ888413</td>	FMR 8541	Soil, Buenos Aires, Argentina	AJ889605	AJ890128	AJ890215	AJ888413	
FMR 8620 Keratitis, Brazl Al889586 Al890116 Al890193 Al888117 FMR 8621 Cystic fibrosis, Barcelona, Spain Al889586 Al890117 Al8890117 Al8890115 Al8890115 Al8890115 Al8890115 Al8890115 Al8890125 Al8890125 Al8890125 Al8890125 Al8890126 Al889127 FMR 8623 Leukemic patient, Zaragoza, Spain Al889576 Al88913 Al890126 Al88940 Al88840 FNR 8630 Ulcer of ankle, Santiago de Compostela, Spain Al889571 Al889579 Al890161 Al88840 FNR 8630 Ulcer of ankle, Santiago de Compostela, Spain Al889579 Al890161 Al888440 FNEM 8630 Ulcer of ankle, Santiago de Compostela, Spain Al889579 Al890161 Al888430 HIEM 14263 Cystic fibrosis, patient 7, Giens, France Al888964 Al889990 Al890165 Al888339 HIEM 14451 Cystic fibrosis, patient 8, Tours, France Al888966 Al889996 Al890166 Al88339 HIEM 14462 Cystic fibrosis, patient 8, Tours, France Al888967 Al880997 Al890166 <	FMR 8619	Keratitis, Brazil	AJ889584	AJ890115	AJ890192	AJ888416	
FMR 8621 Cystic fibrosis, Barcelona, Spain Al889587 Al889117 Al880194 Al888119 FMR 8623 Leukemic patient, Barcelona, Spain Al889587 Al8901125 Al880101 Al888410 FMR 8623 Leukemic patient, Barcelona, Spain Al889586 Al890113 Al880210 Al888440 FMR 8630 Ulcer of ankle, Santiago de Compostela, Spain Al889597 Al880133 Al880219 Al888440 RV 43605 Human, Zaire Al8889561 Al889597 Al880161 Al888436 HEM 14263 Cystic fibrosis, patient 1, Angers, France Al888963 Al889992 Al890162 Al888438 HEM 14354 Cystic fibrosis, patient 9, Tours, France Al888964 Al889993 Al890164 Al888438 HEM 14452 Cystic fibrosis, patient 8, Tours, France Al888966 Al889996 Al8890166 Al8883016 HEM 14462 Cystic fibrosis, patient 1, Angers, France Al888967 Al889996 Al890166 Al888301 HEM 14464 Cystic fibrosis, patient 1, Angers, France Al88970 Al8990166 Al888307 HEM	FMR 8620	Keratitis, Brazil	AJ889585	AJ890116	AJ890193	AJ888417	
FMR 8622 Foot skin, Barcelona, Spain Al889556 Al890125 Al888127 FMR 8625 Leukemic patient, Barcelona, Spain Al889596 Al890125 Al890126 Al890126 Al890126 Al890126 Al890126 Al890126 Al890126 Al890126 Al890126 Al890141 Al888420 FMR 8630 Ulcer of ankle, Santiago de Compostela, Spain Al889571 Al889571 Al880121 Al888431 Al888451 Al889571 Al890161 Al888433 HEM 14265 Cystic fibrosis, patient 1, Angers, France Al888962 Al889990 Al890163 Al888433 HEM 14354 Cystic fibrosis, patient 9, Tours, France Al888965 Al889993 Al890165 Al88843 HEM 14451 Cystic fibrosis, patient 8, Tours, France Al888966 Al889995 Al890166 Al888996 HEM 14462 Cystic fibrosis, patient 1, Angers, France Al888966 Al889997 Al890168 Al889991 HEM 14463 Cystic fibrosis, patient 1, Angers, France Al88970 Al8890170 Al888439 HEM 1467 Cystic fibrosis, patient 1, Angers, France <	FMR 8621	Cystic fibrosis, Barcelona, Spain	AJ889586	AJ890117	AJ890194	AJ888418	
FMR 8623 Leukemic patient, Barcelona, Spain AI88959 AJ890125 AJ89010 AJ889210 AJ888420 FMR 8623 Leukemic patient, Zaragoza, Spain AI88957 AI890133 AJ890219 AJ888420 RV 43605 Human, Zaire AJ88957 AJ88957 AJ890161 AJ888436 HEM 14263 Cystic fibrosis, patient 1, Angers, France AJ888961 AJ889991 AJ890161 AJ888436 HEM 14263 Cystic fibrosis, patient 7, Giens, France AJ888962 AJ889992 AJ890163 AJ888437 HEM 14354 Cystic fibrosis, patient 9, Tours, France AJ888966 AJ889993 AJ890166 AJ888395 HEM 14462 Cystic fibrosis, patient 8, Tours, France AJ888966 AJ889996 AJ890166 AJ888395 HEM 14462 Cystic fibrosis, patient 8, Tours, France AJ888966 AJ889999 AJ890166 AJ888395 HEM 14467 Cystic fibrosis, patient 1, Angers, France AJ888966 AJ889999 AJ890168 AJ888395 HEM 1458 Cystic fibrosis, patient 1, Angers, France AJ889846 AJ890000 AJ890170 AJ	FMR 8622	Foot skin, Barcelona, Spain	AJ889587	AJ890118	AJ890195	AJ888419	
FMR 8625 Leukemic patient, Zaragoza, Spain Al88958 AJ890119 AJ890196 AJ888440 RV 43605 Human, Zaire AJ889511 AJ889579 AJ890131 AJ880219 AJ888443 RV 43605 Human, Zaire AJ889511 AJ889579 AJ890161 AJ888433 IHEM 14263 Cystic fibrosis, patient 1, Argers, France AJ888962 AJ889992 AJ890162 AJ888333 IHEM 14354 Cystic fibrosis, patient 7, Giens, France AJ888963 AJ889992 AJ890165 AJ888438 IHEM 14451 Cystic fibrosis, patient 3, Giens, France AJ888965 AJ889993 AJ890166 AJ888393 IHEM 14464 Cystic fibrosis, patient 3, Giens, France AJ888966 AJ889996 AJ890167 AJ888396 IHEM 14464 Cystic fibrosis, patient 8, Tours, France AJ888966 AJ889997 AJ890166 AJ888390 IHEM 14754 Cystic fibrosis, patient 8, Tours, France AJ888970 AJ889000 AJ890170 AJ88840 IHEM 14754 Cystic fibrosis, patient 7, Giens, France AJ88979 AJ890171 AJ88840 <t< td=""><td>FMR 8623</td><td>Leukemic patient, Barcelona, Spain</td><td>AJ889596</td><td>AJ890125</td><td>AJ890210</td><td>AJ888427</td></t<>	FMR 8623	Leukemic patient, Barcelona, Spain	AJ889596	AJ890125	AJ890210	AJ888427	
FMR 8630 Ulcer of ankle, Santiago de Compostela, Spain AJ88957 AJ890133 AJ890219 AJ888440 RV 43605 Human, Zaire AJ888951 AJ889970 AJ890341 AJ888333 IHEM 14263 Cystic fibrosis, patient 1, Angers, France AJ888961 AJ889990 AJ890161 AJ888333 IHEM 14354 Cystic fibrosis, patient 7, Giens, France AJ888962 AJ889992 AJ890163 AJ888438 IHEM 14354 Cystic fibrosis, patient 9, Tours, France AJ888966 AJ889993 AJ890165 AJ888438 IHEM 14462 Cystic fibrosis, patient 8, Tours, France AJ888966 AJ889995 AJ890166 AJ888396 IHEM 14464 Cystic fibrosis, patient 8, Tours, France AJ888966 AJ889996 AJ890167 AJ888397 IHEM 14638 Cystic fibrosis, patient 1, Angers, France AJ888966 AJ889999 AJ890169 AJ888399 IHEM 14754 Cystic fibrosis, patient 1, Angers, France AJ889846 AJ890000 AJ890170 AJ888490 IHEM 14754 Cystic fibrosis, patient 8, Tours, France AJ889946 AJ890001 AJ890170	FMR 8625	Leukemic patient, Zaragoza, Spain	AJ889588	AJ890119	AJ890196	AJ888420	
RV 43605 Human, Zaire AJ88951 AJ889579 AJ890341 AJ88333 HHEM 1426 Cystic fibrosis, patient 1, Angers, France AJ888962 AJ889990 AJ800161 AJ888333 HHEM 14268 Cystic fibrosis, patient 7, Ciens, France AJ888963 AJ889991 AJ80163 AJ888437 HHEM 14358 Cystic fibrosis, patient 3, Cours, France AJ888965 AJ889993 AJ80165 AJ888394 HHEM 14451 Cystic fibrosis, patient 3, Cours, France AJ888966 AJ889996 AJ80166 AJ888395 HHEM 14464 Cystic fibrosis, patient 8, Tours, France AJ888967 AJ889996 AJ80166 AJ888395 HHEM 14464 Cystic fibrosis, patient 1, Angers, France AJ888968 AJ889998 AJ890169 AJ888397 HHEM 14754 Cystic fibrosis, patient 8, Tours, France AJ888970 AJ889000 AJ890171 AJ888439 HHEM 14754 Cystic fibrosis, patient 5, Giens, France AJ88974 AJ890001 AJ890171 AJ888402 HHEM 15144 Cystic fibrosis, patient 5, Giens, France AJ889560 AJ890002 AJ890172 AJ888	FMR 8630	Ulcer of ankle, Santiago de Compostela, Spain	AJ889597	AJ890133	AJ890219	AJ888440	
IHEMI4263Cystic fibrosis, patient 1, Angers, FranceAJ88961AJ889990AJ890161AJ888432IHEMI4268Cystic fibrosis, patient 7, Giens, FranceAJ88963AJ889992AJ890163AJ888437IHEMI4354Cystic fibrosis, patient 7, Giens, FranceAJ888964AJ889993AJ890164AJ888394IHEMI4451Cystic fibrosis, patient 8, Tours, FranceAJ888966AJ889995AJ890166AJ888395IHEMI4462Cystic fibrosis, patient 8, Tours, FranceAJ888966AJ889996AJ890166AJ888395IHEMI4467Cystic fibrosis, patient 9, Tours, FranceAJ888967AJ889996AJ890168AJ888397IHEMI4467Cystic fibrosis, patient 8, Tours, FranceAJ888967AJ889999AJ890168AJ888399IHEMI4535Cystic fibrosis, patient 1, Angers, FranceAJ889867AJ889999AJ890170AJ888439IHEMI4545Cystic fibrosis, patient 1, Angers, FranceAJ889847AJ890000AJ890171AJ888400IHEMI4545Cystic fibrosis, patient 5, Giens, FranceAJ889847AJ890001AJ890173AJ888402IHEMI544Cystic fibrosis, patient 4, Giens, FranceAJ889649AJ890003AJ890173AJ888439IHEMI5542Cystic fibrosis, patient 4, Giens, FranceAJ889649AJ890003AJ890173AJ888439IHEMI5542Cystic fibrosis, patient 4, Giens, FranceAJ8895809AJ890003AJ890174AJ888439IHEMI5542	RV 43605	Human, Zaire	AJ888951	AJ889579	AJ890341	AJ888383	
IHEM14268Cystic fibrosis, patient 4, Giens, FranceAJ88962AJ889991AJ890162AJ888437IHEM14354Cystic fibrosis, patient 7, Giens, FranceAJ88964AJ889992AJ890164AJ888438IHEM14358Cystic fibrosis, patient 8, Tours, FranceAJ88965AJ889994AJ890165AJ888395IHEM14461Cystic fibrosis, patient 8, Tours, FranceAJ88966AJ889995AJ890166AJ888395IHEM14464Cystic fibrosis, patient 8, Tours, FranceAJ88966AJ889997AJ890166AJ888396IHEM14464Cystic fibrosis, patient 1, Angers, FranceAJ88966AJ889997AJ890169AJ888398IHEM14754Cystic fibrosis, patient 1, Angers, FranceAJ889866AJ889999AJ890170AJ888399IHEM14754Cystic fibrosis, patient 8, Tours, FranceAJ889846AJ890000AJ890171AJ888409IHEM14754Cystic fibrosis, patient 8, Tours, FranceAJ88947AJ890001AJ890172AJ888409IHEM1548Cystic fibrosis, patient 5, Giens, FranceAJ889599AJ890135AJ890221AJ888441IHEM1548Cystic fibrosis, patient 2, Angers, FranceAJ889500AJ890135AJ890221AJ888439IHEM1542Cystic fibrosis, patient 2, Angers, FranceAJ889509AJ890003AJ890173AJ888429IHEM1542Cystic fibrosis, patient 2, Angers, FranceAJ889500AJ890003AJ890174AJ888439IHEM15579Cystic	IHEM 14263	Cystic fibrosis, patient 1, Angers, France	AJ888961	AJ889990	AJ890161	AJ888436	
