Boletín Micológico Vol. 19 : 63 - 69 2004

MICROFUNGI FROM DIFFERENT SUBSTRATA IN SOUTH WEST AFRICA (NAMIBIA)

(Microhongos de diferentes substratos, en el oeste de Africa(Namibia))

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Key words: Microfungi, Namibia soil, seed, herbs, bark, dung

Palabras clave: Microhongos, Namibia suelo, semillas, hierbas, corteza, excretas.

ABSTRACT

This paper can be considered as a little contribution to knowledge of fungal biodiversity in Namibia. Soils, seed dung and some vegetal substrata such as herb, leaves and bark were collected at random in order to investigate the presence of tropical microfungi and to determine the significance of some fungal taxa in the Namibian ecosystem.

A total of 49 genera and 80 taxa predominantly anamorph of Ascomycota was recorded, most of them (51%) were isolated from soil and many were considered common tropical microfungi. The most representative isolate taxa in all substrata analized or in some of them were: ***Alternaria, Aspergillus, Aureobasidium, Bipolaris, Chaetomium, Fusarium, Penicillium*** and ***Phoma****. It is interesting to note in vegetables the presence of* ***Lasiobolidium spirale****, quite a rare species.*

RESUMEN

Este trabajo es una pequeña contribución al conocimiento de la biodiversidad fúngica en Namibia. Se colectaron al azar, muestras de suelos, semillas, excrementos y algunos substratos vegetales, tales como pastos, hojas y cortezas, para poder investigar la presencia de microhongos tropicales y determinar el significado de algunos taxa fúngicos en el ecosistema de Namibia.

Se registraron un total de 49 géneros y 80 taxa, predominantemente anamorfos de Ascomycota, la mayoría de ellos (51%) fueron aislados del suelo y muchos considerados como microhongos tropicales comunes. Los taxa más representativos en todos los substratos analizados o en algunos de ellos fueron: ***Alternaria, Aspergillus, Aureobasidium, Bipolaris, Chaetomium, Fusarium, Penicillium*** *y* ***Phoma.*** *Es interesante destacar que en los vegetales se detectó la presencia de una rara especie,* ***Lasiobolidium spirale.***

INTRODUCTION

In the latest 10 years it can be noted an increasing interest and progress in tropical mycology and particularly in fungal diversity (Hawksworth, 2002). It is believed that most of the unknown species are in the tropics where some fungal groups or habitat are not yet investigated.

During a travel for tourism in Namibia, one of the authors (E.S) collected some vegetal substrata and soils at random, in order to investigate the presence of tropical microfungi and to determine the significance of some fungal *taxa* in the Namibian ecosystem.

Namibia is situated in south-eastern Africa in the latitude of the tropic of Capricorn (Fig.1), wedged between the Kalahari desert (in the east) and the chilly South Atlantic Ocean (west coast). It has many contrasting landscapes: thorn-bush savannah in the central highlands; dense bushveld, woodland savannah and the endless plains of the Etosha Pan in the north; the Fish River Canyon in the south and the world's oldest desert, the Namib, in the west of the country, on the Atlantic seaboard. The northern border is flush with rivers that provide water to most part of Namibia. Although it's predominantly desert, it enjoys

regional climatic variations. Most of Namibia has a sub-tropical 'desert' climate, characterised by a wide range in temperature (from day to night and from summer to winter), and by low rainfall and humidity. The northern strip follows the same pattern, but has a more moderate, less dry climate.

Namibia is the first country in the world to include protection of the environment and sustainable utilization of wildlife in its constitution. About 15,5% of the country has been set aside as National Parks. In these areas, rare and endangered species of animals, birds and plant life are preserved and protected, including virtually the entire Namib Desert coastal strip.

There are no estimates of fungal diversity in southern Africa (Barnard, 1998), studies on biodiversity of South western African fungi regard mostly interaction among these organisms and plants (Berndt *et al.,* 2003; Uhlmann, 2004) or fungi as agents of Biological Control. Tropical plants support dense and complex fungal populations on indigenous as well as cultivated plants. Among the 1226 new fungi described from 1981-1991 in Africa, 43 *taxa* were from Namibia (Hawksworth, 1993).

This work can be considered as a little contribution to knowledge of fungal biodiversity in this area.

MATERIALS AND METHODS

Sampling sites and substrata

The sampling area are shown in Figure1: it must be that the material was collected at random and not as a specific scientific project.

Five soil were sampled, during summer 2000, at Fish River Canyon, just around a kokerboom tree (*Aloe dichotoma*); in the desertic areas of Soussuvlei and Weltwitschia Drive; inside the Okakuejo and the Waterberg camps, nearby the lodges.

