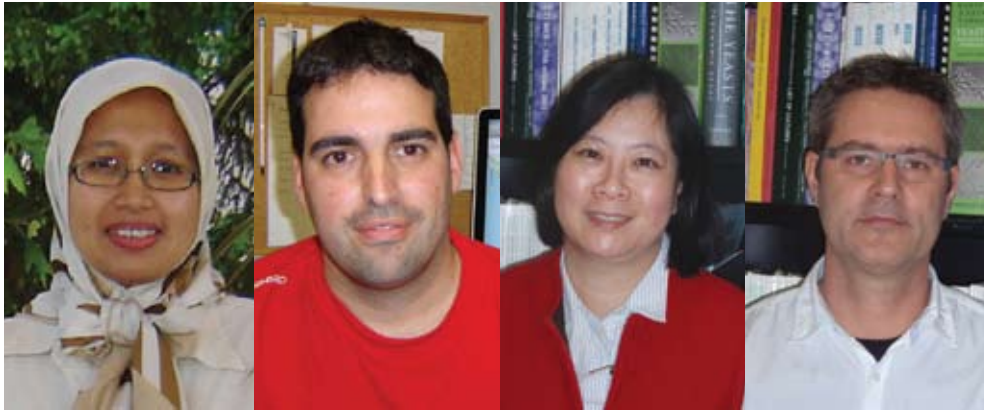


Environmental isolation of *Scedosporium* species from the greater Sydney region: a link to the emergence of infections in Australia?



Azian Harun, Felix Gilgado, Sharon Chen & Wieland Meyer

Molecular Mycology Research Laboratory, Centre for Infectious Disease and Microbiology, The University of Sydney Western Clinical School at Westmead Millennium Institute, Westmead Hospital, Westmead NSW 2145
Tel (02) 9845 6895
Fax (02) 9891 5317
Email w.meyer@usyd.edu.au

***Scedosporium* species are emerging fungal pathogens in Australia and elsewhere. The reason for this increase in infections is unclear. Since it is assumed that the infections are caused by inhalation of fungal spores, we sampled the urban and rural environment of the greater Sydney region for the presence of the human pathogenic species. Our findings indicate that there may be species-specific associations with areas of high human activity, hinting of a possible link between the environment and the emergence of infections.**

Species of the genus *Scedosporium* are emerging fungal pathogens that cause a wide range of clinical disease, from superficial skin/soft tissue infections to disseminated infections¹. Until recently, *S. prolificans* and *S. apiospermum* (asexual state of the species *Pseudallescheria boydii*) were the only clinically important species in this genus. *S. apiospermum*/*P. boydii* has a worldwide distribution, whereas *S. prolificans* is most prevalent in Spain, Australia and the United States.

Recent phylogenetic analysis showed that *S. apiospermum*/*P. boydii* is a complex of species, which is now known to consist of at least seven different species^{2, 3}. These studies also demonstrated that *P. boydii* and *S. apiospermum* are two separate species³. Currently, the following species have been accepted; *Pseudallescheria angusta*, *P. boydii sensu stricto*, *Pseudallescheria desertorum*, *Pseudallescheria ellipsoidea*,

Pseudallescheria fusioidea, *Pseudallescheria minutispora*, *S. apiospermum*, *Scedosporium aurantiacum* and *Scedosporium deboogii*³.

Of these species, *P. boydii*, *S. apiospermum* and the recently described species *S. aurantiacum*, have been recovered in clinical specimens^{2, 3}. Both species affect immunocompromised and immunocompetent individuals and as such are not only fungal opportunists but are primary pathogens. Predisposing factors include organ transplantation and malignancy, and also encompass chronic lung disease and diabetes mellitus [Heath *et al.* unpublished data]. In addition, these organisms are capable of long-term colonisation of the respiratory tract of patients with compromised airways, including those with cystic fibrosis⁴.

Infections caused by *Scedosporium* species, particularly *S. prolificans*, are associated with mortality rates of up to 75-80%⁵. Therefore, early diagnosis and prompt effective antifungal treatment is mandatory to improve patient outcomes. Given that treatment is problematic as these pathogens are inherently less susceptible or resistant to currently available antifungal agents, a better understanding of the epidemiology, mode of transmission and ecological niche of *Scedosporium* spp. is important for the implementation of alternative pre-emptive and preventive strategies.

