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Epidemiology of Human Sporotrichosis Investigated by Amplified Fragment Length Polymorphism

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Amplified fragment length polymorphism (AFLP) was used to analyze the genetic diversity of Peruvian strains of *Sporothrix schenckii* and to compare them to a panel of non-Peruvian strains. AFLP analysis suggests that the Peruvian strains can be divided into two homogeneous clusters with no reference to geographical origin or the clinical form of sporotrichosis. The strains from abroad present heterogeneous profiles, with the Bolivian strain and the Colombian strains related to one of the Peruvian population. Sequencing of internal transcribed spacer 2, used to examine the relationships over a longer distance, confirmed the division of Peruvian strains into two populations that can be identified on the basis of a single but specific sequence divergence. This paper introduces automated AFLP analysis as a valuable tool for further investigation of the epidemiology and ecology of *S. schenckii*.

Sporothrix schenckii is the causative agent of human sporotrichosis. The most common form of infection is a localized cutaneous-subcutaneous disease characterized by ulcerative lesions, associated with lymphangitis and lymphadenopathy. Cases of sporotrichosis have been reported worldwide since the original description by Schenck at Johns Hopkins in 1898. However, North and South America have been the main sources for descriptions of new cases over the last two decades. The disease is considered to be the most common subcutaneous mycosis in South America (3, 4, 15, 18). In Peru, sporotrichosis has recently been shown to be hyperendemic in the Southern Andean mountains (17). Pappas et al. (17) reviewed records in a large referral clinic and found an annual sporotrichosis incidence of 48 to 60 cases per 100,000 population in Abancay, Peru.

Unequivocal diagnosis is obtained by culturing the fungus. *S. schenckii* is a dimorphic fungus in the class *Hyphomycetes*, isolated and cultivated from lesions under the anamorph state only. Mycelial culture develops at 25°C, whereas a characteristic shift to the yeast form is obtained in brain heart infusion (BHI) broth at 37°C.

The plant pathogen *Ophiostoma stenoceras* is the most closely related perfect fungus, with a quasi-undistinguishable conidial state. The primary habitats for *S. schenckii* are soil and plants. In contrast with the *Ophiostoma* species, no preferential biological association or pathogenicity for plants has been described for *S. schenckii* (12).

According to the classical description, resulting from analysis of many outbreaks among miners, forestry workers, gardeners, florists, etc., cutaneous and subcutaneous sporotrichoses are acquired through the skin by traumatic implantation of the fungus from the soil and plants (12, 15, 23). Sporotrichosis is mainly an occupational disease; additionally, a minor trauma may play an important role in introducing *S. schenckii* into the skin, as suggested by the association between sporotrichosis and ownership of a cat (14). Infected cats have been implicated in several small outbreaks or case reports of sporotrichosis in humans, suggesting an alternative route of infection (1, 6, 7). Cats represent a significant zoonotic potential, because their lesions are rich in parasites and they are close to humans.

Several molecular epidemiology studies have addressed the question of the genetic diversity of *S. schenckii*. The resulting clusters of strains have been found to be related to geographic origin and not to pathological or ecological features (5, 8–11, 13, 16, 20, 21, 22).

The experiments presented in this paper were carried out with a view to describing the genetic diversity of *S. schenckii*. The main group of strains investigated here comprised clinical strains of *S. schenckii* from different regions of Peru, a country characterized by a large ecological diversity between the coast, highlands, and tropical forest. A DNA fingerprinting method (amplified fragment length polymorphism [AFLP]) was used to identify different populations of strains. DNA sequencing of the internal transcribed spacer 2 (ITS2) was performed in order to determine the evolutionary relationship between the different populations.

MATERIALS AND METHODS

A total of 43 *S. schenckii* strains from Peru ($n = 32$), Mexico ($n = 7$), Colombia ($n = 1$), Bolivia ($n = 1$), Italy ($n = 1$), and South Africa ($n = 1$) were analyzed. Two Belgian environmental strains of the related species *Ophiostoma piceae* and *Sporothrix catenata* were added as external references (Table 1).