IHEM14354Cystic fibrosis, patient 7, Giens, FranceAJ888963AJ889992AJ890163AJ888438IHEM14451Cystic fibrosis, patient 3, Giens, FranceAJ888964AJ889994AJ890165AJ888394IHEM14451Cystic fibrosis, patient 8, Tours, FranceAJ888967AJ889995AJ890166AJ888396IHEM14464Cystic fibrosis, patient 7, Tours, FranceAJ888967AJ889996AJ890167AJ888396IHEM14467Cystic fibrosis, patient 9, Tours, FranceAJ888967AJ889998AJ890168AJ888398IHEM1463Cystic fibrosis, patient 1, Angers, FranceAJ888970AJ889999AJ890170AJ888399IHEM14754Cystic fibrosis, patient 1, Angers, FranceAJ888970AJ890000AJ890171AJ888401IHEM1578Cystic fibrosis, patient 5, Giens, FranceAJ889847AJ890000AJ890173AJ888402IHEM1548Cystic fibrosis, patient 6, Giens, FranceAJ88959AJ890135AJ890222AJ888439IHEM1548Cystic fibrosis, patient 6, Giens, FranceAJ88956AJ890003AJ890174AJ888403MUCL1549Cystic fibrosis, patient 4, Giens, FranceAJ88955AJ890003AJ890174AJ888403MUCL1646Forest soil, Haasrode, BelgiumAJ88955AJ890004AJ890175AJ888403MUCL1502Forest soil, Jagambi, ZaireAJ88955AJ890005AJ890175AJ888423MUCL14092Forest soil, Jagambi, Zaire	IHEM 14268	Cystic fibrosis, patient 4, Giens, France	AJ888962	AJ889991	AJ890162	AJ888393	
IHEM 14358 Cystic fibrosis, patient 9, Tours, France AJ88996 AJ889993 AJ890165 AJ888394 IHEM 14451 Cystic fibrosis, patient 8, Tours, France AJ888965 AJ889995 AJ800165 AJ888395 IHEM 14461 Cystic fibrosis, patient 8, Tours, France AJ888966 AJ889996 AJ890166 AJ888397 IHEM 14467 Cystic fibrosis, patient 1, Angers, France AJ888968 AJ889996 AJ890169 AJ888397 IHEM 14754 Cystic fibrosis, patient 1, Angers, France AJ888969 AJ890000 AJ890170 AJ888409 IHEM 14754 Cystic fibrosis, patient 8, Tours, France AJ889846 AJ890000 AJ890171 AJ888400 IHEM 15144 Cystic fibrosis, patient 7, Angers, France AJ889599 AJ890102 AJ890172 AJ888401 IHEM 1545 Cystic fibrosis, patient 5, Giens, France AJ889599 AJ890135 AJ888401 IHEM 15454 Cystic fibrosis, patient 1, Angers, France AJ889599 AJ890136 AJ888439 IHEM 15458 Cystic fibrosis, patient 7, Giens, France AJ889540 AJ890002 AJ890173 AJ888442 IHEM 15579 Cystic fibrosis, patient 4, Giens, France <t< td=""><td>IHEM 14354</td><td>Cystic fibrosis, patient 7, Giens, France</td><td>AJ888963</td><td>AJ889992</td><td>AJ890163</td><td>AJ888437</td></t<>	IHEM 14354	Cystic fibrosis, patient 7, Giens, France	AJ888963	AJ889992	AJ890163	AJ888437	
IHEM 14451 Cystic fibrosis, patient 3, Giens, France AJ888965 AJ889994 AJ890165 AJ888394 IHEM 14462 Cystic fibrosis, patient 8, Tours, France AJ888966 AJ889995 AJ890167 AJ888396 IHEM 14464 Cystic fibrosis, patient 9, Tours, France AJ888968 AJ889997 AJ890168 AJ888397 IHEM 14647 Cystic fibrosis, patient 1, Angers, France AJ888969 AJ889999 AJ890170 AJ888398 IHEM 14754 Cystic fibrosis, patient 8, Tours, France AJ889846 AJ890000 AJ890171 AJ888400 IHEM 1575 Cystic fibrosis, patient 5, Giens, France AJ889847 AJ890001 AJ890172 AJ888400 IHEM 15149 Cystic fibrosis, patient 5, Giens, France AJ889848 AJ890002 AJ890173 AJ888441 IHEM 15458 Cystic fibrosis, patient 4, Giens, France AJ889840 AJ890003 AJ890174 AJ888434 IHEM 15642 Cystic fibrosis, patient 4, Giens, France AJ889855 AJ890003 AJ890175 AJ888434 MUCL 6106 Forest soil, Haasrode, Belgium AJ889855 AJ890003 AJ8901	IHEM 14358	Cystic fibrosis, patient 9, Tours, France	AJ888964	AJ889993	AJ890164	AJ888438	
IHEM 14462 Cystic fibrosis, patient 8, Tours, France Al888966 AJ889995 Al890166 AJ888396 IHEM 14464 Cystic fibrosis, patient 9, Tours, France AJ888967 AJ889996 AJ890168 AJ888397 IHEM 14467 Cystic fibrosis, patient 1, Angers, France AJ888968 AJ889997 AJ890168 AJ888397 IHEM 14754 Cystic fibrosis, patient 1, Angers, France AJ888966 AJ890000 AJ890170 AJ888400 IHEM 14754 Cystic fibrosis, patient 8, Tours, France AJ889846 AJ890001 AJ890172 AJ888400 IHEM 15144 Cystic fibrosis, patient 5, Giens, France AJ889599 AJ890135 AJ890173 AJ888402 IHEM 15579 Cystic fibrosis, patient 2, Angers, France AJ889600 AJ890136 AJ890174 AJ888403 MUCL 8302 Soil, Germany AJ889855 AJ890004 AJ890174 AJ888404 MUCL 8302 Soil, Germany AJ889858 AJ890009 AJ890180 AJ888429 MUCL 8302	IHEM 14451	Cystic fibrosis, patient 3, Giens, France	AJ888965	AJ889994	AJ890165	AJ888394	
IHEM 14404Cystic fibrosis, patient S, Tours, FranceAJ888967AJ889996AJ8910167AJ888397IHEM 1467Cystic fibrosis, patient 1, Angers, FranceAJ888968AJ889997AJ890168AJ888398IHEM 14638Cystic fibrosis, patient 1, Angers, FranceAJ888969AJ889998AJ8901170AJ888398IHEM 14754Cystic fibrosis, patient 8, Tours, FranceAJ888970AJ889000AJ890171AJ888400IHEM 14758Cystic fibrosis, patient 8, Tours, FranceAJ889846AJ890001AJ890172AJ888400IHEM 15144Cystic fibrosis, patient 5, Giens, FranceAJ889847AJ890001AJ890173AJ888402IHEM 15149Cystic fibrosis, patient 5, Giens, FranceAJ889600AJ890136AJ890221AJ888439IHEM 1557Cystic fibrosis, patient 4, Giens, FranceAJ889600AJ890136AJ890222AJ888439IHEM 15642Cystic fibrosis, patient 4, Giens, FranceAJ889650AJ890003AJ890174AJ888403MUCL 6106Forest soil, Haasrode, BelgiumAJ889850AJ890004AJ890175AJ888402MUCL 8302Soil, GermanyAJ889589AJ890010AJ890136AJ888421MUCL 14009Forest soil, Yangambi, ZaireAJ88956AJ890010AJ890131AJ888422MUCL 120263Greenhouse soil, Herverlee, BelgiumAJ889585AJ890010AJ890177AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889598AJ890134AJ890217AJ888423MUCL 29263Greenhouse soil, Herverlee, Belgium <td>IHEM 14462</td> <td>Cystic fibrosis, patient 8, Tours, France</td> <td>AJ888966</td> <td>AJ889995</td> <td>AJ890166</td> <td>AJ888395</td>	IHEM 14462	Cystic fibrosis, patient 8, Tours, France	AJ888966	AJ889995	AJ890166	AJ888395	
IHEM 1446/Cystic fibrosis, patient 9, Tours, FranceAl888968Al889997Al890168AJ888398IHEM 14638Cystic fibrosis, patient 1, Angers, FranceAJ888969AJ889998AJ890170AJ888398IHEM 14754Cystic fibrosis, patient 1, Angers, FranceAJ88970AJ889999AJ890171AJ888399IHEM 14754Cystic fibrosis, patient 1, Angers, FranceAJ889846AJ890000AJ890171AJ888401IHEM 15144Cystic fibrosis, patient 5, Giens, FranceAJ889848AJ890002AJ890173AJ888402IHEM 1545Cystic fibrosis, patient 5, Giens, FranceAJ889599AJ890135AJ890221AJ888402IHEM 1545Cystic fibrosis, patient 2, Angers, FranceAJ889600AJ89003AJ890174AJ888403IHEM 15579Cystic fibrosis, patient 4, Giens, FranceAJ889850AJ890003AJ890175AJ888404MUCL 8106Forest soil, Haasrode, BelgiumAJ889850AJ890009AJ890180AJ888442MUCL 14009Forest soil, Baarn, the NetherlandsAJ88951AJ890013AJ890136AJ888422MUCL 14092Forest soil, Yangambi, ZaireAJ88956AJ890130AJ890176AJ888429MUCL 120263Greenhouse soil, Herverlee, BelgiumAJ88952AJ890123AJ890171AJ888424MUCL 120254Fuel oud (<i>Coelocarpon preussi</i>), Ivory CoastAJ88958AJ890134AJ890174AJ888435MUCL 20255Fuel oil, Antwerpen, BelgiumAJ889598AJ890134AJ890217AJ888435MUCL 20254Fuel oil, Antwe	IHEM 14464	Cystic fibrosis, patient 8, Tours, France	AJ888967	AJ889996	AJ890167	AJ888396	
IHEM 14638 Cystic fibrosis, patient 1, Angers, France AJ88999 AJ889998 AJ890169 AJ88398 IHEM 14754 Cystic fibrosis, patient 8, Tours, France AJ889846 AJ890000 AJ890171 AJ888499 IHEM 15144 Cystic fibrosis, patient 8, Tours, France AJ889847 AJ890001 AJ890173 AJ888401 IHEM 15149 Cystic fibrosis, patient 6, Giens, France AJ88959 AJ890135 AJ890221 AJ888402 IHEM 15579 Cystic fibrosis, patient 4, Giens, France AJ88950 AJ890003 AJ890174 AJ888403 MUCL 6106 Forest soil, Haasrode, Belgium AJ889855 AJ890003 AJ890180 AJ888421 MUCL 8302 Soil, Germany AJ889855 AJ890005 AJ890130 AJ888421 MUCL 14009 Forest soil, Yangambi, Zaire AJ889850 AJ890005 AJ890176 AJ888425 MUCL 1402 Forest soil, Herverlee, Belgium AJ889502 AJ890006 AJ890176 AJ888425 MUCL 1402 Forest soil, Yangambi, Zaire AJ889502 AJ890010	IHEM 14467	Cystic fibrosis, patient 9, Tours, France	AJ888968	AJ889997	AJ890168	AJ888397	
IHEM 14/54Cystic fibrosis, patient 8, Tours, FranceAJ8889/0AJ88999AJ8901/0AJ888400IHEM 14758Cystic fibrosis, patient 1, Angers, FranceAJ889846AJ890000AJ890171AJ888400IHEM 15144Cystic fibrosis, patient 5, Giens, FranceAJ889847AJ800001AJ890173AJ888400IHEM 15149Cystic fibrosis, patient 5, Giens, FranceAJ889599AJ80002AJ890173AJ888402IHEM 15458Cystic fibrosis, patient 6, Giens, FranceAJ889599AJ890135AJ890221AJ888439IHEM 15642Cystic fibrosis, patient 4, Giens, FranceAJ889849AJ890003AJ890174AJ888403MUCL 6106Forest soil, Haasrode, BelgiumAJ889550AJ890004AJ890175AJ888442MUCL 8302Soil, GermanyAJ889558AJ890009AJ890180AJ888422MUCL 14009Forest soil, Yangambi, ZaireAJ88955AJ890010AJ890176AJ888422MUCL 14092Forest soil, Yangambi, ZaireAJ889852AJ890130AJ890177AJ888423MUCL 120263Greenhouse soil, Herverlee, BelgiumAJ889593AJ890106AJ890177AJ888423MUCL 22258Fuel oil, Antwerpen, BelgiumAJ889593AJ89013AJ890217AJ888432RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ88958AJ89013AJ890217AJ888432RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ88950AJ89013AJ890217AJ888432CBS 101.22Mycetoma, TexasAJ889501AJ890131AJ890217 <td>IHEM 14638</td> <td>Cystic fibrosis, patient 1, Angers, France</td> <td>AJ888969</td> <td>AJ889998</td> <td>AJ890169</td> <td>AJ888398</td>	IHEM 14638	Cystic fibrosis, patient 1, Angers, France	AJ888969	AJ889998	AJ890169	AJ888398	
