

# Epidemiological aspects and genotypic characterization of strains of *Microsporum audouinii* isolated in the context of a Belgian National survey on anthropophilic tinea

Sacheli R.<sup>1,2</sup>, Dekkers C.<sup>2</sup>, Géron B.<sup>2</sup>, Graide H.<sup>2</sup>, Darfouf R.<sup>1,2</sup>, Adjetej C.<sup>2</sup>, Seidel L.<sup>4</sup>, Meex C.<sup>2</sup>, Descy J.<sup>2</sup>, Huynen P.<sup>2</sup>, Melin P.<sup>1</sup>, André J.<sup>5</sup>, Arrese J.<sup>3</sup>, Hayette M.-P.<sup>1,2</sup>.

<sup>1</sup>National Reference Center for Mycosis, <sup>2</sup>Department of Clinical Microbiology, <sup>3</sup>Department of Dermatopathology, <sup>4</sup>Department of biostatistics, University Hospital of Liège; Belgium  
<sup>5</sup>Department of Dermatology, Brugmann University Hospital, Bruxelles, Belgium.

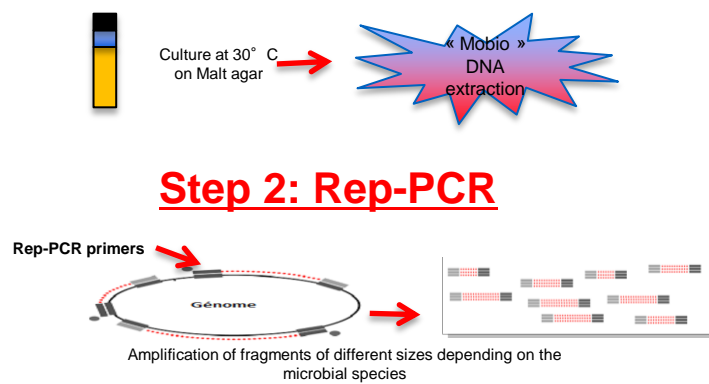
## Objectives

- To investigate the epidemiological determinants responsible for the high number of anthropophilic dermatophytes received by the National Reference Centre for Mycosis of Liege (NRCL), during the last years. Indeed, the last two years, clinical cases of tinea capitis caused by *Microsporum audouinii* (*M. audouinii*), have increased in Belgium.
- To perform a genotypic characterization by the Diversilab<sup>®</sup> system focusing on *M. audouinii*.
- To present results of the national survey launched between February 2013 and March 2014.

## Methods

**Population:** A total of 117 strains of *M. audouinii* (116 clinical+ 1 reference strains) collected between March 2013 and February 2014 were included in the study. The strains were collected from different laboratories through Belgium but mainly were coming from Brussels. **Figure 1** describes the Diversilab<sup>®</sup> system (bioMérieux) used for genomic analysis of *M. audouinii* strains.

### Step 1: Fungal culture + DNA extraction



### Step 3: Diversilab<sup>®</sup> analysis

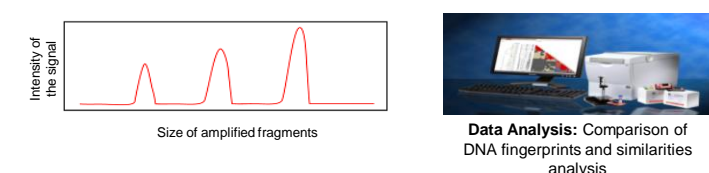


Figure 1: Description of the Diversilab<sup>®</sup> method.

## Results

The 117 strains have been genotypically characterised by Diversilab<sup>®</sup> fingerprinting to visualize genomic variants between *M. audouinii* species. **Figure 2** represents the DNA fingerprints of these strains (Strains 1 to 26). **Figure 3** represents the scatterplot of all strains analysed showing the differentiation into 6 different groups.

