ARTICLE / INVESTIGACIÓN

Molecular analysis of Fungi: *Malasseziarestricta* from Felidae

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DOI. 10.21931/RB/2023.08.01.46

Abstract: A total of 9 samples of wild cat *Felischausfurax* (de Winton, 1898) and 13 (11 positives) samples of domestic cat *Feliscattus* (Linnaeus, 1758) belong to Family Felidae. All cats were trapped and used hair and skin scrapings by forceps and surgical blades. The areas of the collection were: Mosul province (north of Iraq); Baghdad, Al-Rashidiya, Tharthar, Nahrawan, AL-Mahmoudiya (middle of Iraq) and AL-Haretha (south of Iraq). The current study revealed that the sensitive and specific PCR assay allowing rapid and reliable identification of *Malasseziarestricta* by the fragment size amplified was 500bp in the ITS1 gene in one sample of wild cats. The current study recorded a new strain of *Malasseziarestricta* that called AF2013 strain "small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1", complete sequence; and 5.8S ribosomal RNA gene, partial sequence. Which was inserted in GenBank: MW376484.1 from wild cat *Felischausfurax* for the first time in Iraq. Sequencing revealed close matching of the phylogenetic tree to an isolate from Korea (CP030254). The compression was performed using NCBI – the based nucleotides website.

Key words: Dermatitis, Cutaneous microflora, fungi, Genotype, *Malasseziarestricta*.

Introduction

The taxonomy of *Malasseziarestricta* (E.Guého, J.Guillot&Midgley) according to GBIF[1]:

Kingdom: Fungi
Phylum: Basidiomycota
Class: Malasseziomycetes
Order: Malassezialess
Family: Malasseziaceae
Genus: *MalasseziaBaill*, (1889)
Synonyms: *PityrosporumSabour* (1904)

The genus of *Malassezia* includes seven species comprising the three former taxa *M. furfur, M. pachydermatis* and *M.sympodialis*, and four new taxa *M. globosa*, *M. obtuse, M. restricta* and *M. slooffiae*[2].

Molecular techniques have increased research in this field, such that revealed the infection with *Malassezia* spp. to man for over 150 years, and seven new species have been added to this genus[3].

The skin colonization with *Malassezia* spp. depends on the skin's body site, the host's age, and other comorbid skin conditions, as the geographic area. It is found in the highest density in sebaceous regions such as the scalp, face, and upper trunk. It is seen in higher densities in young adults, who tend to have relatively oily skin[4].

Malassezia spp. is naturally found on the skin surfaces of many animals, including humans. It can cause hypopigmentation or hyperpigmentation on the trunk and other locations in humans[5]. The knowledge about *Malassezia* spp. has expanded remarkably since the 1990s in dogs and cats[6]. *Malassezia* furfurwas isolated from cats in 1999 by Crespo[7].

The epidemiological map of the spread of fungi Malassezia from animals to humans is unclear, as Morris[8] indicated that *M. pachydermatis* was transmitted from pet dogs to humans. This is an important indicator that prompts us to research further the transmission of *Malassezia* spp. from domestic and wild animals. In Poland, along with surveys from 2008 to 2018 for many groups of animals like dogs, cats, rodents, riding horses, birds and other pet animals (reptiles and mammals), Bozena[9] revealed to fungal species involved and evaluated the risk of their transmission to humans.

The study aims to spotlight the species of Malasseziarestrictathat infected domestic and wild cats through molecular analysis for the first time in Iraq.

Materials and methods

Collection of samples

A total of 9 samples of wild cat *Felischausfurax* (de Winton, 1898) and 13 (11 positives) samples of domestic cat *Feliscattus* (Linnaeus, 1758) of Felidae Family were trapped and used as hair and skin scrapings by forceps and surgical blades. The scales were collected in sterile empty Petri dishes[10]. The areas of the collection were: Mosul province (north of Iraq); Baghdad, Al-Rashidiya, Tharthar, Nahrawan, AL-Mahmoudiya (middle of Iraq) and AL-Haretha (south of Iraq).

DNA Extraction

Genomic DNA was isolated from two samples according to the QIAamp DNA Mini Kit protocol, QIAGEN.