The strains were kept freeze-dried in the Belgian Coordinated Collections of Microorganisms—Institute of Hygiene and Epidemiology, Mycology (BCCM-IHEM) collection of medical fungi (http://www.belspo.be/bccm/ihem.htm). All the Peruvian strains were isolated and identified in the Mycology Laboratory of the Instituto de Medicina Tropical "Alexander von Humboldt," Universidad Peruana Cayetano Heredia, in Lima, Peru. All Mexican strains were kindly

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TABLE 1. *Sporothrix schenckii* strains used in this study

BCCM-IHEM no.[a]	Acronym	Date and author of first identification	Source[b]	Location[c]
695		Nolard, 1981	Air from factory	Belgium
2390		Beguin	Seeds of cereals	Belgium
3774	B40225	Restrepo, 1984	Sporotrichosis	Colombia
3787	B39515	Bayles; 1984	NK	South Africa
4178	IUM86431	Viviani, 1986	Skin nodule	Italy
15477		Yargas, 1998	Skin nodule	Bolivia
15486	1621	Bustamante, 1983	NK	Lima, Peru
15487	3287	Bustamante, 1983	FC sporotrichosis	Amazonas, Peru
15488	9788	Bustamante, 1989	LC sporotrichosis	Loreto, Peru
15489	9789	Bustamante, 1989	LC sporotrichosis	Loreto, Peru
15490	9963	Bustamante, 1989	LC sporotrichosis	Apurimac, Peru
15491	9965	Bustamante, 1989	LC, sporotrichosis	Lima, Peru
15492	10012	Bustamante, 1990	LC sporotrichosis	Amazonas, Peru
15495	11549	Bustamante, 1991	LC sporotrichosis	Puno, Peru
15499	13462	Bustamante, 1992	FC sporotrichosis	Apurimac, Peru
15500	14036	Bustamante, 1992	LC sporotrichosis	Amazonas, Peru
15501	14679	Bustamante, 1992	LC sporotrichosis	Amazonas, Peru
15502	14955	Bustamante, 1993	LC sporotrichosis	S. Martin, Peru
15503	16678	Bustamante, 1994	LC sporotrichosis	Ayacucho, Peru
15508	20825	Bustamante, 1995	LC sporotrichosis	Amazonas, Peru
15509	21259	Bustamante, 1996	LC sporotrichosis	Amazonas, Peru
15511	22770	Bustamante, 1996	LC sporotrichosis	Lima, Peru
15512	23432	Bustamante, 1997	FC sporotrichosis	Cuzco, Peru
15513	23489	Bustamante, 1997	LC sporotrichosis	Lima, Peru
15516	25013	Bustamante, 1997	FC sporotrichosis	Apurimac, Peru
15518	P19-A	Bustamante, 1988	FC sporotrichosis	Apurimac, Peru
15522	P81-A	Bustamante, 1988	FC sporotrichosis	Apurimac, Peru
15524	SSC-1	Bustamante, 1993	DC sporotrichosis	Cuzco, Peru
15525	SSC-2	Bustamante, 1993	NK	Cuzco, Peru
15526	SSC-3	Bustamante, 1993	DC sporotrichosis	Cuzco, Peru
15527	SST-7	Bustamante, 1997	Adobe wall	Apurimac, Peru
15528	SST-2	Bustamante, 1997	Adobe wall	Apurimac, Peru
15529	SS95	Bustamante, 1995	LC sporotrichosis	Loreto, Peru
15534	GP0230004	Bustamante, 1997	LC sporotrichosis	Apurimac, Peru
15536	JI0230006	Bustamante, 1997	FC sporotrichosis	Lima, Peru
15541	EFD230013	Bustamante, 1997	LC sporotrichosis	Lima, Peru
15542	27521	Bustamante, 1997	LC sporotrichosis	Cajamarca, Peru
Not available	36836	Bustamante, 2001	DC sporotrichosis	Lima, Peru
19716	EH143	Arenas, 1992	LC sporotrichosis	Mexico FD
19717	EH176	Arenas, 1992	FC sporotrichosis	Mexico FD
19718	EH179	Arenas, 1992	LC sporotrichosis	Mexico FD
19719	EH182	Arenas, 1992	DC sporotrichosis	León, Mexico
19720	EH190	Arenas, 1992	LC sporotrichosis	Oaxaca, Mexico
19723	EH200	Arenas, 1992	LC sporotrichosis	Puebla, Mexico
19724	EH260	Arenas, 1992	FC sporotrichosis	Chiapas, Mexico

