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First analysis of the faecal microbiota of the Barbary macaque (*Macaca sylvanus*) reveals differences between a wild group and a tourist-provisioned group in the region of Bejaia, Northeast of Algeria

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Article

First Descriptive Analysis of the Faecal Microbiota of Wild and Anthropized Barbary Macaques (*Macaca sylvanus*) in the Region of Bejaia, Northeast Algeria

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Etude de l'impact des activités humaines sur les populations de Magot de Barbarie "*Macaca sylvanus*" peuplant la région de béjaia, au Nord-Est de l'Algérie – Boumenir Mourad PhD student. (Université of Liège: 2000-202x)

Table of Contents



01

Introduction

Literature review
Objectives

03

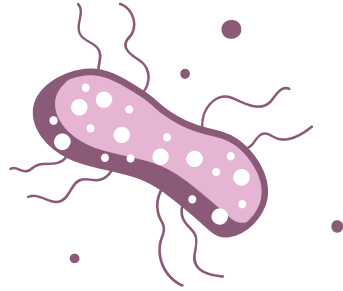
Results

02

Methodology

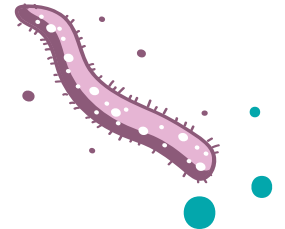
04

Conclusions



“The gastrointestinal microbiota, or the faecal microbiota, is defined as the set of microbial communities –viruses, bacteria, archaea, fungi, protists and their genetic material– that colonise the gastrointestinal tract of animals.”

— Clayton *et al.*, 2008





Microbiota

Play an important role in various physiological functions of the host



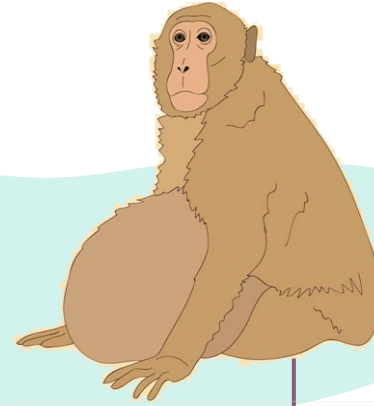
Digestion

Primates can obtain 30–57% of their daily energy budget



Immunological defence

Maintenance of the immune response



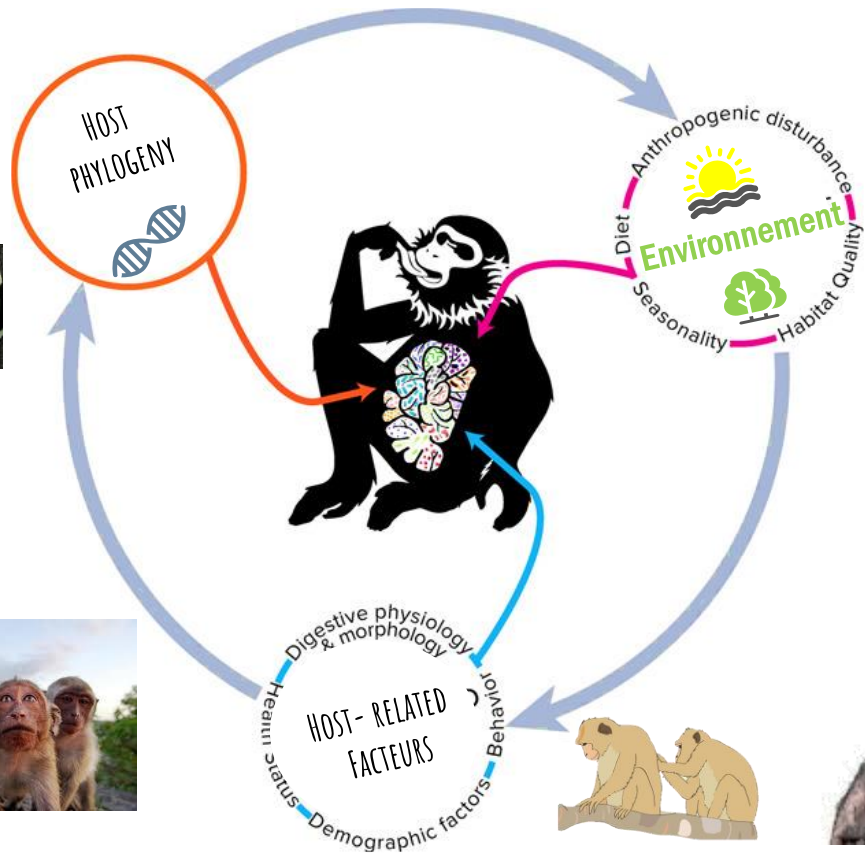
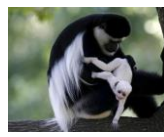
Patho - physiology