Primer preparation

Lyophilized primers (Macrogen Company) were dissolved in nuclease-free water to give a final concentration of 100 pmol/µl as a stock solution. A working solution of these primers was prepared by adding 10µl of primer stock solu-

Citation: Muslim Hadi A, Saber Khalif H. Molecular analysis of Fungi: Malasseziarestricta from Felidae. Revis Bionatura 2023;8 (1)46. http://dx.doi.org/10.21931/RB/2023.08.01.46

Received: 23 December 2022 / **Accepted:** 30 January 2023 / **Published:** 15 March 2023

tion (stored at freezer -20 C) to 90µl of nuclease-free water to obtain a working primer solution of 10 pmol/µl. Tables 1, 2 and 3.

Primer Name	Vol. of nuclease-free water (µl)	Concentration (pmol/µl)
ITS1-F	300	100
ITS1-R	300	100
ITS2-F	300	100
ITS2-R	300	100

Table 1. Primer preparation as Macrogen Company protocol.

PCR Component Calculation				
No. of Reaction	**2**	rxn	Annealing temperature of primers	**54**
Reaction Volume /run	**25**	µl	Length of PCR product (bp)	**≈500**
Safety Margin	**5**	%	No. of PCR Cycles	**30**

Table 2. Reaction Setup and Thermal Cycling Protocol.

Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
Master Mix	2	X	1	X	12.5
Forward primer	10	µM	1	µM	1
Reverse primer	10	µM	1	µM	1
Nuclease Free Water					5.5
DNA		ng/µl		ng/µl	5
Total volume					25
Aliquot per single rxn	20	µl of Master mix per tube and add			5 µl of Template

Table 3. PCR Program.

Agarose Gel Electrophoresis was adopted to confirm the presence of PCR amplification.PCR products were loaded directly. The Ethidium bromide-stained bands in gel were visualized using a Gel imaging system. Standard Sequencing APPLIED IN KOREA (MACROGEN CORPORATION) BY SANGER USING (ABI3730XL).

Results and discussion

Summary of Data Production: the results of DNA concentration for two samples were concluded in table 4.

Sample	Conc.
01	1
02	1.26

Table 3. DNA Concentration (ng/µl).

PCR amplification of two samples, ITS1 and ITS2, as in Figures 1 and 2.

The current study revealed that the sensitive and specific PCR assay allowing rapid and reliable identification of Malasseziarestricta by the fragment size amplified was 500bp in ITS1 gene in one sample of the wild cat as in Figure 1. And there is no results shown in the ITS2 gene as in figure 2.

Data Analysis

The current study recorded a new strain of *Malasseziarestricta* that called AF2013 strain "small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1", complete sequence; and 5.8S ribosomal RNA gene, partial sequence, which inserted in GenBank: MW376484.1 from wild cat *Felischausfuraxfor* the first time in Iraq—fig. 3.

The current result of recording *Malasseziarestricta* cat indicates that it is a common disease between humans and animals, where it was found by Sugita[11] revealed that *M. restrict* commonly colonizes both AD (Atopic Dermatitis) patients and healthy subjects. And more, Annabelle[12] described the case of a pediatric oncology patient with splenic lesions secondary to *Malasseziarestricta*. Males and females are affected by the infection of (PV) PityriasisVersicolor, especially individuals between (10-20) years[13]. No significant correlation was reported between economic status; type of job; or water source with the infection of *Malassezia* spp.[14], which increases the risk.

Awad[14] revealed that patients who have contact with dogs only were equally exposed to *Malassezia spp.*, which reflects that dogs are not only the source of infection. Bond[6] reviewed 18 types of fungus *Malassezia* and their locations between animals and humans and mentioned *Malasseziarestricta* only in humans.

Phylogenetic tree

The current sequencing data has reported *Malasseziarestricta* from Iraq (MW376484); their host is a wild cat; this species revealed close matching on the phylogenetic tree to an isolate of *Malasseziarestricta* strain KCTC 27527 chromosome IV, complete sequence from Korea (CP030254); their host is Homo sapiens. On the other hand, it matches the isolation of *Malasseziarestricta* strain Y. H. Yeh I0610 "small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1", "5.8S ribosomal RNA gene and