[a] BCCM-IHEM, Belgian Coordinated Collections of Microorganisms—Institute of Hygiene and Epidemiology Mycology. 695, *O. piceae;* 2390, *S. catenata.*
[b] LC, lymphocutaneous; FC, fixed cutaneous; DC, disseminated cutaneous; NK, not known.
[c] FD, Federal District.

provided by H. Torres-Guerrero (Universidad Nacional Autónoma de México, Mexico D.F., Mexico).

The pigmentation of the colonies was observed after 7 and 14 days of incubation at 25°C for subcultures performed in Sabouraud dextrose agar. The ability to shift to the yeast phase was examined microscopically after 7 days for subcultures performed at 37°C in BHI broth.

For DNA extraction, cells were cultured in BHI broth for 7 days with constant agitation at 37°C for the *S. schenckii* strains or 25°C for *Ophiostoma*. Cells were recovered by centrifugation and then disrupted by using glass beads and vortexed. The DNA was purified by using the Dneasy Plant Maxi Kit (QIAGEN, Hilden, Germany). The AFLP procedure was performed according to the AFLP Analysis System for Microorganisms protocol (AFLP microorganism primer kit; Invitrogen Life Technologies, Carlsbad, Calif.). The first PCR was performed with the two preselective primers EcoRI core sequence and MseI core sequence, under the following conditions: 20 cycles of 30 s at 94°C, 60 s at 56°C, and 60 s at 72°C. A second PCR was performed using selective fluorescent primers EcoRI-AA and different primer MseI core sequences, MseI-A and MseI-G. Conditions were as follows: a 30-s denaturation step at 94°C, a 1-min annealing step, and a 1-min extension step at 72°C. The annealing temperature in the first cycle was 65°C; for each of the next 12 cycles, the annealing temperature was reduced further by 0.7°C; for the remaining 23 cycles, it was kept at 56°C.

Two microliters of each sample was mixed with 1 μl of a DNA size standard CEQ 600 (Beckman Coulter, Fullerton, Calif.) and 18 μl of sample loading solution in each well of a 96-well plate provided by the manufacturer. Capillary electrophoresis was performed in a CEQ 2000XL DNA analysis system (Beckman Coulter) using the fragment-2 parameters (inject voltage, 2 kV for 60 s; denaturation temperature, 90°C for 120 s; capillary temperature, 35°C; separation voltage, 6 kV for 60 min).

The list of fragments generated by AFLP wzx exported to Gel Compare software (version 4.1; Applied Maths, Sint-Martens-Latem, Belgium) and analyzed by the unweighted-pair group method using arithmetic means (UPGMA) with the Dice coefficient.

A DNA fragment covering the 3′ end of the 18S rRNA, the ITS, and the 5′ end of the 26S rRNA was amplified with primers IT1 (5′-GTCGCTACTACCCGA TTGAATGGCT-3′) and IT2 (5′-CCTCCGCTTATTGATATGCTTAAG-3′). PCR was performed for 35 cycles of denaturation at 92°C for 30 s, annealing at