IHEM 14/58Cystic fibrosis, patient 1, Angers, FranceAJ88946AJ890000AJ890171AJ888401IHEM 15144Cystic fibrosis, patient 5, Giens, FranceAJ889847AJ890001AJ890172AJ888401IHEM 15149Cystic fibrosis, patient 5, Giens, FranceAJ889599AJ890135AJ890221AJ888402IHEM 15458Cystic fibrosis, patient 2, Angers, FranceAJ889600AJ890136AJ890222AJ888441IHEM 15579Cystic fibrosis, patient 2, Angers, FranceAJ889600AJ890136AJ890174AJ888403MUCL 6106Forest soil, Haasrode, BelgiumAJ889850AJ890004AJ890175AJ888404MUCL 8302Soil, GermanyAJ889855AJ890005AJ890176AJ888442MUCL 14009Forest soil, Yangambi, ZaireAJ889851AJ890005AJ890176AJ888429MUCL 14092Forest soil, Yangambi, ZaireAJ889856AJ890010AJ890174AJ888402MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ889856AJ890101AJ890177AJ888423MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889593AJ890134AJ890217AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889598AJ890134AJ890220AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890134AJ890220AJ888432KI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889590AJ890134AJ8902207AJ888432CBS 106.53Goat dung, Aligarh, IndiaAJ889501	IHEM 14/54	Cystic fibrosis, patient 8, Tours, France	AJ888970	AJ889999	AJ890170	AJ888399	
IHEM 15144Cystic fibrosis, patient 5, Tours, FranceAJ899847AJ890001AJ890172AJ888401IHEM 15149Cystic fibrosis, patient 5, Giens, FranceAJ889848AJ890002AJ890173AJ888402IHEM 15458Cystic fibrosis, patient 6, Giens, FranceAJ889599AJ890135AJ890221AJ888439IHEM 15579Cystic fibrosis, patient 2, Angers, FranceAJ889600AJ890136AJ890122AJ888443MUCL 6106Forest soil, Haasrode, BelgiumAJ889850AJ890004AJ890175AJ888404MUCL 8302Soil, GermanyAJ889855AJ890009AJ890180AJ888424MUCL 14009Forest soil, Yangambi, ZaireAJ889851AJ890005AJ890176AJ888422MUCL 14092Forest soil, Yangambi, ZaireAJ889856AJ890010AJ890176AJ888423MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ889852AJ890006AJ890177AJ888423MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889593AJ890134AJ890220AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889598AJ890134AJ890207AJ888432RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890131AJ890207AJ888432CBS 106.53Goat dung, Aligarh, IndiaAJ889501AJ890121AJ890207AJ888442CBS 254.72Half-digested sewage tank, OhioAJ889601AJ890129AJ890214AJ888428CBS 418.73Soil, TadzjikistanAJ88959AJ890124AJ889211 <t< td=""><td>IHEM 14/58</td><td>Cystic fibrosis, patient 1, Angers, France</td><td>AJ889846</td><td>AJ890000</td><td>AJ8901/1</td><td>AJ888400</td></t<>	IHEM 14/58	Cystic fibrosis, patient 1, Angers, France	AJ889846	AJ890000	AJ8901/1	AJ888400	
IHEM 15149Cystic fibrosis, patient 5, Giens, FranceAJ889848AJ890002AJ891175AJ888402IHEM 15458Cystic fibrosis, patient 6, Giens, FranceAJ889599AJ890135AJ890221AJ888449IHEM 15579Cystic fibrosis, patient 2, Angers, FranceAJ889600AJ890003AJ890174AJ888449IHEM 15642Cystic fibrosis, patient 4, Giens, FranceAJ889850AJ890003AJ890174AJ888403MUCL 6106Forest soil, Haasrode, BelgiumAJ889850AJ890004AJ890175AJ888440MUCL 8302Soil, GermanyAJ889855AJ890009AJ890180AJ888421MUCL 14009Forest soil, Yangambi, ZaireAJ889589AJ890130AJ890213AJ888422MUCL 14092Forest soil, Yangambi, ZaireAJ889602AJ890130AJ8901181AJ888429MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889852AJ890006AJ890177AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890123AJ890217AJ888424RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890134AJ890217AJ888432CBS 101.22Mycetoma, TexasAJ889500AJ890131AJ890212AJ888432CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890131AJ890212AJ888434CBS 11.72Brown sandy soil, Tsintsabis, NamibiaAJ889603AJ890132AJ890214AJ888426FMR 7294Blood, AustraliaAJ889591AJ890127AJ880223AJ880424FMR	IHEM 15144	Cystic fibrosis, patient 8, Tours, France	AJ889847	AJ890001	AJ890172	AJ888401	
IHEM 15436Cystic fibrosis, patient 6, Giens, FranceAJ889599AJ890135AJ890221AJ888439IHEM 15579Cystic fibrosis, patient 2, Angers, FranceAJ889600AJ890003AJ890174AJ888439MUCL 6106Forest soil, Haasrode, BelgiumAJ88950AJ890004AJ890175AJ888404MUCL 8302Soil, GermanyAJ88955AJ890009AJ890180AJ888424MUCL 8522Humic soil, Baarn, the NetherlandsAJ889589AJ890005AJ890176AJ888422MUCL 14009Forest soil, Yangambi, ZaireAJ889602AJ890130AJ890213AJ888429MUCL 14092Forest soil, Yangambi, ZaireAJ889650AJ890100AJ890113AJ888429MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ88952AJ890010AJ890181AJ888429MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889593AJ890123AJ890177AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890134AJ890217AJ888432RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890114AJ890217AJ888435CBS 101.22Mycetoma, TexasAJ88950AJ890121AJ890217AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890131AJ890212AJ888428CBS 254.72Half-digested sewage tank, OhioAJ889603AJ890129AJ890214AJ888426CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889595AJ890124AJ890214AJ888425 <td>IHEM 15149</td> <td>Cystic fibrosis, patient 5, Giens, France</td> <td>AJ889848</td> <td>AJ890002</td> <td>AJ890173</td> <td>AJ888402</td>	IHEM 15149	Cystic fibrosis, patient 5, Giens, France	AJ889848	AJ890002	AJ890173	AJ888402	
IHEM 15379Cystic fibrosis, patient 2, Angers, FranceAJ889000AJ890130AJ890222AJ888439IHEM 15642Cystic fibrosis, patient 4, Giens, FranceAJ889849AJ890003AJ890174AJ888403MUCL 6106Forest soil, Haasrode, BelgiumAJ889850AJ890004AJ890175AJ888442MUCL 8302Soil, GermanyAJ88955AJ890009AJ890180AJ888422MUCL 8522Humic soil, Baarn, the NetherlandsAJ889589AJ890120AJ890208AJ888422MUCL 14009Forest soil, Yangambi, ZaireAJ889651AJ890005AJ890176AJ888422MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ889856AJ890010AJ890181AJ888429MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889593AJ890123AJ890121AJ888423MUCL 20258Fuel oil, Antwerpen, BelgiumAJ889593AJ890134AJ890220AJ888432RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889590AJ890134AJ890217AJ888442RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889500AJ890131AJ890217AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890131AJ890212AJ888428CBS 254.72Half-digested sewage tank, OhioAJ889603AJ890132AJ890214AJ888442CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889603AJ890124AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888425	IHEM 15458	Cystic librosis, patient 0, Glens, France	AJ889399	AJ890133	AJ890221	AJ888441	
ITEM 15642Cystic horosis, patient 4, Giens, FranceAJ889849AJ889005AJ890005AJ890174AJ888405MUCL 6106Forest soil, Haasrode, BelgiumAJ889850AJ890004AJ890175AJ888404MUCL 8302Soil, GermanyAJ889855AJ890009AJ890180AJ888422MUCL 8522Humic soil, Baarn, the NetherlandsAJ889559AJ890120AJ890176AJ888421MUCL 14009Forest soil, Yangambi, ZaireAJ889602AJ890130AJ890213AJ888422MUCL 14092Forest soil, Yangambi, ZaireAJ889856AJ890010AJ890181AJ888429MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ889852AJ890006AJ890177AJ888423MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889593AJ890123AJ890217AJ888424RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890114AJ890220AJ888425RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ88965AJ890114AJ890217AJ888425CBS 101.22Mycetoma, TexasAJ889601AJ889601AJ890121AJ88422AJ88422CBS 254.72Half-digested sewage tank, OhioAJ889601AJ8890129AJ890214AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890132AJ890214AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888425FMR 7294Blood, AustraliaAJ889591AJ890127AJ880223AJ884444	IHEM 15579	Cystic librosis, patient 2, Angers, France	AJ889000	AJ890130	AJ890222	AJ888439	
MUCL 0100Forest soil, Haastode, BeightinAJ889850AJ890004AJ890175AJ888442MUCL 8302Soil, GermanyAJ889855AJ890009AJ890180AJ888442MUCL 8522Humic soil, Baarn, the NetherlandsAJ889559AJ890120AJ890208AJ888422MUCL 14009Forest soil, Yangambi, ZaireAJ889602AJ890130AJ890176AJ888422MUCL 14092Forest soil, Yangambi, ZaireAJ889602AJ890130AJ890213AJ888429MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ889856AJ890010AJ890177AJ888429MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889593AJ890123AJ890217AJ888424RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890134AJ890220AJ888432RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889590AJ890114AJ890207AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890121AJ8890212AJ888425CBS 216.72Half-digested sewage tank, OhioAJ889601AJ890131AJ890214AJ888425CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889595AJ890132AJ890218AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888425FMR 7294Blood, AustraliaAJ889591AJ890127AJ880211AJ884444	MUCL 6106	Expect coil Hoograde Bolgium	AJ009049	AJ890003	AJ090174	AJ000403	