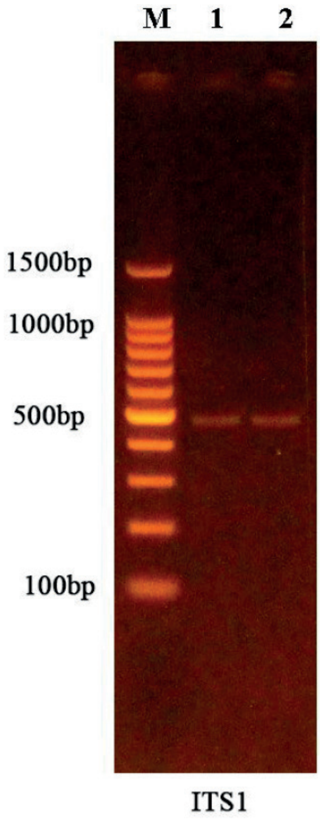


Figure 1. Results of the amplification of *ITS1* gene were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-2 resemble ≈500bp PCR products.

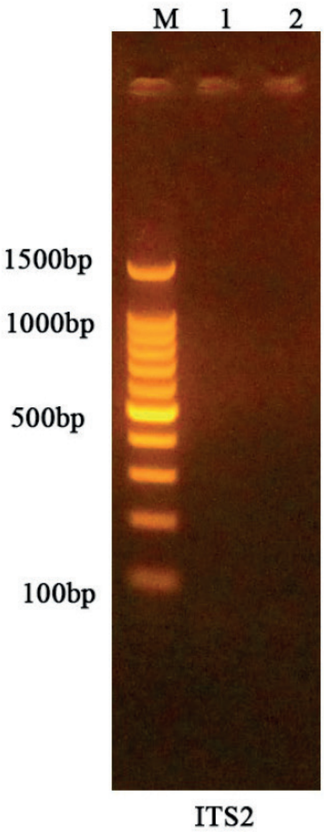
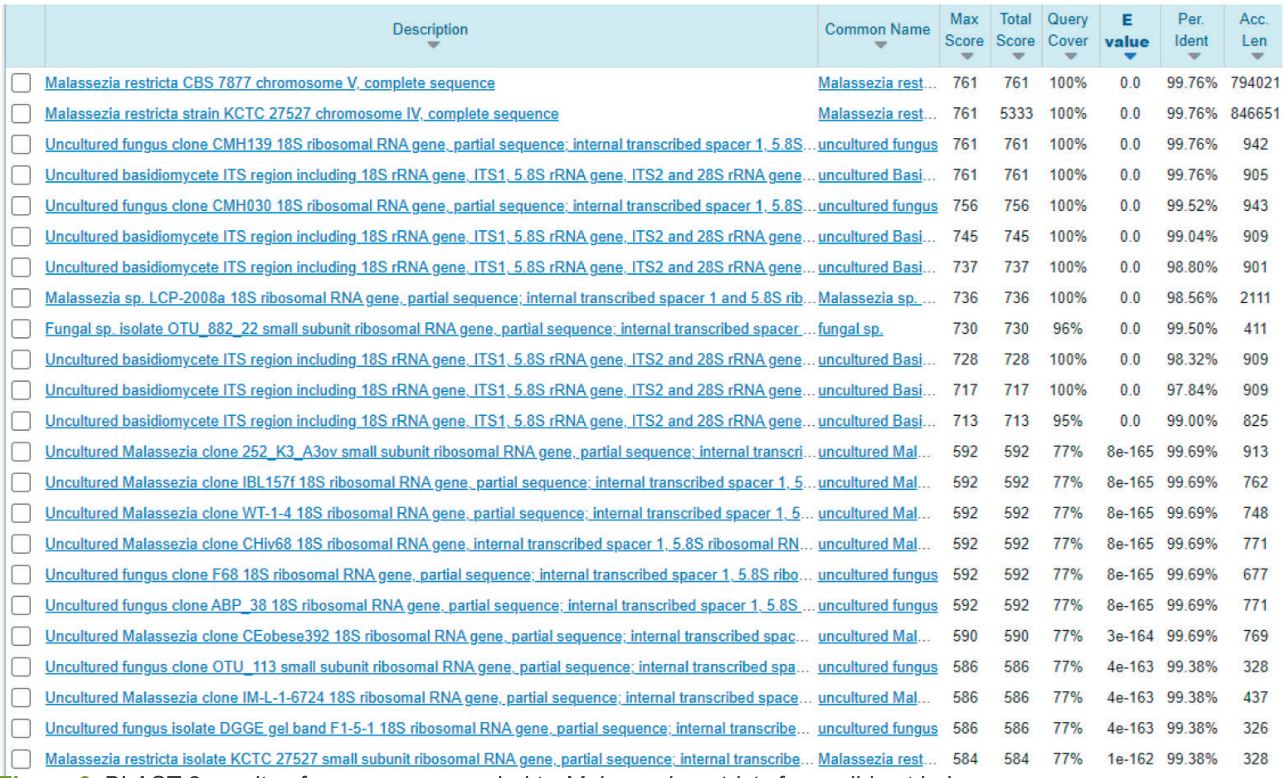