The importance of scedosporiosis in Australia has been highlighted by recent reports, which describe the epidemiology and clinical

features as well as outcomes of infection. A single-centre study including 59 patients highlighted the emergence of *Scedosporium* spp. as causative agents of human mycoses⁶. In a large Australia-wide population-based study involving 180 occasions of isolations, distinct risk factors for invasive disease and clinical features of *Scedosporium* infection were analysed. A key finding of this survey was the identification of a substantial number of the new species *S. aurantiacum* and its ability to cause a wide range of invasive infections [Heath *et al.* unpublished data].

The emergence of *Scedosporium* infections also has public health significance. In this regard, understanding of factors that have contributed to their emergence would assist in implementing appropriate early intervention strategies. Given that *Scedosporium* spp. are ubiquitous in nature, and that infection is thought to be acquired via inhalation of airborne

fungus spores, it is logical to postulate that exposure to these organisms in the environment may have contributed to the increase in human infections. Thus far, a limited number of studies have indicated that the main ecological niche of the *P. boydii* complex include environments heavily exploited by agricultural activities or human impacted environments such as cities and industrial areas^{7,8}. However, the association between the occurrence of *Scedosporium* spp. in the environment and the prevalence of scedosporiosis remains uncertain and the ecological niche of *S. prolificans* and *S. aurantiacum* are poorly defined.

As the first step towards finding such an association, a preliminary survey was undertaken to study the occurrence of *Scedosporium* spp. in the environment, specifically from the soil in the urban, high-populated areas in the greater Sydney region. In this