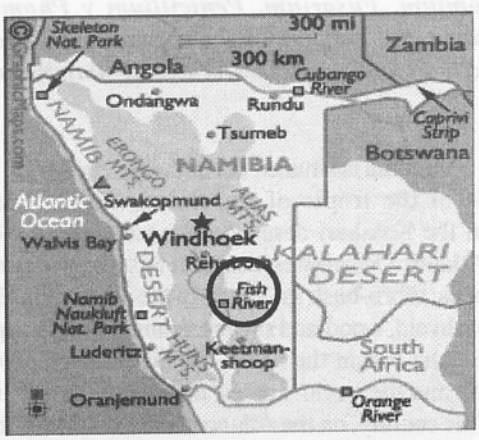

Figure 1.-Namibia map an geographic zones of sampling in circle

Vegetables, or part of them, were collected in the same area of soil samples:

a) Bark belonging to *Aloe dichotoma* (common name: quiver tree or kokerboom) (3 samples)

b) Leaves of *Colosphermum mopane* (common name: Mopane) and *Terminlia prunoides* (purplepod terminalia)(4 samples of 6 leaves each one)

c) Herbaceous plants: *Stipagrostis uniplumis* and *Tricholoma teneriffae* (*Poaceae*) and one not identified collected nearby the dunes in Soussuvlei desert (3 samples of each specimen)

d) Seeds of: *Acacia erioloba* (Camel thorn), *Acacia tortilis* subsp. *heterocantha* (umbrella thorn), *Terminlia prunoides* (3 samples with 10-15 seed of each specimen).

Sampling procedures

Samples of different material types were kept in separate sterile plastic bags and transferred to Italy for analyses of fungal communities. They were different depending on the *substratum*.

I) Soil. The five soil samples, about 200g each, collected every 100m along a 500m lineal transect, resulted from a 2-5 cm deep superficial scraping made with a metal spoon. Each samples stood for a pool made up of 5 subsamples collected at random in a surface about 30 m^2 in size. It was processed by the dilution plate technique that is the most frequently used procedure to determine a great variety of sporulating fungi (Gams *et al.,* 1998). Moreover, when the material was enough, it was divided in parts to be processed in different way to point out fungi belonging to unlike ecological groups:

a – Dilution plate technique. At first soil was diluted with sterile water 1: 10, further the suspension was diluted again till 1: 10.000;

b – Hairbating technique (Vanbreuseghem, 1952), which is the standard method for isolation of keratinophilic fungi from soil. Three Petri dishes for each soil sample were prepared, using human hair and bird feather as keratinic source.

c – Moist chamber. We used this method in order to identify coprophilous fungi as, in a few samples, soil was mixed with dung. After soil washing with sterile water, to separate the two component, the dung (possibly from cow or horse) was placed on most sterile filter-paper in Petri dishes and observed at regular intervals at environment temperature for 30 days (Bell, 1983; Caretta *et al.*, 1998).

II) Part of vegetables (bark, leaves, whole plants) was processed by direct plating method using Water Agar Medium (Gams et al., 1998; Caretta *et al.*, 1999).