Figure 2. No Results of the amplification of the *ITS2* gene were fractionated on 1.5% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker.



	Description	Common Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len
☐	Malassezia restricta CBS 7877 chromosome V, complete sequence	Malassezia rest...	761	761	100%	0.0	99.76%	794021
☐	Malassezia restricta strain KCTC 27527 chromosome IV, complete sequence	Malassezia rest...	761	5333	100%	0.0	99.76%	846651
☐	Uncultured fungus clone CMH139 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	uncultured fungus	761	761	100%	0.0	99.76%	942
☐	Uncultured basidiomycete ITS region including 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene...	uncultured Basi...	761	761	100%	0.0	99.76%	905
☐	Uncultured fungus clone CMH030 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S...	uncultured fungus	756	756	100%	0.0	99.52%	943
☐	Uncultured basidiomycete ITS region including 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene...	uncultured Basi...	745	745	100%	0.0	99.04%	909
☐	Uncultured basidiomycete ITS region including 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene...	uncultured Basi...	737	737	100%	0.0	98.80%	901
☐	Malassezia sp. LCP-2008a 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S rib...	Malassezia sp. ...	736	736	100%	0.0	98.56%	2111
☐	Fungal sp. isolate OTU_882_22 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer ...	fungal sp.	730	730	96%	0.0	99.50%	411
☐	Uncultured basidiomycete ITS region including 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene...	uncultured Basi...	728	728	100%	0.0	98.32%	909
☐	Uncultured basidiomycete ITS region including 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene...	uncultured Basi...	717	717	100%	0.0	97.84%	909
☐	Uncultured basidiomycete ITS region including 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene...	uncultured Basi...	713	713	95%	0.0	99.00%	825
☐	Uncultured Malassezia clone 252_K3_A3ov small subunit ribosomal RNA gene, partial sequence; internal transcri...	uncultured Mal...	592	592	77%	8e-165	99.69%	913
☐	Uncultured Malassezia clone IBL157f 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...	uncultured Mal...	592	592	77%	8e-165	99.69%	762
☐	Uncultured Malassezia clone WT-1-4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...	uncultured Mal...	592	592	77%	8e-165	99.69%	748
☐	Uncultured Malassezia clone CHiv68 18S ribosomal RNA gene, internal transcribed spacer 1, 5.8S ribosomal RN...	uncultured Mal...	592	592	77%	8e-165	99.69%	771
☐	Uncultured fungus clone F68 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribo...	uncultured fungus	592	592	77%	8e-165	99.69%	677
☐	Uncultured fungus clone ABP_38 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ...	uncultured fungus	592	592	77%	8e-165	99.69%	771
☐	Uncultured Malassezia clone CEobese392 18S ribosomal RNA gene, partial sequence; internal transcribed spac...	uncultured Mal...	590	590	77%	3e-164	99.69%	769
☐	Uncultured fungus clone OTU_113 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa...	uncultured fungus	586	586	77%	4e-163	99.38%	328
☐	Uncultured Malassezia clone IM-L-1-6724 18S ribosomal RNA gene, partial sequence; internal transcribed space...	uncultured Mal...	586	586	77%	4e-163	99.38%	437
☐	Uncultured fungus isolate DGGE gel band F1-5-1 18S ribosomal RNA gene, partial sequence; internal transcribe...	uncultured fungus	586	586	77%	4e-163	99.38%	326
☐	Malassezia restricta isolate KCTC 27527 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Malassezia rest...	584	584	77%	1e-162	99.38%	328

Figure 3. BLAST 2 results of sequences revealed to *Malasseziarestricta* from wild cat in Iraq.

internal transcribed spacer 2 , the complete sequence" and large subunit ribosomal RNA gene, partial sequence from Taiwan (MK336446) their host is a goat (Ipomoea pes-caprae). Then, it matches the isolation of *Malasseziarestricta* CBS 7877 chromosome V, complete sequence from the United Kingdom: Bristol (CP033152); their host is Homo sapiens. Fig. 4.[13] revealed that the evidence shown by the phylogenetic tree showed that all species of *Malasseziaspp*. have high similarity with each other.