MUCL 8502Soft, GernanyAJ889553AJ890005AJ890180AJ880442MUCL 8522Humic soil, Baarn, the NetherlandsAJ889589AJ890120AJ890208AJ888421MUCL 14009Forest soil, Yangambi, ZaireAJ889589AJ890005AJ890130AJ890213AJ888422MUCL 14092Forest soil, Yangambi, ZaireAJ889602AJ890110AJ890181AJ888429MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ88956AJ890006AJ890177AJ888423MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889523AJ890123AJ890217AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890134AJ890220AJ888424RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890114AJ890220AJ888432RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889590AJ890121AJ890207AJ888435CBS 101.22Mycetoma, TexasAJ889601AJ889601AJ890121AJ88422CBS 216.53Goat dung, Aligarh, IndiaAJ889601AJ890129AJ880212AJ88424CBS 254.72Half-digested sewage tank, OhioAJ889603AJ890132AJ890218AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888425FMR 7294Blood, AustraliaAJ889591AJ890127AJ890213AJ88424	MUCL 0100	Soil Cormany	AJ009030 A 1990955	AJ890004	AJ090173	AJ000404	
MUCL 8322Fulling soft, Barlin, the RetheritationAJ389339AJ389120AJ3890203AJ3890203AJ3890203MUCL 14009Forest soil, Yangambi, ZaireAJ889851AJ890130AJ890113AJ888429MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ889856AJ890010AJ890181AJ888405MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889852AJ890006AJ890177AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890123AJ890217AJ888424RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890114AJ890220AJ888432RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889655AJ890114AJ890217AJ888445CBS 101.22Mycetoma, TexasAJ889601AJ889601AJ890121AJ8890217AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890129AJ880212AJ888424CBS 254.72Half-digested sewage tank, OhioAJ889603AJ890132AJ880214AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888425FMR 7294Blood, AustraliaAJ889591AJ890127AJ889211AJ888424	MUCL 8502	Humie soil Beern, the Netherlands	A 1880280	AJ890009 AJ800120	AJ090100 A 1000200	AJ000442	
MUCL 14092Forest soil, Yangambi, ZaireAJ809031AJ800100AJ800130AJ8004223MUCL 14092Forest soil, Yangambi, ZaireAJ889602AJ890130AJ890131AJ890181MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ889856AJ890010AJ890181AJ888405MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ88952AJ890006AJ890177AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890123AJ890217AJ888424RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890134AJ890220AJ888432RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889655AJ890114AJ890191AJ888415CBS 101.22Mycetoma, TexasAJ889601AJ889601AJ890121AJ880220AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ889601AJ890121AJ8890212AJ888428CBS 254.72Half-digested sewage tank, OhioAJ889604AJ890132AJ889421AJ888425CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889595AJ890132AJ890218AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888425FMR 7294Blood, AustraliaAJ889591AJ890127AJ890223AJ888444	MUCL 14000	Forest soil Vangambi Zaire	A 1880851	A 1800005	A 1800176	A 1888/22	
MUCL 14022Forest son, Fanganor, ZancAJ8002AJ800120AJ800130AJ800131AJ800429MUCL 18784Treated wood (<i>Coelocarpon preussi</i>), Ivory CoastAJ889856AJ890010AJ890117AJ888405MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889852AJ890006AJ890177AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890123AJ890217AJ888423RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890134AJ890220AJ888432RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889655AJ890114AJ890191AJ888435CBS 101.22Mycetoma, TexasAJ889500AJ890121AJ890207AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890131AJ890212AJ888428CBS 254.72Half-digested sewage tank, OhioAJ889604AJ890132AJ890214AJ888425CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889595AJ890124AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888425FMR 7294Blood, AustraliaAJ889591AJ890127AJ880223AJ888444	MUCL 14009	Forest soil, Yangambi, Zaire	A 1880602	A 1800130	A 1800213	A 1888/20	
MUCL 20263Greenhouse soil, Herverlee, BelgiumAJ889850AJ890016AJ890177AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890123AJ890217AJ888423MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889598AJ890134AJ890220AJ888423RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889865AJ890114AJ890220AJ888432RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889655AJ890114AJ890207AJ888435CBS 101.22Mycetoma, TexasAJ889590AJ890121AJ890207AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890131AJ8890212AJ888428CBS 254.72Half-digested sewage tank, OhioAJ889604AJ890132AJ880214AJ888425CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889603AJ890132AJ880218AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888426FMR 7294Blood, AustraliaAJ889591AJ890127AJ890223AJ888444	MUCL 18784	Treated wood (Coelocarpon preussi) Ivory Coast	A 1889856	Δ 1890010	Δ 1890181	A 1888405	
MUCL 20205Fuel oil, Antwerpen, BelgiumAJ805022AJ805000AJ80177AJ805027MUCL 29258Fuel oil, Antwerpen, BelgiumAJ889593AJ890123AJ890217AJ888424RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ889598AJ890134AJ890220AJ888432RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889865AJ890114AJ890110AJ888415CBS 101.22Mycetoma, TexasAJ889590AJ890121AJ800207AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890131AJ890212AJ888428CBS 254.72Half-digested sewage tank, OhioAJ889604AJ890132AJ890214AJ888414CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889603AJ890132AJ890218AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ88420AJ888426FMR 7294Blood, AustraliaAJ889591AJ890127AJ890223AJ888444	MUCL 20263	Greenhouse soil Herverlee Belgium	A 1889852	A 1890006	Δ 1890177	Δ 1888423	
RKI 2782/95Trauma and sepsis, Hamburg, GermanyAJ809750AJ809120AJ8890220AJ888432RKI 2782/95Bronchoalveolar lavage fluid, Berlin, GermanyAJ889598AJ890134AJ890220AJ888432RKI 2956/93Bronchoalveolar lavage fluid, Berlin, GermanyAJ889865AJ890114AJ890191AJ888435CBS 101.22Mycetoma, TexasAJ889590AJ890121AJ890207AJ888435CBS 106.53Goat dung, Aligarh, IndiaAJ889601AJ890131AJ890212AJ888428CBS 254.72Half-digested sewage tank, OhioAJ889604AJ890129AJ890214AJ888414CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889603AJ890132AJ890218AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ888426AJ889221AJ888426FMR 7294Blood, AustraliaAJ889591AJ890127AJ890223AJ888444	MUCL 29258	Fuel oil. Antwerpen. Belgium	AJ889593	AJ890123	AJ890217	AJ888474	
RKI 2956/93 Bronchoalveolar lavage fluid, Berlin, Germany AJ809595 AJ809114 AJ800220 AJ800432 RKI 2956/93 Bronchoalveolar lavage fluid, Berlin, Germany AJ889865 AJ890114 AJ890121 AJ890127 AJ888415 CBS 101.22 Mycetoma, Texas AJ889590 AJ890131 AJ890207 AJ888428 CBS 254.72 Half-digested sewage tank, Ohio AJ889604 AJ890129 AJ890214 AJ888414 CBS 311.72 Brown sandy soil, Tsintsabis, Namibia AJ889595 AJ890132 AJ890218 AJ888425 CBS 418.73 Soil, Tadzjikistan AJ889595 AJ890124 AJ888426 FMR 7294 Blood, Australia AJ889591 AJ890127 AJ890223 AJ888444	RKI 2782/95	Trauma and sensis Hamburg Germany	A 1889598	A 1890134	A 1890220	A 1888437	
CBS 101.22Mycetoma, TexasAJ805000AJ8050114AJ8050171AJ8050171CBS 106.53Goat dung, Aligarh, IndiaAJ889500AJ890121AJ890212AJ888435CBS 216.53Goat dung, Aligarh, IndiaAJ889601AJ890131AJ890212AJ888428CBS 254.72Half-digested sewage tank, OhioAJ889604AJ890129AJ800214AJ888414CBS 311.72Brown sandy soil, Tsintsabis, NamibiaAJ889603AJ890132AJ890218AJ888425CBS 418.73Soil, TadzjikistanAJ889595AJ890124AJ880211AJ888426FMR 7294Blood, AustraliaAJ889591AJ890127AJ890223AJ888444	RKI 2956/93	Bronchoalveolar lavage fluid Berlin Germany	A 1889865	A 1890114	A 1890191	A J888415	
CBS 106.53 Goat dung, Aligarh, India AJ889601 AJ890121 AJ890212 AJ888428 CBS 216.53 Goat dung, Aligarh, India AJ889601 AJ890131 AJ890212 AJ888428 CBS 254.72 Half-digested sewage tank, Ohio AJ889603 AJ890129 AJ890214 AJ888414 CBS 311.72 Brown sandy soil, Tsintsabis, Namibia AJ889603 AJ890132 AJ890218 AJ888425 CBS 418.73 Soil, Tadzjikistan AJ889595 AJ890124 AJ888426 FMR 7294 Blood, Australia AJ889591 AJ890127 AJ890223 AJ888444	CBS 101 22	Mycetoma, Texas	AJ889590	AJ890121	AJ890207	AJ888435	
CBS 254.72 Half-digested sewage tank, Ohio AJ889604 AJ890129 AJ890214 AJ888414 CBS 311.72 Brown sandy soil, Tsintsabis, Namibia AJ889603 AJ890132 AJ890218 AJ888425 CBS 418.73 Soil, Tadzjikistan AJ88955 AJ890124 AJ888426 FMR 7294 Blood, Australia AJ889591 AJ890127 AJ890223 AJ888444	CBS 106 53	Goat dung, Aligarh, India	AJ889601	AJ890131	AI890212	A 1888478	
CBS 311.72 Brown sandy soil, Tsintsabis, Namibia AJ889603 AJ890132 AJ890218 AJ888425 CBS 418.73 Soil, Tadzjikistan AJ88955 AJ890124 AJ890211 AJ888425 FMR 7294 Blood, Australia AJ889591 AJ890127 AJ890223 AJ888444	CBS 254.72	Half-digested sewage tank. Ohio	AJ889604	AJ890129	AJ890214	AJ888414	
CBS 418.73 Soil, Tadzjikistan AJ889595 AJ890124 AJ890211 AJ888426 FMR 7294 Blood, Australia AJ889591 AJ890127 AJ890223 AJ888444	CBS 311.72	Brown sandy soil. Tsintsabis, Namibia	AJ889603	AJ890132	AJ890218	AJ888425	
FMR 7294 Blood, Australia AJ889591 AJ890127 AJ890223 AJ888444	CBS 418.73	Soil. Tadziikistan	AJ889595	AJ890124	AJ890211	AJ888426	
	FMR 7294	Blood, Australia	AJ889591	AJ890127	AJ890223	AJ888444	