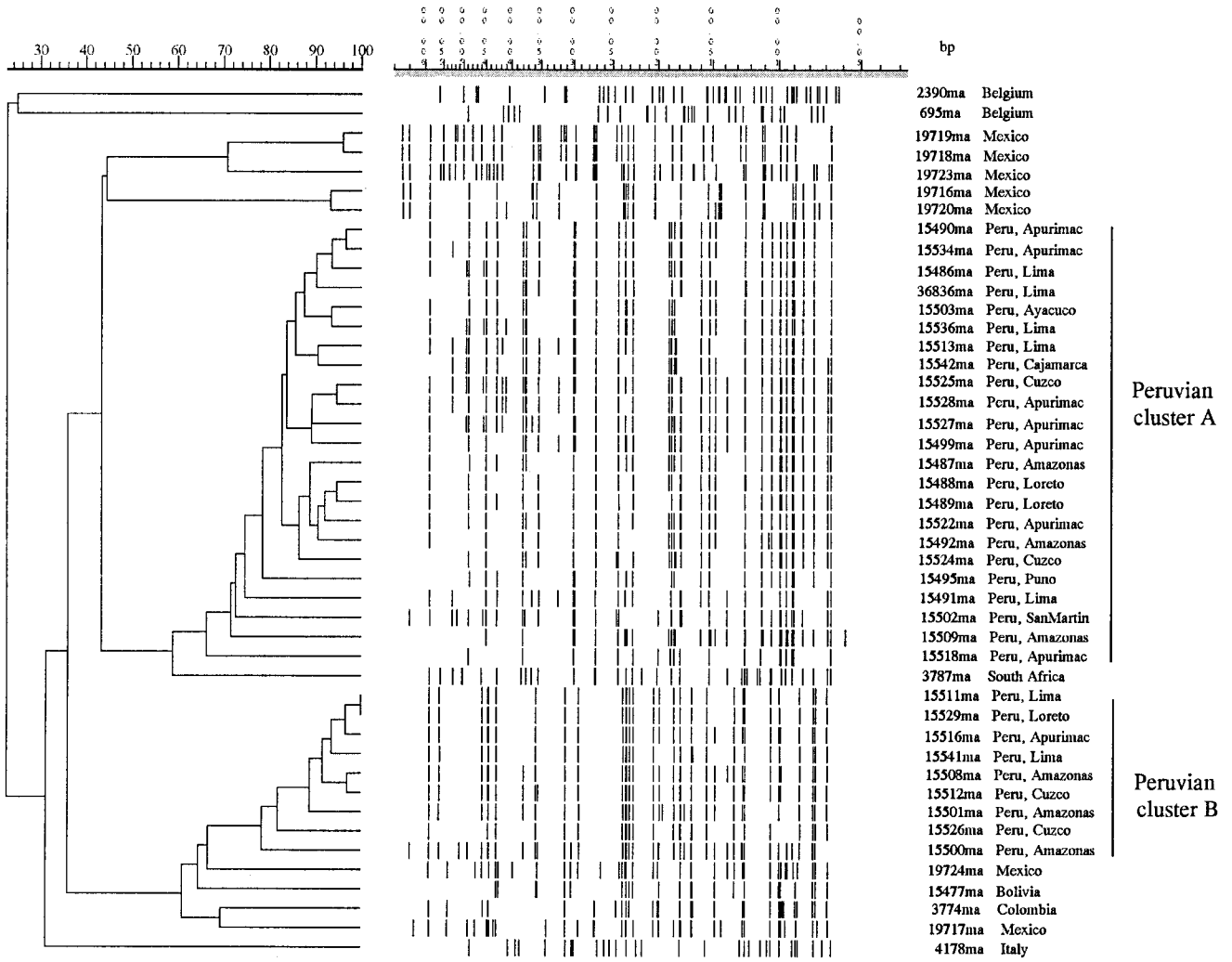

FIG. 1. Dendrogram generated by UPGMA analysis with the Dice coefficient from AFLP profiles obtained for strains of *S. schenckii* by using primers EcoRI-AA and MseI-A, showing Peruvian clusters A and B.

52°C for 30 s, and extension at 72°C for 1 min. Cycle sequencing of the ITS2 region was performed with primers ITS3 (5′-GCATCGATGAAGAACGCAG C-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) using the Beckman Coulter TM CEQ dye terminator cycle sequencing quick start kit. Further editing and multiple alignments were performed by using Lasergene (version 5; DNASTAR, Inc., Madison, Wis.), and the distance method UPGMA was used to create the tree (see Fig. 3) using the PAUP program (version 4.0b10, 2001; Sinauer Associates, Inc., Sunderland, Mass.).