Conclusions

The current study spotlighted the species of *Malasseziarestricta* that infected domestic and wild cats by mo-

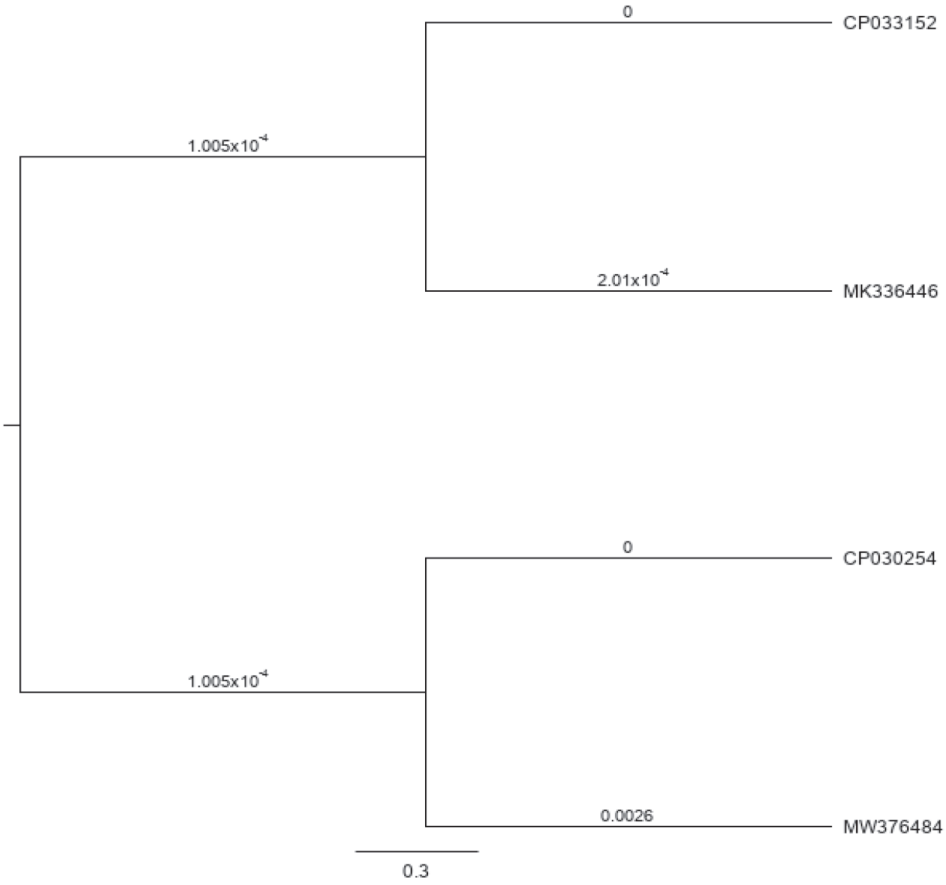


Figure 4. Phylogenetic tree analysis relied on the *ITS1* gene-specific region of *Malasseziarestrictafrom* Iraq (MW376484); their host is a wild cat. Sequencing revealed close matching of the phylogenetic tree to an isolate from Korea (CP030254). The compression was performed using NCBI – the based nucleotides website.

lecular analysis for the first time in Iraq, which was inserted in GenBank: MW376484.1 from wild cat *Felischausfurax*. This is a significant result as it proves that the fungus *Malasseziarestricta* is a common infection between humans and animals (zoonotic infection) and that cats can play this role.

Ethical approval

The trial was registered in "The Iraq Natural History Research Center and Museum INHM" (Email: info@nhm.uobhdad.edu.iq). The research proposal was approved by the Scientific Affairs Department of Baghdad University (SH.A.923/17/2/2021).

Conflicts of Interest

The authors declare no conflict of interest related to the work in a manuscript.

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