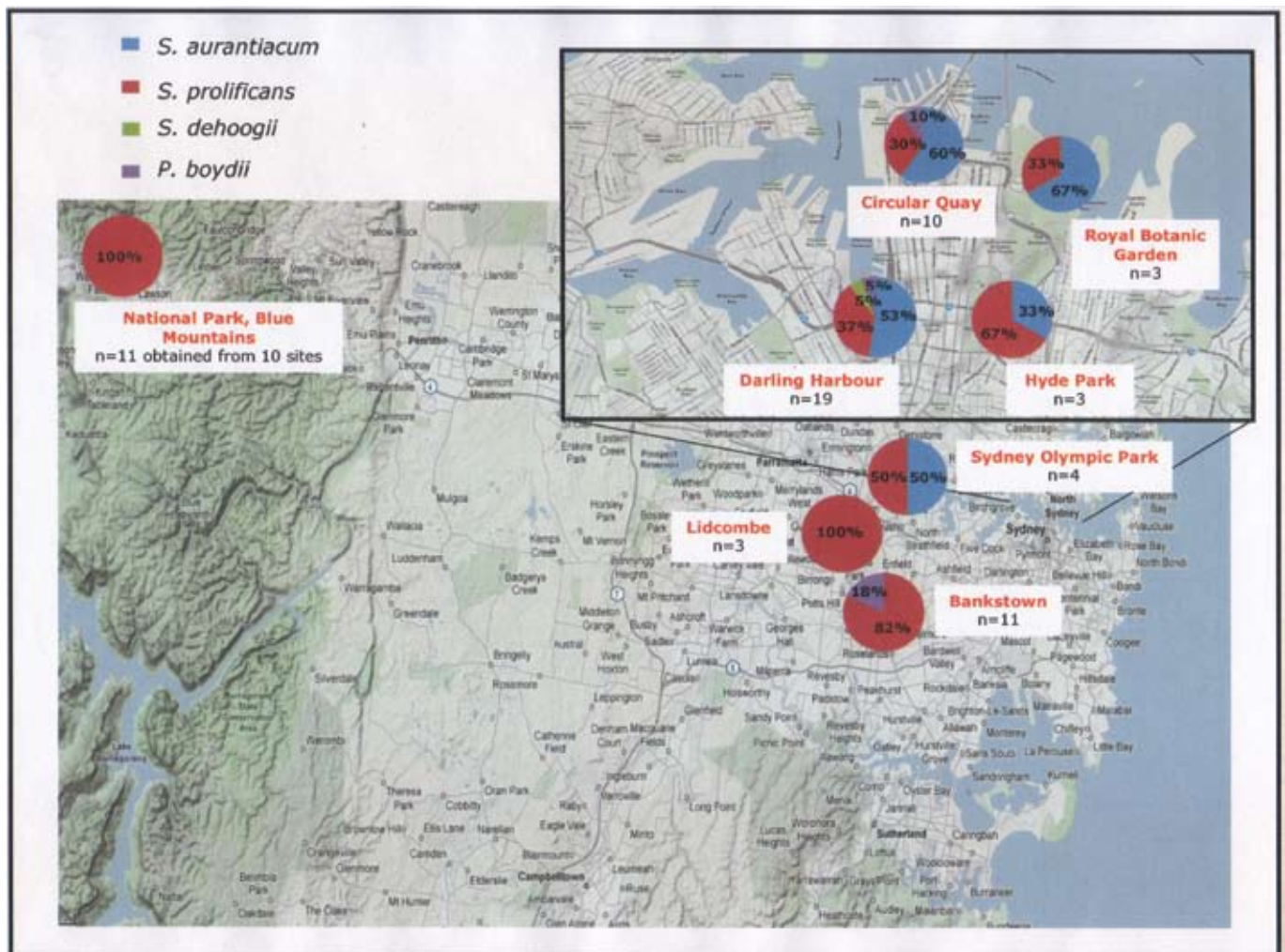


Figure 1. Environmental sampling sites in the greater Sydney region indicating the number of isolates recovered from soil and the percentage of isolates belonging to either *S. aurantiacum*, *S. dehoogii*, *S. prolificans* or *P. boydii*.

survey, two soil samples were each taken from a number of randomly selected locations around urban areas in the greater Sydney region (Figure 1). For comparison, sites in suburban and rural areas around Sydney were also sampled. Ten sites

were sampled in the Blue Mountains National Park, which were subsequently treated as a single site because of the low numbers of isolates recovered from those sites. The samples were collected from the superficial soil layers, transferred into