TABLE 1. Isolates included in the study and their origins

DNA extraction, amplification, and sequencing. DNA was extracted and purified directly from fungal colonies according to the Fast DNA kit protocol (Bio101, Vista, Calif.), with a minor modification that consisted of the homogenization step repeated three times with a FastPrep FP120 instrument (Thermo Savant, Holbrook, N.Y.). The DNA was quantified with GeneQuant *pro* (Amersham Pharmacia Biotech, Cambridge, England). The internal transcribed spacer (ITS) region of the nuclear rRNA gene was amplified with the primer pair ITS5 and ITS4 (21), a fragment of the nuclear gene calmodulin (CAL) was amplified with the degenerated primer pair CL1 and CL2A (14), and two regions within the β -tubulin gene, BT2 and TUB, were amplified using the degenerated primer pair BT2-F (5'-GG(CT)AACCA(AG)AT(ATC)GGTGC(CT) GC(CT)-3') and BT2-R (5'-ACCCTC(AG)GTGTAGTGAACCATGGC-3') and TUB-F/TUB-R (6), respectively.

The PCR mixture (25 µl) included 20 to 60 ng of fungal DNA template, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 2.5 mM MgCl₂ (10× Perkin-Elmer buffer II plus MgCl₂ solution; Roche Molecular Systems, Branchburg, N.J.), 100 µM each deoxynucleoside triphosphate (Promega, Madison, Wis.), 1 µM of each primer, and 1.5 U of Ampli*Taq* DNA polymerase (Roche). The amplification program included an initial denaturation step at 94°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing for 1 min at 50°C (ITS), 55°C (CAL and TUB), or 60°C (BT2), and extension for 1 min at 72°C. A final extension step at 72°C for 7 min was included at the end of the amplification. After PCR, the products were purified with a GFXTM PCR DNA purification kit (Pharmacia Biotech, Cerdanyola, Spain) and stored at -20°C until they were used in sequencing.

The protocol for sequencing was the *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Gouda, The Netherlands). Reactions were run with a 310 DNA sequencer (Applied Biosystems). The consensus sequences were obtained using the Autoassembler program (Applied Biosystems).