Nucleotide sequence accession numbers. Representative *S. schenckii* ITS2 sequences were submitted to the EMBL Sequence Database and were assigned accession numbers AJ850920 to AJ850924.

RESULTS

Under our experimental conditions, the molecular masses of the bands generated by AFLP ranged from 57 to 650 bp. We found two clusters among the Peruvian strains by UPGMA and DICE analysis. These clusters were very stable by using the combination of six primers (EcoRI-AA and MseI, EcoRI-AA and MseI-A, EcoRI-AA and MseI-G).

The reproducibility of this technique was high but not perfect; however, the presence of nonreproducible bands did not modify the clustering. Figure 1 shows that we found two clusters for the Peruvian strains: cluster A (23 strains) and cluster B (9 strains). The South African strain was more related to Peruvian cluster A. The Colombian and Bolivian strains were closer to Peruvian cluster B.

The seven Mexican strains are highly heterogeneous, with two strains found to be related to Peruvian cluster B and the other five strains grouped into two subclusters separated from the Peruvian strains.

The Italian *S. schenckii* strain was separated from the other *S. schenckii* strains, and *O. piceae* and *S. catenata* were outside of the *S. schenckii* clusters. There was no correlation between the AFLP types of the Peruvian strains and their geographical origins; *S. schenckii* strains from Amazonas, Apurimac, Ayacucho, Cuzco, Lima, Loreto, San Martin, and Puno (Fig. 2) are dispersed into the two genetic clusters A and B. No correlation was found between AFLP type and the clinical form of the disease ($P = 0.037$).

Direct DNA sequence analysis of the ITS2 region was performed for 18 *S. schenckii* strains: 10 from Peru, 5 from Mex-

FIG. 2. Map of Peru showing the geographic locations of *S. schenckii* strains used in this study. The AFLP cluster B/AFLP cluster A ratio is given in parentheses for each location.

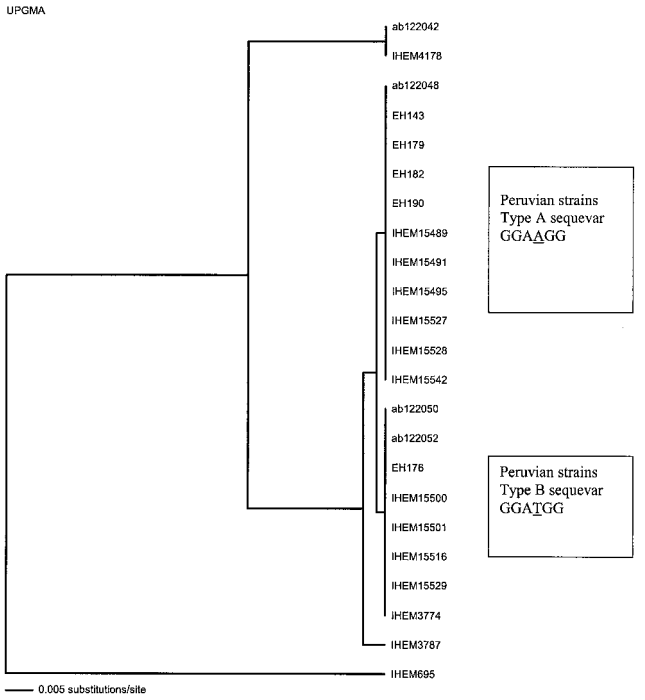

FIG. 3. Phylogram expressing distances (UPGMA) between ITS2 sequences of different strains typed by AFLP (this study) or mtDNA-RFLP (22). DNA sequences aligned cover part of the 5.8S sequence and the complete ITS2 sequence (316 positions aligned). Sequences from the work of Watanabe et al. (22) are identified by GenBank accession no. AB122042 is an mtDNA group B strain from Japan. AB122048, AB0122050, and AB0122052 are mtDNA group A strains from Argentina, Venezuela, and South Africa, respectively.

ico, and 1 each from Colombia, South Africa, and Italy. The *O. piceae* strain was used as an outgroup. Comparison of our ITS2 sequences with previously published sequences from the GenBank-EMBL database confirmed the identification of all strains. Representative sequences were submitted to the EMBL Sequence Database (see Materials and Methods).