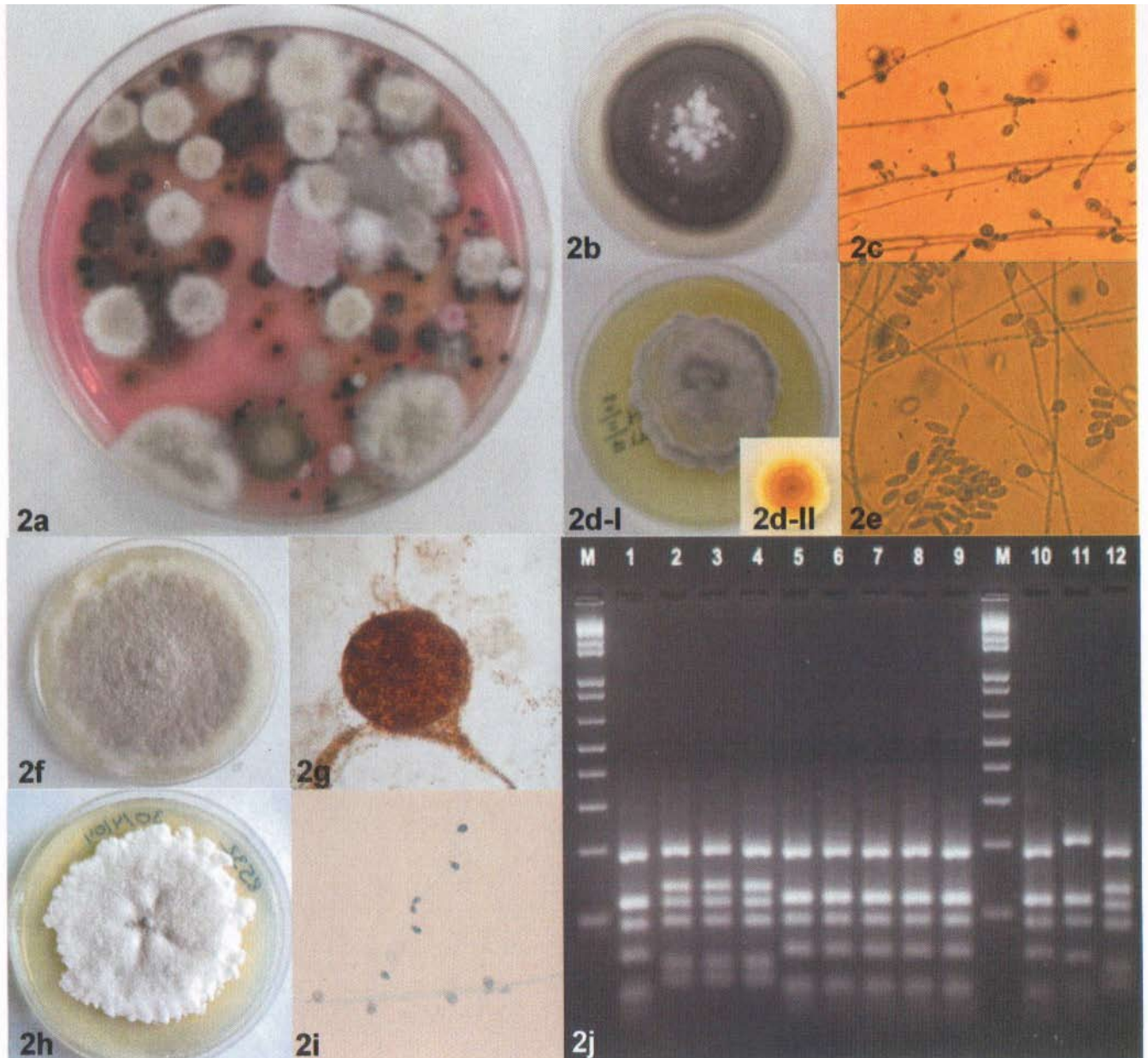


Figure 2. Species identification.

(2a) primary isolation plate (selective medium: Dichloran Rose-Bengal chloramphenicol agar supplemented with benomyl); (2b) *S. prolificans* macro colony morphology on PDA; (2c) *S. prolificans* micro-morphology; (2d-I) *S. aurantiacum* macro colony morphology on PDA; (2d-II) *S. aurantiacum* reverse site macro colony morphology on PDA, showing yellow diffusible pigment; (2e) *S. aurantiacum* micro-morphology; (2f) *P. boydii* macro colony morphology on PDA; (2g) *P. boydii* micro-morphology with ascoma and ascospores; (2h) *S. deboogii* macro colony morphology on PDA; (2i) *S. deboogii* micro-morphology; (2j) Examples of RFLP patterns obtained after double digestion of the ITS1/2 region of the rDNA gene cluster with the enzymes *Sau96I* and *HbaI*^o.

M=1kb plus molecular weight marker (Invitrogen, USA), lanes 1 till 9 environmental samples obtained from Darling Harbour, Lane 1 = WM09.12 (*S. aurantiacum*), Lane 2 = WM09.30 (*S. prolificans*), Lane 3 = WM09.31 (*S. prolificans*), Lane 4 = WM09.32 (*S. prolificans*), Lane 5 = WM09.13 (*S. aurantiacum*), Lane 6 = WM09.14 (*S. aurantiacum*), Lane 7 = WM09.15 (*S. aurantiacum*), Lane 8 = WM09.16 (*S. aurantiacum*), Lane 9 = WM09.17 (*S. aurantiacum*) Lanes 10 till 12 standard strains, Lane 10 = WM06.482 (*S. aurantiacum*), Lane 11 = WM06.472 (*S. apiospermum*), Lane 12 = WM06.525 (*S. prolificans*).

sterilised containers and processed as previously described². In short, 1g of soil was suspended in 10ml sterile water, and 2ml were cultured on the selective medium Dichloran Rose-Bengal chloramphenicol agar [Oxoid, United Kingdom] supplemented with benomyl at a concentration of 10µg/ml agar [Sigma-Aldrich, USA]. The plates were incubated at 35°C, and were regularly examined for growth of fungi with typical *Scedosporium* colony morphology. Colonies suspicious for *Scedosporium* spp. were isolated, cultured on Potato Dextrose Agar (PDA) [Oxoid, United Kingdom], and species identification was performed by routine morphological as well as by molecular methods (Figure 2), using restriction fragment length polymorphism analysis⁹ (Figure 2j). The identification of isolates with a presumptive morphological identification as *S. deboogii* or *P. boydii* were confirmed via ITS sequence analysis⁹.