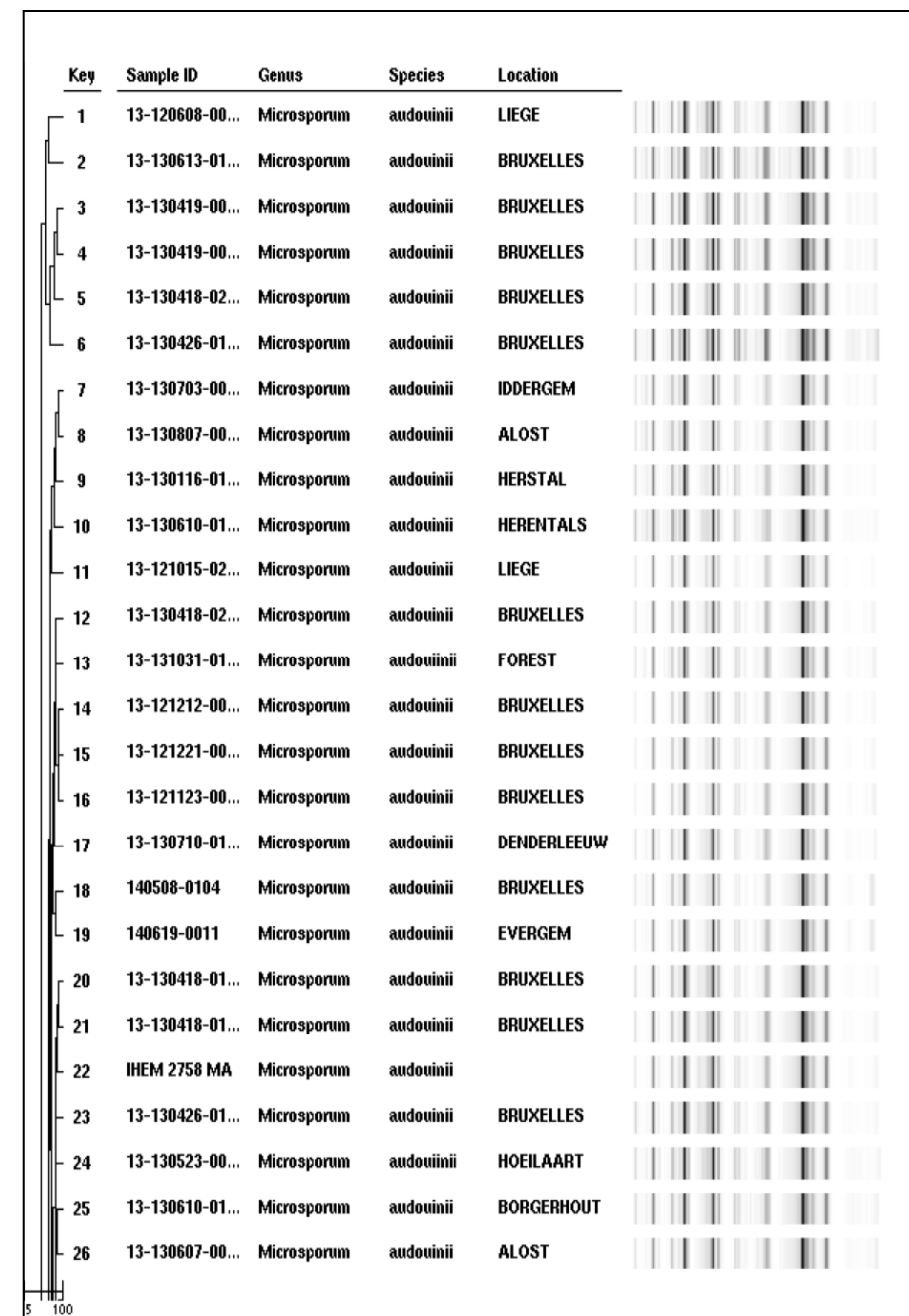


Figure 2: Dendrogram obtained after Diversilab<sup>®</sup> analysis of the strains N° 1 to 26.

The genotypic analysis led thus to the distinction of several genotypic variants of *M. audouinii*. One of these variants was exclusively recovered from South Belgium (11 strains). The major group included 96 strains, well distributed in different Belgian locations.

Two other groups of three strains each were close to the major group but the analysis of the spectral superposition showed some differences between these groups (**See Figure 4**). The three last groups were clearly different from the major group but species identification was confirmed by ITS sequencing.

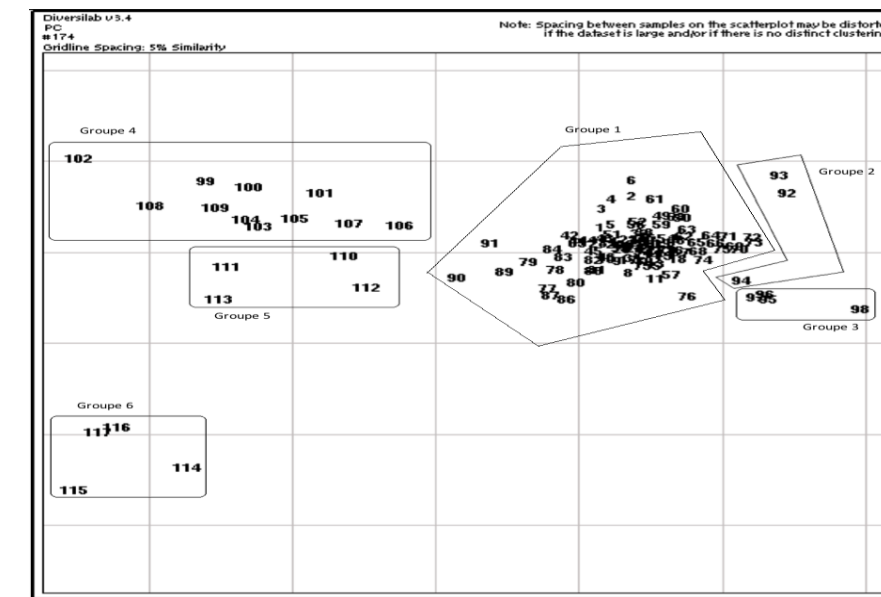


Figure 3: Scatter plot obtained after genomic analysis of strains by Diversilab<sup>®</sup> showing 6 different groups amongst *M. audouinii* strains.