Tab. I Fungal taxa from Namibia	Soils	Vegetables	Seeds
Acremonium strictum W. Gams		x	
Acremonium sp.	x		
Aphanoascus durus (Zukal) Cano & Guarro	x		
Aphanoascus fulvescens (Cooke) Apinis	x		
Aphanoscus sp.	x		
Alternaria alternata complex (Fr.) Keissler	x	x	x
Alternaria tenuissima (Kunze) Wiltshire	x		x
Ascobolus crenulatus Karst.	x		
Ascobolus immersus Pers. per Pers.	x		
Aspergillus flavus Link		x	x
Aspergillus niger Van Tieghem	x	x	x
Aspergillus ochraceus Wilhelm		x	x
Aspergillus sidowii (Bain. & Sart.) Thom & Church		x	x
Aspergillus terreus Thom			x
Aspergillus ustus (Bain.) Thom & Church	x		
Aspergillus sp.	x		
Aureobasidium pullulans var melanigenum (De Bary) Arnaud	x	x	x
Beauveria bassiana (Balls.) Vuill.	x		
Bipolaris australiensis (M.B. Ellis) Tsuda & Ueda	x		
Bipolaris cynodontis (Marignoni) Shoem.			x
Bipolaris kusanoi (Nisikado) Shoem.		x	
Bipolaris indica Rai, Wadhwani & Tewari			x
Bipolaris papendorfii (van der Aa) Alcorn			x
Chaetomium bostrychodes Zopf	x		
Chaetomium globosum Kunze:Fries	x		
Chaetomium murorum Corda		x	
Chaetomium spp.	x	x	
Chrysosporium indicum (H.S. Randhawa & R.S. Sandhu) Garg	x		
Chrysosporium sp.	x		
Circinella circinelloides Van Thieghem	x		
Cladosporium cladosporioides (Fresn.) de Vries	x		x
Cladosposrium sp.	x		
Coprinus sp.	x		
Curvularia eragrostidis (Henn.) J. A. Meyer		x	
Doratomyces columnaris Swart	x		
Doratomyces stemonitis (Pers: Fries)Morton & Smith.			x
Emericella nidulans (Eidam) Vuill.		x	x
Epicoccum nigrum Link	x	x	
Fusarium dimerum Penzig	x		x
Fusarium oxysporum Schlecht.:Fr.	x		
Fusarium semitectum Wollenw.	x		
Fusarium solani (Mart.) Sacc.	x		
Fusarium sp.	x		
Harzia verrucosa (Tognini) Holu.-Jechová			x
Lasiobolus microsporus Bezerra & Kimbrough	x		
Lasiobolidium spirale Malloch & Cain		x	
Memnoniella echinata (Riv.) Galloway		x	
Microsphaerospsis olivacea (Bon.) Hohnel		x	
Mucor hiemalis Wehmer	x		

Table 1.- (continued)

Myceliophthora sp.	x		
Oedocephalum pallidum (Berk. & Broome) Cost.	x		
Paecilomyces lilacinus (Thom) Samson	x		
Penicillium expansum Link	x		
Penicillium spp.	x	x	x
Persiciospora africana Krug			x
Pestalotiopsis maculans (Desm.) Steayert			x
Phialopora sp.	x		
Phoma herbarum Westend.	x		x
Phoma glomerata (Corda) Wollenw. & Hochapfel			x
Phoma spp.	x	x	x
Pilobolus crystallinus (F.H. Wiggers : Fries) Tode	x		
Pithomyces chartarum (Berk. & Curt.) M.B.Ellis			x
Pithomyces sacchari (Speg.) M.B. Ellis			x
Podospora setosa (Winter) Niessl	x		
Rhizopus stolonifer (Ehnreb.:Fr.) Vuill.			x
Saccobolus minimus Vel.	x		
Saccobolus thaxteri Brumm.	x		
Sarcinomyces crustaceus Lindner	x		
Scopulariopsis candida (Guéguen) Vuillemin		x	
Sincephalastrum racemosus Cohn		x	
Sordaria fimicola (Rob.) Ces. et de Not.	x		
Sporormia fimetaria de Not.	x		
Sporormiella minima Auersw. Ahmed & Cain	x		
Stachybotrys atra Corda		x	
Stemphylium sp.	x		
Talaromyces flavus (Klocker) Stolk & Samson			x
Thielavia terricola (Gilman & Abbott) Emmons		x	
Trichoderma viride Pers.	x		
Trichoderma spp.	x		
Ulocladium consortiale (Thuem.) Simmons		x	
Micelia sterila	x		
Yeasts (not identified)	x		
TOTAL NUMBER OF TAXA	**51**	**23**	**26**

III) Seeds. In order to isolate endophytic fungi, the seeds were sterilized by bleaching with 5% hydrogen peroxide for 5 minutes, after that they were washed with running water (Bisseger & Sieber, 1994).

All Petri dishes were kept at 25°C and observed microscopically regularly for two months. Mainly, the cultural *medium* used for fungal isolation, identification and manteinance was Potato Dextrose Agar (PDA) with chloramphenicol added (200 mg/l). Identification at the species level was carried out according to the diagnostic morphological criteria found mainly in publications by Bell, 1983; Malloch & Cain, 1971; Domsh *et al.* (1980); Ellis (1971, 1976), Sutton (1980); Sivanesan (1987).

Considering the reduced area under study as well as the scarce number of samples analysed in each substrate, ocurrence percentages of taxa found were not estimated, therefore only their presence or absence was taken into account in the analysis.