Phylogenetic analysis. The sequences were aligned using the Clustal X (version 1.8) computer program (19) followed by manual adjustments with a text editor. Most-parsimonious tree (MPT) analyses were performed by using PAUP* version 4.0b10 (18). One hundred heuristic searches were conducted with random sequence addition and tree bisection reconnection branch-swapping algorithms, collapsing zero-length branches and saving all minimal-length trees (MulTrees) on different data sets. Scedosporium prolificans (FMR 7294) and Pseudallescheria africana (CBS 311.72) were chosen as the outgroup. Regions of sequences with ambiguous alignments were excluded from all analyses (ITS, positions 58 and 59; BT2, positions 90 to 126), and gaps were treated as missing data. Support for internal branches was assessed using a heuristic parsimony search of 500 bootstrapped data sets. The combined data set was tested for incongruence with the partition homogeneity test (PHT), as implemented in PAUP*. To avoid detecting incongruence that is expected within lineages, partition homogeneity tests were restricted to data sets containing only 20 individuals that represented the main lineages (CBS 254.72, FMR 4072, FMR 4167, FMR 6694, FMR 6697, FMR 6920, FMR 6921, FMR 7884, FMR 8532, FMR 8540, FMR 8541, FMR 8623, FMR 8625, FMR 8630, IHEM 14268, IHEM 14467, IHEM 15458, MUCL 14009, RKI 2956/93, RKI 2782/95, and RV 43605). To test alternative phylogenetic relationships, the Kishino-Hasegawa maxi-

mum-likelihood ratio test (11) was performed, as implemented in PAUP*.
Morphological study. The fungi were subcultured on potato dextrose agar

(PDA; Difco Laboratories, Detroit, Mich.) for macroscopic examination and growth rates at 25, 37, 40, 42, 45, and 50°C in darkness. For the study of microscopic characteristics, they were cultivated on oatmeal agar (OA) (30 g oat flakes, 1 g MgSO₄ · 7H₂O, 1.5 g KH₂PO₄, 15 g agar, 1 liter tap water). The microscopic features were determined by making wet mounts with lactic acid, which were then examined under a light microscope (Leitz Dialux 20).

Nucleotide sequence accession numbers. All the sequences obtained were deposited in the GenBank database. Accession numbers are shown in Table 1.

RESULTS

Phylogeny. Sixty isolates of *P. boydii* and relatives were chosen to examine species limits and evolutionary relationships among them. With the primers used, we were able to amplify and sequence 522 bp, 419 bp, 549 bp, and 570 bp of the ITS, BT2, TUB, and CAL loci, respectively. Of the 2,060 nucleotides sequenced, 386 characters (18.73%) were parsimony informative in the different *P. boydii* isolates. The lowest number was 42 in the ITS region, and the highest was 160 in CAL region. Sequences of the four region genes were analyzed phylogenetically as separate and combined data sets.

Phylogenetic analysis of the 419-bp BT2 data set yielded 12 MPT, which resulted in a total of 25 haplotypes (Fig. 1). There were 246 constant, 93 parsimony-informative, and 77 variable parsimony-uninformative characters in this fragment. The type strains of *P. fusoidea*, *P. ellipsoidea* and *P. angusta* were interspersed with the isolates of *P. boydii*. Four main highly supported clades (100%) were shown: the basal one comprising 4 European clinical isolates; two other small clades made up of 5 and 2 almost exclusively environmental isolates; and the biggest one, which comprised the 49 remaining isolates. However, inside the latter, another six terminal branches were present, each of them supported by a 100% bootstrap.

Parsimony analysis of the TUB data set yielded 420 MPT with 203 steps in length, in which 18 nodes received 100% bootstrap support. There were 420 constant, 90 parsimony-informative, and 39 variable parsimony-uninformative characters in this set. These trees resulted in a total of 22 different haplotypes (Fig. 2). Although the tree topology was slightly different from that of the previously mentioned locus, the three small, most-basal clades formed were also maintained here. Two of these clades, formed by four clinical and two environmental isolates, respectively, were the most phylogenetically distant.

Analysis of CAL and ITS sequences yielded a single MPT of 377 steps in length and 5,000 MPT of 97 steps in length, respectively. The numbers of haplotypes observed were 21 in the CAL tree (Fig. 3) and 19 in the tree based on ITS sequences (Fig. 4). Both trees showed similar topologies to that of the BT2 tree. The two above-mentioned basal clades were also placed here away from the other isolates. Overall, the ITS rRNA gene data set is considerably less informative for phylogenetic reconstruction than the other three markers.

The result of the partition homogeneity test showed that the sequence data sets for the four loci were congruent (P = 0.07) and could therefore be combined. A total of 2,496 MPT were produced from a heuristic search using the combined data set of 2,060 characters from the four loci (Fig. 5). From these characters, 1,440 were constant, 386 were parsimony informative, and 234 were variable parsimony noninformative. Clustering was similar to that observed in the particular trees of the different genes analyzed. A total of 44 haplotypes were shown. Most nodes in the combined analysis showed increased clade support as measured by bootstrapping (20 nodes with 100%) bootstrap support). As within the ITS, TUB, and CAL gene trees, two clades were identified as the basal-most lineages (clades 1 and 2), each of them with a bootstrap support of 100%. Phylogenetic analysis of the remaining monophyletic ingroup taxa (bootstrap, 100%) identified a basal clade (clade 3) and two bigger clades (clades 4 and 5), all them with 100%bootstrap support. The type strains of P. boydii, P. ellipsoidea, P. fusoidea, and P. angusta were placed in clade 5.

Morphology. All the 60 isolates that constituted the ingroups in the different trees obtained in our phylogenetic analyses were clearly identified by the presence of a characteristic *Scedosporium* anamorph. Although no relevant morphological differences were observed among them, the isolates that constitute clade 1 showed narrower conidia (2 to 5 μ m wide) than the rest (3 to 6 μ m wide). In addition, the members of this



FIG. 1. One of the 12 most-parsimonious trees obtained from heuristic searches based on BT2 sequence. Bootstrap support values above 70% are indicated at the nodes. Type strains are indicated with boldface type. *P. africana* and *S. prolificans* were used as outgroups. *Pb, P. boydii*; *Pe, P. ellipsoidea*; *Pf, P. fusoidea*; *Pa, P. angusta*; *Pm, P. minutispora*; *Sa, S. aurantiacum*; *Paf, P. africana*; *Sp, S. prolificans*; CI, consistency index; RI, retention index; HI, homoplasy index.

clade produced a yellow diffusible pigment, absent in the rest, and showed a maximum growth at 45°C compared to 40°C for the rest of isolates. Practically all isolates also developed conidia growing directly on vegetative hyphae, which were usually brown, thick-walled, and ellipsoidal to obovoidal. This latter feature was an important characteristic in differentiating the members of the subclade where the type strain of *P. boydii* is included, in which such conidia were globose to subglobose



— 1 change

FIG. 2. One of the 420 most-parsimonious trees obtained from heuristic searches based on TUB sequence. Bootstrap support values above 90% are indicated at the nodes. Type strains are indicated with boldface type. *P. africana* and *S. prolificans* were used as outgroups. *Pb, P. boydii*; *Pe, P. ellipsoidea*; *Pf, P. fusoidea*; *Pa, P. angusta*; *Pm, P. minutispora*; *Sa, S. aurantiacum*; *Paf, P. africana*; *Sp, S. prolificans*; CI, consistency index; RI, retention index; HI, homoplasy index.

(Table 2). Another typical feature, common to the half of the isolates included in the study, was the development of a second type of anamorph, namely, *Graphium* sp. *Graphium* is characterized by the production of synnemata terminated in a slimy head of conidia. The size of the synnemata was very variable

(80 to 750 μ m long) and depended on the culture medium used and the age of the culture. The production of *Graphium* was not exclusive of any clade since the isolates that produced them were distributed in all the five clades (Fig. 5). However, it was most common in clade 4 (in 14 of the 22 isolates) and in the



FIG. 3. The single most-parsimonious tree obtained from heuristic searches based on CAL sequence. Bootstrap support values above 70% are indicated at the nodes. Type strains are indicated with boldface type. *P. africana* and *S. prolificans* were used as outgroups. *Pb, P. boydii*; *Pe, P. ellipsoidea*; *Pf, P. fusoidea*; *Pa, P. angusta*; *Pm, P. minutispora*; *Sa, S. aurantiacum*; *Paf, P. africana*; *Sp, S. prolificans*; CI, consistency index; RI, retention index; HI, homoplasy index.



— 1 change

FIG. 4. One of the 5,000 most-parsimonious trees obtained from heuristic searches based on ITS sequence. Bootstrap support values above 80% are indicated at the nodes. Type strains are indicated with boldface type. *P. africana* and *S. prolificans* were used as outgroups. *Pb, P. boydii*; *Pe, P. ellipsoidea*; *Pf, P. fusoidea*; *Pa, P. angusta*; *Pm, P. minutispora*; *Sa, S. aurantiacum*; *Paf, P. africana*; *Sp, S. prolificans*; CI, consistency index; RI, retention index; HI, homoplasy index.

two isolates of clade 2. The presence of the teleomorph (the sexual state) was less frequent than the above-mentioned structures; it was produced only by the members of clades 5 (in 18 of the 27 isolates) and 2 (in the two isolates). Interestingly, the teleomorph of the isolates of the latter clade showed ascospores clearly different from those of the other species of *Pseudallescheria* included in the study. On the basis of the morphological differences, supported by the molecular data, we think



FIG. 5. One of the 2,496 most-parsimonious trees obtained from heuristic searches based on analysis produced from the combined data set. Bootstrap support values of 100% are indicated at the nodes. Type strains are indicated with boldface type. *P. africana* and *S. prolificans* were used as outgroups. *Pb, P. boydii; Pe, P. ellipsoidea; Pf, P. fusoidea; Pa, P. angusta; Pm, P. minutispora; Sa, S. aurantiacum; Paf, P. africana; Sp, S. prolificans;* S, source; T, teleomorph; *G, Graphium* anamorph; CI, consistency index; RI, retention index; HI, homoplasy index; c, clinical; e, environmental; +, presence; -, absence.