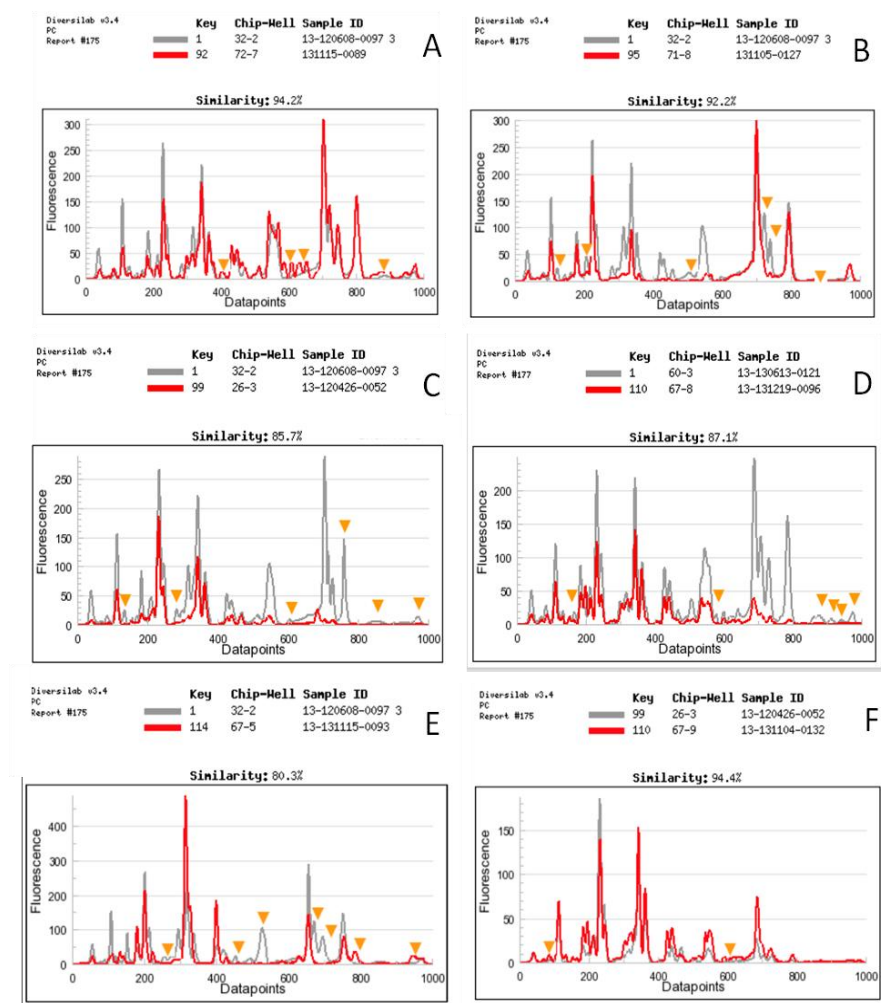


Figure 4: Rep-stacking of peaks obtained after Diversilab<sup>®</sup> analysis showing differences (yellow arrows) between strains of different defined groups.

Analysis of the epidemiological characteristics of the infected population (**see Table 1**) shows that the main age category concerns 5-9 year-old children (55,7%) with a sex-ratio M/F of 1.97. Data concerning the geographic origin of the family have been obtained in only 32,7% of the cases. It reveals that strains have been mainly isolated from patients with a Belgian nationality (44,7%) suggesting bias in the data collection. The geographic origin of the remaining group includes several African countries such as Congo (21,1%), Guinea(13,2%) and Cameroun (5,3%).

Variable	Geographical origin	<i>M. audouinii</i>		p-value
		N	Number (%)	
Localisation	Liège	116	29 (25.0)	<0.0001
	Brussels	58 (50.0)		
	Dutch Brabant	5 (4.3)		
	Limbourg	2 (1.7)		
	Antwerpen	7 (6.0)		
	West Flanders	1 (0.9)		
	East Flanders	11 (9.5)		
	Hainaut	1 (0.9)		
	Walloon Brabant	2 (1.7)		
Age (years)	0-4	115	29 (25.2)	<0.0001
	5-9	64 (55.7)		
	10-14	17 (14.8)		
	>15	5 (4.3)		
Sex	Woman	116	39 (33.6)	0,0004
	Man	77 (66.4)		
Ethnical origin	Belgium	38	17 (44.7)	<0.0001
	Burundi	1 (2.6)		
	Congo	8 (21.1)		
	Guinea	5 (13.2)		
	Cameroun	2 (5.3)		
	Morocco	2 (5.3)		
	Mauritania	1 (2.6)		
	Rwanda	1 (2.6)		
	South Africa	1 (2.6)		
Ethiopia	0 (0.0)			

Table 1: Epidemiological analysis of the data obtained during the national survey.

## Conclusions

The Diversilab<sup>®</sup> system proved to be an efficient method to investigate the molecular epidemiology of dermatophytes infections. These results show that several groups of *M. audouinii* isolates co-exist in Belgium providing evidence of genetic heterogeneity. However, no clear correlation could be established between the appearance to a group and epidemiological factors, such as the age or ethnical origin.