RESULTS AND DISCUSSION

The results are reported in Table I. A total number of taxonomic entities isolated from soil, vegetables and seeds at different sites was 80, respectively 51 from soils, 23 from vegetables and 26 from seeds. Among these entities, representative of 49 genera, some species occurred in soils, vegetable and seeds as: ***Alternaria***

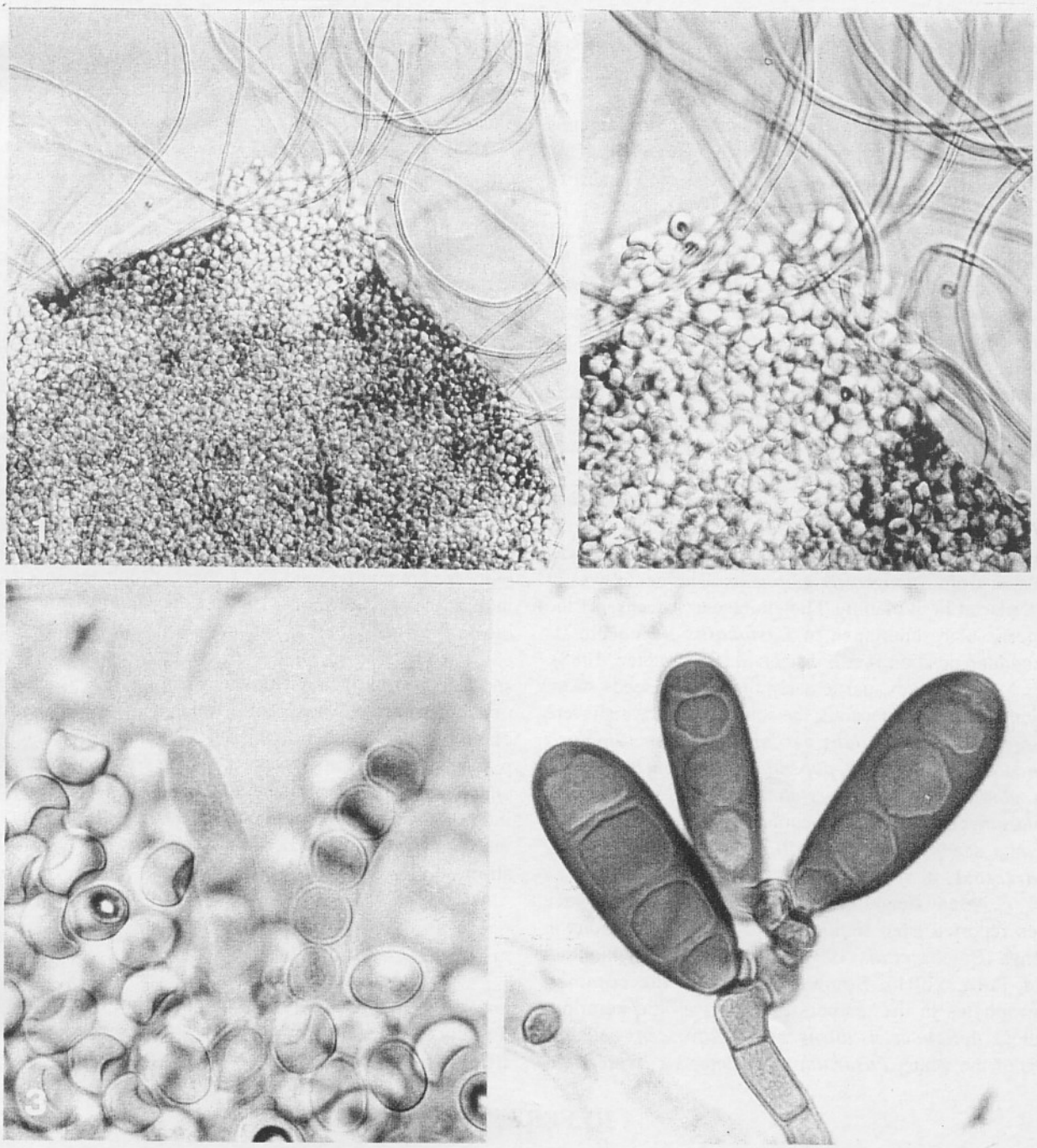

Figure 2. 1,2,3.*Lasiobolidium spirale*. 1.Cleistothecium and spiralateappendages, 200x. 2.Liberated ascospores from ascoma peridium, 400x. 3 Asci and ascospores,1000x. 4. *Bipolaris kusanoi*, 1000x

alternata*, *Apergillus niger*, *Aureobasidium pullulans var. ***melanigenum*, *Penicillium*** spp. and ***Phoma*** spp. The highest presence of genera was found to occur on soil; some showed substantial species diversity, as ***Aphanoascus*, *Ascobolus*, *Chaetomium*, *Fusarium* (*F. dimerum*, *F. oxysporum*, *F. semitectum*, *F. solani*), *Saccobolus* (*S. minimus*, *S. thaxteri*)** and other coprophilous as ***Sordaria fimicola*, *Sporormia fimiteria*** and ***Sporormiella minima***. Among the fungi occurring as individual *taxon* the commonest of these were: ***Beauveria bassiana*, *Bipolaris australiensis*, *Chrysosporium indicum*, *Circinella circinelloides*, *Epicoccum nigrum*,**