Species	Diffusible pigment on PDA at 25°C	Maximum growth temp (°C)	Teleomorph		Anamorph	
			Development of ascomata	Shape of ascospores	Shape of conidiogenous cells	Most common shape of conidia borne on vegetative hyphae
P. boydii	_	40	<u>±</u>	Broadly fusiform	Cylindrical	Globose
P. ellipsoidea	_	40	+	Ellipsoidal	Cylindrical	Ellipsoidal
S. aurantiacum	+ (yellow)	45	_	I	Cylindrical or slightly flask-shaped	Obovoid
S. prolificans	_	40	_		Flask-shaped	Globose

TABLE 2. Relevant features to differentiate the clinical species of *Pseudallescheria/Scedosporium* spp.

that clades 1 and 2 represent two different species from those up to now accepted in *Scedosporium* and *Pseudallescheria*, respectively, and are consequently here proposed as new.

Scedosporium aurantiacum. Gilgado, Cano, Gené, et Guarro, sp. nov. (Fig. 6).

Etym.: referred to the yellow color of the diffusible pigment of the colonies. Coloniae in agaro dense, plerumque gossypinae, luteolus vel brunneolus griseae; reversum brunneolus aurantiacum. Pigmentis flavis in culturis formantis. Conidiophora solitaria vel synnematica. Synnemata erecta, 330 to 750 μ m alta, cum caule atro griseo, cylindrico, 7.5 to 17.5 μ m lato, et capitulo mucoso usque ad 60 μ m alto, 70 to 140 μ m lato. Conidia obovoidea, subcylindrica vel claviformia, 5 to 14 by 2 to 5 μ m. Conidia sessilis copiosae, laterales, unicellularia, brunnea, plerumquam obovoidea, 6 to 10 by 3 to 5 μ m. Teleomorphosis ignota. Holotypus, IMI 392886, ex mycosis humanum (cultura viva, FMR 8630, IHEM 21147, CBS 116910).

The colonies on PDA attained a diameter of 40 to 50 mm after 14 days at 25°C. They were dense and usually cottony, but in some isolates they were lanose, especially at the center, frequently showing a concentric growth of aerial mycelium of different colors, yellowish gray combined with brownish gray areas, usually with a whitish, irregularly lobate and fimbriate margin, and the reverse was brownish orange at the center and brown to colorless towards the periphery. All isolates produced a light yellow diffusible pigment on PDA and OA after a few days of incubation. Conidiophores were solitary on aerial mycelium or grouped to form synnemata (Graphium) mainly on the agar surface. Solitary conidiophores were often reduced to a conidiogenous cell growing laterally on undifferentiated mycelium or branched, usually bearing verticils of two to three conidiogenous cells. Synnemata were present only in the isolates IHEM 15458 and RKI 2782/95. They were erect, 330 to 750 μ m long, consisting of a cylindrical stipe 7.5 to 17.5 μ m wide, dark gray, smooth-walled, and slightly roughened apically, and they terminated in a slimy head of conidia, up to 60 μ m long by 70 to 140 μ m wide. The conidiogenous cells were percurrent, lateral, or terminal, subhyaline, smooth-walled, cylindrical, or slightly flask shaped, 10 to 37 µm long by 1.5 to 2.5 µm wide, less frequent intercalary as a lateral projection on hyphae, and up to 5 μ m long by 2 μ m wide. There were three types of conidia: (i) those produced on solitary conidiophores were subhyaline, smooth-walled, obovoid, or subcylindrical, and 5 to 14 μ m by 2 to 5 μ m; (ii) those produced on synnemata were predominantly cylindrical or claviform, 6 to 12 µm by 3 to 5 μ m with a wide truncate base; (iii) those developed mainly from the undifferentiated hyphae of the substrate were sessile

or on short protrusions, solitary, lateral, brown, smooth, and thick-walled, usually obovoid, 6 to 10 μ m long by 3 to 5 μ m wide. The latter were abundantly produced by all isolates. Teleomorph was unknown.

The optimum growth temperature was from 37° C to 40° C with colonies on PDA attaining a diameter up to 60 to 67 mm after 14 days. Maximum growth was at 45° C. The fungus did not grow at 50° C.

Pseudallescheria minutispora. Gilgado, Gené, Cano, et Guarro, sp. nov. (Fig. 7).

Etym.: referred to the small size of the ascospores. Coloniae in agaro dense, gossypinae vel lanosae, aurantium griseae vel brunneolus griseae; reversum incoloratum. Pigmentis in culturis non formantis. Ascomata solitaria, non ostiolata, globosa vel subglobosa, 50 to 150 µm, luteolus vel brunneolus grisea, cum peridium ex textura epidermoidea. Asci octospori, globosi vel subglobosi, 12 to 15 by 10 to 13 µm, evanescentes. Ascosporae unicellulares, subhyalinae vel dilute brunneae, laeves, tenuitunicateae, ellipsoideae, 5 to 7 by 3 to 4 µm. Conidiophora solitaria vel synnematica. Synnemata erecta, 180 to 300 μm alta, cum caule atrobrunneo, cylindrico, 7.5 to 17.5 μm lato, et capitulo mucoso, 60 to 100 µm alto et 80 to 170 µm lato. Conidia obovoidea, ellipsoidea, cylindrica vel claviformia, 5 to 14 by 2.5 to 4.5 µm. Conidia sessilis parcusae, laterales, unicellularia, subhyalina, plerumquam obvoidea, 7 to 10 by 2.5 to 5 µm. Holotypus, IMI 392887, ex sedimentis fluvialibus (cultura viva, FMR 4072, IHEM 21148, CBS 11691).

The colonies on PDA attained a diameter of 50 to 57 mm after 14 days at 25°C. They were dense, cottony to lanose, orange gray combined with brownish gray areas, with a whitish, lobate or irregular, and fimbriate margin; the reverse was colorless. Diffusible pigment was absent. All isolates developed abundant ascomata on OA. The ascomata were solitary, nonostiolate, globose to subglobose, and 50 to 150 µm in diameter, with a peridium of textura epidermoidea, yellowish gray to brownish gray, and often covered with brown, thick-walled septate, 2.2 to 3 µm wide. The asci were eight-spored, globose to subglobose, and 12 to 15 µm long by 10 to 13 µm wide with evanescent walls. Ascospores were unicellular, subhyaline to light brown, smooth and thin-walled, ellipsoidal, and 5 to 7 µm long by 3 to 4 μ m wide, with a germ pore at each pole and usually with oil drops. Both isolates developed the two typical anamorph simply or scarcely branched conidiophores, up to 35 µm long. Its conidia were subhyaline to light brown, smoothwalled, obovoid, ellipsoidal or subclaviform, and 6 to 11 µm long by 3 to 4 µm wide. The Graphium anamorph produced synnemata which were erect and 180 to 300 µm long, with a



FIG. 6. *Scedosporium aurantiacum* isolates (A, D, E) IHEM 15458 and (B, C) FMR 8630. (A) Colony growing on PDA after 14 days of incubation at 25°C. (B, C) A conidiogenous cell and conidia from solitary conidiophores. (D) A synnema of the *Graphium* anamorph. (E) Apical part of a synnema producing conidia.



FIG. 7. *Pseudallescheria minutispora* strain FMR 4072. (A) Colony growing on PDA after 14 days of incubation at 25°C. (B) Conidiogenous cells and conidia of the *Scedosporium* anamorph. (C) Apical part of a synnema of the *Graphium* anamorph producing conidia. (D) Ascoma. (E) Conidia borne on undifferentiated hyphae. (F) Ascospores.

cylindrical stipe that was 7.5 to 17.5 μ m wide, and they were smoke brown, smooth-walled, slightly roughened apically, slightly inflated at the base, and up to 25 μ m wide and terminated in a slimy head of conidia that was 60 to 100 μ m long by 80 to 170 μ m wide. The conidia were predominantly cylindrical or claviform, 5 to 14 μ m long by 2 to 4.5 μ m wide, with a wide truncate base. The conidiogenous cells were percurrent, lateral or terminal, subhyaline, smooth-walled, usually cylindrical, 10 to 35 μ m long by 1.5 to 2 μ m wide, and less frequently intercalary as a lateral projection on hyphae, up to 6 μ m long by 2 μ m wide. Conidia from undifferentiated hyphae were scarcely produced. They were lateral, usually sessile, subhyaline, smooth and thick-walled, ellipsoidal to obovoid, and 7 to 10 μ m long by 3.5 to 5 μ m wide.

The optimum growth temperature was from 25°C to 30°C with colonies on PDA attaining a diameter up to 50 to 61 mm after 14 days. Maximum growth was at 40°C. The fungus was unable to grow at 42°C.

DISCUSSION

DNA sequences from four loci were analyzed to investigate phylogenetic relationships and species limits within the P. boydii species complex. Until recently, the genus Pseudallescheria was considered to comprise the following seven species (20): P. africana, P. angusta, P. boydii, P. desertorum, P. ellipsoidea, P. fimeti, and P. fusoidea. All of these species are morphologically very similar, and the main distinction among them is based on the size of the cleistothecia and ascospores (20). Recently, Rainer et al. (16), using different molecular techniques, concluded that P. ellipsoidea, P. fusoidea, and P. angusta are probable synonyms of P. boydii. In our study, we have included the type strains of these three species, and the results confirmed that all of them are genetically and morphologically different from P. boydii. We have also detected a high number of phylogenetic species, but only two of them can be clearly recognized morphologically.