The sequences obtained for Peruvian strains from AFLP clusters A and B were nearly identical; however, a 1-bp difference at position 189 allows us to distinguish between the type A strains (strains 15542, 15528, 15527, 15495, 15491, and 15489) and the type B strains (strains 15500, 15501, 15516, and 15529) (Fig. 3). The Colombian strain (strain 3774) had the same ITS2 sequence as the Peruvian strains with the type B AFLP profile. Among Mexican strains, 19717 (EH176) presented the type B ITS2 sequence, while 19720 (EH190), 19716 (EH143), 19719 (EH182), and 19718 (EH179) presented the more-frequent type A sequence. The South African strain (strain 3787) was very close to the Peruvian types A and B (>99% identity). The Italian strain (strain 4178) was more distant (95% identity). The Belgian *O. piceae* strain (strain 695) was clearly distinct at the species level (75% identity).

All clinical *S. schenckii* strains shifted to the yeast phase at 37°C in BHI broth. The two environmental *S. schenckii* cultures exhibited filamentous growth at 37°C. *O. piceae* and *S. catenata* did not grow at 37°C.

Dark-brown pigmentation appeared in 100% of genotype B cultures ($n = 9$) and in 56% (12 out of 23) of genotype A cultures; the difference found was statistically significant ($P < 0.05$). No association was found between the clinical sources of the strains and their pigmentation ($P = 0.33$).

DISCUSSION

The result of AFLP analysis is clear for the Peruvian *S. schenckii* strains. Two main genotypes were identified. Cluster A was significantly more frequent (23 strains) than cluster B (9 strains). The two genotypes of Peruvian strains were clearly distinct (Fig. 1). The strains from Mexico presented more-variable genotypes, with two strains related to cluster B and five strains distinct from Peruvian strains. The Bolivian and Colombian strains were found to be more closely related to type B. Among the strains isolated from non-American countries, only the South African strain was related to the Peruvian strains, specifically to cluster B. This result is in agreement with those of Ishizaki and colleagues (8–11), who found by using restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) that the South American strains were related to the South African strains. The 24 mtDNA-RFLP types described were classified into two large phylogenetic groups. mtDNA-RFLP group A is predominant in South America and Africa, and mtDNA-RFLP group B is predominant in Australia and Asia. Watanabe et al. have recently published the ITS sequences of *S. schenckii* strains previously typed by mtDNA-RFLP (22). Figure 3 compares the ITS2 sequences obtained for strains analyzed by AFLP with ITS2 sequences published by Watanabe, designated here by their GenBank accession numbers (22). According to the ITS2 sequence, only the Italian strain (strain 4178) is related to mtDNA group B strains from the work of Ishizaki et al. (10). The other *S. schenckii* strains tested here have ITS2 sequences closely related to sequences published for mtDNA group A

strains, which is not surprising considering their geographical origin.

Analysis of the Peruvian strains reveals that their ITS2 sequences are almost identical. The only divergence, a 1-bp difference at position 189 (Fig. 3), correlates perfectly with the division into two groups resulting from AFLP analysis.

The variability observed in the AFLP patterns appear to result from a combination of factors. Geographical separation is certainly important; however, the main division was not related to geography. For example, the main cluster of Peruvian strains, cluster A, is more closely related to the two main clusters of Mexican strains than to the other Peruvian strains of cluster B. More-detailed analysis of Peruvian strains also failed to link genotype and geographical origin (Fig. 1). Type A is dominant and is found all over the country (Ayacucho [$n = 1$], Lima [$n = 5$], Amazonas [$n = 3$], Apurimac [$n = 7$], Cajamarca [$n = 1$], Loreto [$n = 2$], San Martin [$n = 1$], Cuzco [$n = 2$], and Puno [$n = 1$]). Type B was less frequent but was not restricted to defined areas (Lima [$n = 2$], Apurimac [$n = 1$], Amazonas [$n = 3$], Cuzco [$n = 2$], and Loreto [$n = 1$]). There was no association between the clinical form of sporotrichosis and a particular genotype ($P = 0.37$). The origins of cluster A strains are lymphocutaneous ($n = 12$), fixed cutaneous ($n = 5$), disseminated cutaneous ($n = 2$), environmental ($n = 2$), and not recorded ($n = 2$). Cluster B strains are from lymphocutaneous ($n = 6$), fixed cutaneous ($n = 2$), and disseminated cutaneous ($n = 1$) cases. The main factors determining the clinical form are most likely related to the condition of the patient rather than to the genotype of the strains.