***Lasiobolus microsporus*, *Oedocephalum pallidum*, *Pilobolus crystallinus*, *Podospora setosa*, *Sarcinomyces crustaceus*.**

Our data confirm that ***Aspergillus niger*** was abundant (5000 cfu/g) in soil sampled just around *Welwitschia mirabilis*: this fungus is quite dangerous for this plant because reduces severely the seed viability (Cooper-Driver *et al.*, 2000).

The microfungal community showed a gradual change on vegetables. It is interesting to note the occurrence of some fungal *taxa* only in plants as ***Emericella nidulans*** (anamorph ***A.nidulans***) on *Colospermum mopane*, *Terminlia prunoides*, *Stipagrostis uniplumis* and *Tricholoma teneriffae*. This ubiquitous soil fungus has been isolated most frequently from tropical and subtropical climates (Klich, 2002), from desert soils (Piontelli *et al.* 2002), from a wide variety of foods and from indoor environments (Samson *et al.*, 2001).

Another interesting species is ***Lasiobolidium spirale***, the type species of a rare genus classified as *Incertae sedis*, Pezizales, Pezizomycetidae; it was isolated from *Stipagrostis uniplumis*. The genus was at first placed in the family **Thelebolaceae**, because of the superficial resemblance to ***Lasiobolus*** Sacc., but its taxonomic position is still discussed (Brummelen, 1988).

The seeds, particularly the *Acacia* seeds, were colonized by dematiaceous; the commonest of which were ***Aureobasidium pullulans*** var. ***melanigenum*, *Bipolaris cynodontis*, *B. indica*, *B. papendorfii*, *Phoma herbarum*, *Ph. glomerata*, *Pithomyces chartarum*, *P. sacchari*** and ***Talaromyces flavus*.** Other common plurivorous fungi on *Acacia* seeds were ***Aspergillus flavus*, *A. niger*, *A. ochraceous*, *A. sydowii*** and ***A. terreus*.**

Many fungal species recorded in this study have been reported from similar works in tropical regions in Kenya (Caretta *et al.*, 1999) and in Tanzania (Piontelli and Toro, 2001). Some members of the common saprophytes in the temperate regions as the keratinophilous ***Aphanoascus durus*** and ***A. fulvescens*** and species of the genus ***Fusarium*** were detected. ***Myceliophthora*** and ***Oedocephalum pallidum*** appear to be restricted to the tropic soil of Namibia.

It is interesting to note that among the fungi occurring in soil samples in Namibia many were coprophilous ascomycetes such as ***Ascobolus immersus*, *Podospora setosa*, *Saccobolus minimus*, *Sordaria fimicola*** and ***Sporormiella minima*.** They were found to be present in most of soil samples. These fungal taxa were also isolated from herbaceous plants endemic to native Kenyan grassland on the Marula Estate and from the dungs of ruminant and non-ruminant animals of this region. Upon comparing this fungal mycota was absent in samples from diverse leaf litter material collected in Tanzania (Piontelli and Toro,2001) and in soil and seed collected in our study in Namibia. An interdependence of fungi occurring on the dung of herbivorous and the fungi colonizing the phylloplane and vegetative organs of grasses and herbs did not occur at any site.

The results of the present study suggested that the genera ***Alternaria*, *Aureobasidium*, *Bipolaris*, *Chaetomium*, *Fusarium*, *Penicillium*, *Phoma*** are pioneer communities and had richer saprophytes species in African tropical regions. They are the widely distributed biota in soil and in aerial plant parts and many of them are pathogenic on crop plant and implicated in extensive spoilage of crops in the fields and in storage. Many species of these genera are toxigenic strains and are known to elaborate toxic metabolites for humans, animals and plants. Some toxigenic strains are present in tropical area, and others are listed as temperate area. It would be interesting to investigate a comparison about secondary metabolite production between temperate and tropical strains.

AKNOWLEDGMENTS

The authors wish to thank Dr. Vanda Terzo, University of Pavia (Italy) - Dipartimento di Ecologia del Territorio e degli Ambienti Terrestri, for plant identification

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