The information provided by the four loci analyzed was very similar, which proved to be excellent phylogenetic markers for species level systematics within Pseudallescheria. The less informative locus was ITS, which only resolved 9 phylogenetically distinct species, whereas CAL, BT2, and TUB resolved 12, 14, and 15 species, respectively. Apart from P. africana, which was the outgroup of the present analysis, the two phylogenetic species most clearly separated were the two proposed as new, i.e., S. aurantiacum, represented by four clinical isolates, and P. minutispora, represented by two environmental isolates. However, we have recently studied another isolate of clinical origin from Germany (RKI 866/94) that genetically and morphologically matches the features of the latter species. The two clades formed by these species were highly supported in all the phylogenetic trees. Up to know, practically all the described species of *Pseudallescheria*, with the exception of *P*. boydii, P. fusoidea, and P. ellipsoidea, were monotypic; i.e., they are known by only one isolate. In the present study, the type strain of P. angusta nested with another soil isolate (FMR 8541) from Argentina. Both isolates showed identical ITS sequences, and those of the other loci studied were only different in 1 or 2 nucleotides (BT2, 1 nucleotide; TUB, 1 nucleotide; CAL, 2 nucleotides). The type strain of P. fusoidea nested with an environmental isolate from Zaire in three of the four loci analyzed. Both isolates also showed identical ITS sequences and differed in a few nucleotides in the other genes (BT2, 8 nucleotides; TUB, 4 nucleotides; CAL, 2 nucleotides). Our phylogenetic study revealed that P. fusoidea and P. angusta were phylogenetically very close. They only differed in 22 bp in the combined data set. However, P. angusta showed smaller ascomata (up to 110 µm in diameter versus up to 160 µm in diameter for P. fusoidea) and narrower ascospores (3 to 3.5 µm wide versus 4 to 4.5 µm wide for P. fusoidea). In addition, the isolates of P. fusoidea grew faster than those of P. angusta (69 to 70 mm versus 52 to 54 mm at 14 days on PDA at 25°C). The teleomorphs developed by the isolates of P. angusta, P. ellipsoidea, and P. fusoidea were consistent with the morphological features for the species described previously by von Arx et al. (20). However, the isolates that nested with the type strain of P. boydii (CBS 101.22) showed larger ascospores (6 to 9 by 5 to $6 \,\mu m$) than those described previously by von Arx et al. (20) for such species (6 to 7 by 3.5 to 4 μ m). Unfortunately, in our

study, the type strain of *P. boydii* only produced the *Scedosporium* anamorph. It is an old strain that has probably lost the ability to develop the sexual state. *P. ellipsoidea* was the species that was genetically and morphologically closest to the group of isolates that nested in the same branch as the type strain of *P. boydii*. However, *P. ellipsoidea* can be distinguished by its ellipsoidal ascospores, while the ascospores of the members of such groups of isolates are broadly fusiform. Moreover, in the former, the conidia from vegetative hyphae are ellipsoidal to obovoid and scarce, while those of the *P. boydii* branch are abundant and predominantly globose to subglobose (Table 2). Further studies of this group of isolates are required in order to define the morphological features of *P. boydii* sensu stricto.

Up to now, P. boydii had been considered the only pathogenic species of the genus Pseudallescheria, but this study has demonstrated that other phylogenetic species of the P. boydii complex also included clinical isolates. However, the clinical strains are not homogeneously distributed in the different clades, and some correlation between the clades and the clinical origin of the strains could be observed instead. Clinical strains were mainly concentrated in three of the five clades. Scedosporium aurantiacum (clade 1) grouped European clinical strains exclusively. Clades 4 and 5 were the biggest ones and included numerous strains each. However, 72% of the isolates of clade 5 were clinical, while only 50% of the isolates of clade 4 had such origin. It is noteworthy that two of the three strains included in the P. ellipsoidea group (CBS 418.73, FMR 7884, and FMR 8623) had caused disseminated infections, which emphasizes the clinical relevance of this species.

Most of the terminal branches that grouped more than one isolate included clinical and environmental isolates. This seems to demonstrate that any environmental strain can cause infection under the appropriate conditions. Using a murine model of invasive infection by *S. prolificans*, Ortoneda et al. (15) proved that there are no virulence differences between environmental strains and those that caused colonization or infection.

As expected, these results have demonstrated that P. boydii does not represent a single species. It encompasses a high number of phylogenetic species, although only a few of them can be recognized morphologically. One of the most important findings of this work is to provide phenotypic features useful for the distinction of some of these species (Table 2). Considering that not all the hospitals have facilities for molecular diagnosis and that not all these species are equally involved in human infections, these results can be especially useful for clinical microbiologists or laboratorians in order to identify these fungi. Judging by the high clinical relevance of this fungal group, further investigation is expected in the near future. It is especially important to determine if these species, and perhaps others, that could be identified in the future using similar approaches and involving more isolates from different sources and geographical regions are equally pathogenic to humans. Knowledge of the degree of virulence of these species and their response to the antifungal drugs may also be very useful in order to choose the appropriate treatment of the severe and refractory infections attributed to P. boydii sensu lato. Furthermore, taking into account that many of these species can only be reliably separated through molecular phylogenetics of DNA

sequences, finding morphological apomorphies for their laboratory identification would also be very valuable.

ACKNOWLEDGMENTS

We are indebted to the curators of the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands), BCCM/IHEM Biomedical Fungi and Yeasts Collection (Brussels, Belgium), Mycotheque de l'Universite Catholique de Louvain (Belgium), the Institute for Tropical Medicine (Antwerp, Belgium), and the Robert Koch Institute (Berlin, Germany) and to P. Godoy (Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil), J. M. Torres (IMIM, Hospital del Mar, Barcelona, Spain), A. Rezusta (Hospital Universitario Miguel Servet, Zaragoza, Spain), R. Negroni (Hospital de Infecciosas Francisco Javier Muñiz, Buenos Aires, Argentina), J. Llovo (Complejo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain), and A. del Palacio (Hospital Universitario Doce de Octubre, Madrid, Spain) for supplying many of the strains used in the study.

This study was supported by the Spanish Ministerio de Ciencia y Tecnología, grant CGL 2004-00425/BOS.

REFERENCES

- Capilla, J., C. Serena, F. J. Pastor, M. Ortoneda, and J. Guarro. 2004. Efficacy of voriconazole in treatment of systemic scedosporiosis in neutropenic mice. Antimicrob. Agents Chemother. 48:4009–4011.
- Carrillo, A., and J. Guarro. 2001. In vitro activities of four novel triazoles against *Scedosporium* spp. Antimicrob. Agents Chemother. 45:2151–2153.
- Castiglioni, B., D. A. Sutton, M. G. Rinaldi, J. Fung, and S. Kusne. 2002. *Pseudallescheria boydii* (anamorph *Scedosporium apiospermum*) infection in solid organ transplant recipients in a tertiary medical center and review of the literature. Medicine 81:333–348.
- Cazin, J., Jr., and D. W. Decker. 1964. Carbohydrate nutrition and sporulation of *Allescheria boydii*. J. Bacteriol. 88:1624–1628.
- Cazin, J., Jr., and D. W. Decker. 1965. Growth of Allescheria boydii in antibiotic-containing media. J. Bacteriol. 90:1308–1313.
- Cruse, M., R. Telerant, T. Gallagher, T. Lee, and J. Taylor. 2002. Cryptic species in *Stachybotrys chartarum*. Mycologia 94:814–822.
- 7. Defontaine, A., R. Zouhair, B. Cimon, J. Carrère, E. Bailly, F. Symoens, M.

Diouri, J. N. Hallet, and J. P. Bouchara. 2002. Genotyping study of *Scedosporium apiospermum* isolates from patients with cystic fibrosis. J. Clin. Microbiol. 40:2108–2114.

- de Hoog, G. S., J. Guarro, J. Gené, and M. J. Figueras. 2000. Atlas of clinical fungi, 2nd. ed. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, and University Rovira i Virgili, Reus, Spain.
- Gordon, M. A. 1957. Nutrition and sporulation of *Allescheria boydii*. J. Bacteriol. 73:199–205.
- Gueho, E., and G. S. de Hoog. 1991. Taxonomy of the medical species of Pseudallescheria and Scedosporium. J. Mycol. Med. 1:3–9.
- Kishino, H., and M. Hasegawa. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. 29:170–179.
- Koufopanou, V., A. Burt, T. Szaro, and J. W. Taylor. 2001. Gene genealogies, cryptic species, and molecular evolution in the human pathogen *Coccidioides immitis* and relatives (Ascomycota, Onygenales). Mol. Biol. Evol. 18:1246– 1258.
- O'Donnell, K. 2000. Molecular phylogeny of the Nectria haematococca-Fusarium solani species complex. Mycologia 92:919–938.
- O'Donnell, K., H. I. Nirenberg, T. Aoki, and E. Cigelnik. 2000. A multigene phylogeny of the *Giberella fujikuroi* species complex: detection of additional phylogenetically distinct species. Mycoscience 41:61–78.
- Ortoneda, M., F. J. Pastor, E. Mayayo, and J. Guarro. 2002. Comparison of the virulence of *Scedosporium prolificans* strains from different origins in a murine model. J. Med. Microbiol. 51:924–928.
- Rainer, J., G. S. de Hoog, M. Wedde, I. Graser, and S. Gilges. 2000. Molecular variability of *Pseudallescheria boydii*, a neurotropic opportunist. J. Clin. Microbiol. 38:3267–3273.
- Steinbach, W. J., and J. R. Perfect. 2003. Scedosporium species infections and treatments. J. Chemother. 15:16–27.
- Swofford, D. L. 2001. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods) (version 4.0). Sinauer Associates, Sunderland, Mass.
- Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24:4876–4882.
- von Arx, J. A., M. J. Figueras, and J. Guarro. 1988. Sordariaceous ascomycetes without ascospore ejaculation. Beihefte Nova Hedwigia 94:1–104.
- White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to the methods and applications. Academic Press, New York, N.Y.