AFLP is a multilocus genotyping method that has been successfully applied to the study of pathogenic fungi such as *Cryptococcus neoformans*. Boekhout et al. (2), using AFLP, described considerable divergence between the different varieties of *C. neoformans*, a finding supported by independent phenotypic, biochemical, and molecular markers.

The division of Peruvian strains into two AFLP clusters has not been found to be related to the substrate, pathology, or geography. All AFLP type B strains have the ability to produce a dark-brown pigment. This characteristic is also present in 56% of the AFLP-type A strains; the difference was found to be statistically significant ($P < 0.05$). Romero-Martinez et al. (19) showed that melanized cells of *S. schenckii* are more resistant to killing by human monocytes, suggesting a link with pathogenicity. In our study, no association was found between pigmentation of the colonies and clinical form. As mentioned above for AFLP, the condition of the patient is most likely the main factor determining the clinical form.

The relationship between the genetic diversity and ecology of *S. schenckii* has not been studied. *S. schenckii* has been isolated from a variety of environmental sources. Also, sporotrichosis occurs in cats, dogs, and horses, and transmission from animals to humans is known (1, 6, 7). It might seem, therefore, that the two populations identified in Peru occupied two distinct niches in the environment of patients. Further work, including strains from animals and more samples from the environment, is required in order to identify a putative association between the genotype and specific niches. This paper introduces automated AFLP analysis as a valuable tool for investigating the biodiversity of *S. schenckii*. Compared to other methods such as mtDNA-RFLP or rRNA phylogeny, AFLP has the great advantage of targeting the whole genome rather than a part of the genome, thus allowing further studies that could establish a specific link between particular DNA fragments and relevant phenotypic markers.