Results of this preliminary survey showed that *Scedosporium* spp. can be isolated from most of the collection sites (Figure 1). A total of 64 *Scedosporium* isolates were recovered, of which 38 were identified as *S. prolificans*, 21 as *S. aurantiacum*, four as *P. boydii* and one as *S. deboogii*. Soil samples from the urban areas showed a relatively higher density of organism per gram of soil compared to rural areas (Figure 1). *S. aurantiacum* was recovered from urban areas with high human activities, but not from areas with a low human population density (Figure 1). *S. prolificans* was found in both rural and urban areas, but in much higher density in urban areas (Figure 1).

The results of the survey found the occurrence of *Scedosporium* spp. in the soil of urban areas in the greater Sydney region to be common. This raises the possibility that increased exposure to (as yet unidentified) environmental sources may place individuals at risk for developing scedosporiosis. To verify the proposed link between the environmental occurrence and clinical infection, molecular typing studies, using PCR-fingerprinting and multi-locus sequence analysis, comparing the genotypes of environmental isolates with those of clinical isolates are under way. The data generated in this environmental survey has the potential to be used to trace the route of infection and to determine and monitor high-risk environmental areas.

References

- Cortez, K.J. *et al.* (2008) Infections caused by *Scedosporium* spp. *Clin. Microbiol. Rev.* 21, 157-197.
- Gilgado, F. *et al.* (2005) Molecular phylogeny of the *Pseudallescheria boydii* species complex: proposal of two new species. *J. Clin. Microbiol.* 43, 4930-4942.
- Gilgado, F. *et al.* (2008) Molecular and phenotypic data supporting distinct species statuses for *Scedosporium apiospermum* and *Pseudallescheria boydii* and the proposed new species *Scedosporium deboogi*. *J. Clin. Microbiol.* 46, 766-771.
- Cimon, B. *et al.* (2000) Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. *Eur. J. Clin. Microbiol. Infect. Dis.* 19, 53-56.
- Rodriguez-Tudela, J.L. *et al.* (2009) Epidemiology and outcome of *Scedosporium prolificans* infection, a review of 162 cases. *Med. Mycol.* doi: 10.1080/13693780802524506.
- Cooley, L. *et al.* (2007) Infections with *Scedosporium apiospermum* and *Scedosporium prolificans* in Australia. *Emerg. Infect. Dis.* 13, 1170-1177.
- Guarro, J. *et al.* (2006) *Scedosporium apiospermum*: changing clinical spectrum of a therapy-refractory opportunist. *Med. Mycol.* 44, 295-327.
- Kaltseis, J. *et al.* (2009) Ecology of *Pseudallescheria* and *Scedosporium* species in human-dominated and natural environments and their distribution in clinical samples. *Med. Mycol.* doi: 10.1080/13693780802585317
- Delhaes L. *et al.* (2008) Molecular typing of Australian *Scedosporium* isolates showing genetic variability and numerous *S. aurantiacum*. *Emerg. Infect. Dis.* 14, 282-290.

Azian Harun is a third year PhD student from Malaysia in A/Prof. W Meyer's laboratory. Her research focuses on the population genetics of *Scedosporium* species in Australia.

Felix Gilgado is a postdoctoral fellow in A/Prof. W Meyer's laboratory. His research focuses on phylogeny and population genetics of human pathogenic fungi.

Sharon Chen is a senior staff specialist and medical mycologist at the Centre for Infectious Disease and Microbiology at Westmead Hospital. Her major research focus is on clinical mycology and epidemiology of fungal infections.

Wieland Meyer is Associate Prof. at the Faculty of Medicine at the University of Sydney and Chief Scientist of the Molecular Mycology Research Laboratory at Westmead Hospital. His research focuses on phylogeny, molecular identification, ecology, molecular epidemiology and the development of virulence of pathogenic fungi.

ASM Golden Jubilee

ASM's foundations go back to July 1959.

To recognise ASM's achievements there will be special celebrations commencing this July.

Be part of the celebrations.

Celebrations will include:

Release of a commemorative ASM envelope
by Australia Post (available from July 1)

✱

Celebrations at ASM 2009 in Perth

✱

A special extra issue of *Microbiology Australia*
on the History of ASM.
(available at ASM 2009 and to all members)

✱

Feature articles on State Branch histories