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REFERENCES

1. **Barros, M., A. O. Schubach, A. do Valle, G. M. Gutierrez, F. Conceicao-Silva, T. Schubach, R. Reis, B. Wanke, K. Marzochi, and M. Conceicao.** 2004. Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. Clin. Infect. Dis. **38:**529–535.
2. **Boekhout, T., B. Theelen, M. Diaz, J. W. Fell, W. Hop, E. Abeln, F. Dromer, and W. Meyer.** 2001. Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans.* Microbiology **147:**891–907.
3. **Bustamante, B., and P. Campos.** 2001. Endemic sporotrichosis. Curr. Opin. Infect. Dis. **14:**145–149.
4. **Campos, P., R. Arenas, and H. Coronado.** 1994. Epidemic cutaneous sporotrichosis. Int. J. Dermatol. **33:**38–41.
5. **Cooper, C., B. Breslin, D. Dixon, and I. Salkin.** 1992. DNA typing of isolates associated with the 1988 sporotrichosis epidemic. J. Clin. Microbiol. **30:** 1631–1635.
6. **De Lima Barros, M., A. de Oliveira Schubach, M. Galhardo, T. Schubach, R. dos Reis, M. Conceicao, and A. do Valle.** 2003. Sporotrichosis with widespread cutaneous lesions: report of 24 cases related to transmission by domestic cats in Rio de Janeiro, Brazil. Int. J. Dermatol. **42:**677–681.
7. **Fleury, R., P. Taborda, A. Gupta, M. Fujita, P. Rosa, A. Weckwerth, M. Negrao, and I. Bastazini.** 2001. Zoonotic sporotrichosis. Transmission to humans by infected domestic cat scratching: report of four cases in Sao Paulo, Brazil. Int. J. Dermatol. **40:**318–322.
8. **Ishizaki, H., M. Kawasaki, M Aoki, T. Matsumoto, A. Padhye, M Mendoza, and R. Negroni.** 1998. Mitochondrial DNA analysis of S*porothrix schenckii* in North and South America. Mycopathologia **142:**115–118.
9. **Ishizaki, H., M. Kawasaki, M. Aoki, M. Miyaji, K. Nishimura, and J. A. Garcia Fernandez.** 1996. Mitochondrial DNA analysis of *Sporothrix schenckii* in Costa Rica. J. Met. Vet. Mycol. **34:**71–73.
10. **Ishizaki, H., M. Kawasaki, M. Aoki, H. Vismer, and D. Muir.** 2000. Mitochondrial DNA analyses of *Sporothrix schenckii* in South Africa and Australia. Med. Mycol. **38:**433–436.
11. **Ishizaki, H., and M. Kawasaki.** 2000. Molecular epidemiology of *Sporothrix schenckii.* Nippon Ishinkin Gakkai Zasshi **41:**245–249. (In Japanese.)
12. **Kwon-Chung, K., and J. Bennett.** 1992. Medical mycology: the pathogenic fungi and the pathogenic actinomycetes. Lea & Febiger, Philadelphia, Pa.
13. **Lin, J., M. Kawasaki, M. Aoki, H. Ishizaki, G. You, and R. Li.** 1999. Mitochondrial DNA analysis of *Sporothrix schenckii* clinical isolates from China. Mycopathology **148:**69–72.
14. **Lyon, G., S. Zurita, J. Casquero, W. Holgado, J. Guevara, M. Brandt, S. Douglas, K. Shutt, D. Warnock, R. A. Hajjeh, and the Sporotrichosis in Peru Investigation Team.** 2003. Population-based surveillance and a case-control study of risk factors for endemic lymphocutaneous sporotrichosis in Peru. Clin. Infect. Dis. **36:**34–39.
15. **Mandell, G. L., J. E. Bennett, and R. Dolin.**1999. Mandell, Douglas and Bennett's principles and practice of infectious diseases, 5th ed., vol. 2. Churchill Livingstone, Edinburgh, United Kingdom.
16. **Mora-Cabrera, M., R Alonso, R. Ulloa-Arvizu, and H. Torres-Guerrero.** 2001. Analysis of restriction profiles of mitochondrial DNA from S*porothrix schenckii.* Med. Mycol. **39:**439–444.
17. **Pappas, P., I. Tellez, A. Deep, D. Nolasco, H. Holgado, and B. Bustamante.** 2000. Sporotrichosis in Perú: description of an area of hyperendemicity. Clin. Infect. Dis. **30:**65–70.
18. **Rios-Fabra, A., A. Restrepo, and R. Istúriz.** 1994. Fungal infections in Latin American countries. Infect. Dis. Clin. N. Am. **8:**129–154.
19. **Romero-Martinez, R., M. Wheeler, A. Guerrero-Plata, G. Rico, and H. Torres-Guerro.** 2000. Biosynthesis and funtions of melanin in *Sporothrix schenckii.* Infect. Immun. **68:**3696–3703.
20. **Suzuki, K., M. Kawasaki, and H. Ishizaki.** 1988. Analysis of restriction profiles of mitochondrial DNA from S*porothrix schenckii* and related fungi. Mycopathologia **103:**147–154.
21. **Takeda, Y., M. Kawasaki, and H. Ishizaki.** 1991. Phylogeny and molecular epidemiology of S*porothrix schenckii* in Japan. Mycopathologia **116:**9–14.
22. **Watanabe, S., M. Kawasaki, T. Mochizuri, and H. Ishizaki.** 2004. RFLP analysis of the internal transcribed spacer regions of *Sporothrix schenckii.* Jpn. J. Med. Mycol. **45:**165–175.
23. **Werner, A., and B. Werner.** 1994. Sporotrichosis in man and animal. Int. J. Dermatol. **33:**692–700.