Obesity, diabetes, Alzheimer's disease, and Crohn's disease





Primates Microbiota



© (Clayton et al., 2018)

The faecal microbiota of primates is influenced by three key drivers : host phylogeny, transient host-related factors (sex, age, health status, behaviour, etc.) and, finally, environment (diet, seasonality, habitat quality and anthropogenic disturbance)



Anthropogenic Disturbances & Primates Microbiota

Food Provisioning (Chen et al., 2020)



Habitat degradation (Barelli et al., 2015)



Captivity (Hale et al., 2019)





Barbary Macaques Microbiota



Currently, there are few studies examining the microbiota of Barbary macaques. These limited studies have focused on specific **pathogenic microbial** taxa or on those of bacteria with **antibiotic resistance**.

Several known pathogenic microbes such as

Pathogens	Wild/ captive	Study sites	Studies
Bacteria			
<i>Leptospira spp</i>	Wild	Chrea and Gouraya National Parks (Algeria)	(Medkour et al., 2021)
<i>Treponema spp</i>	Wild		
<i>Mycobacterium spp</i>	Wild		
<i>Acinetobacter spp</i>	Wild		
<i>Rickettsia spp</i>	Wild		
Protozoa			
<i>Entamoeba coli</i>	Wild	Ifrane National Park (Morroco)	(Borg et al., 2014)
<i>Entamoeba dispar</i>	Wild		
<i>Entamoeba hartmanni</i>	Wild		
<i>Entamoeba polecki</i>	Wild		
<i>Iodamoeba butschlii</i>	Wild		
Kinetoplastida (<i>Bodo sp.</i>)	Wild	Chrea and Gouraya National Parks (Algeria)	(Medkour et al., 2020)
<i>Toxoplasma gondii</i>	Captive	Zoos in Spain	(Cano-Terriza et al., 2019)



Previous research revealed the presence of **E. coli M076** isolates **resistant** to colistin, b-lactams, aminoglycosides and fluoroquinolones

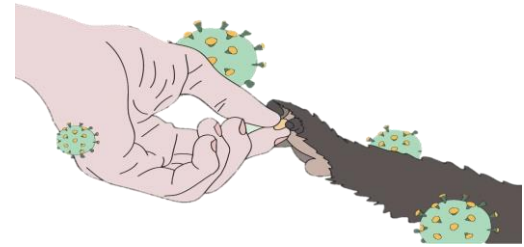
Study Objectives

To the best of our knowledge, both the composition of **all microbial** communities colonising the gastrointestinal tract of Barbary macaques, and the **impact of food provisioning** on these communities were not previously reported.



Barbary macaques Microbiota

to characterize, for the first time, the bacterial communities of the faecal microbiota of *M. Sylvanus*



Impact of food provisioning

to explore its alteration due to tourist-food provisioning.

The present study also included behavioural observations to examine the diet composition of a tourist-fed group



Methodology

Study groups

National park of Gouraya
Akfadou forest

Sample collection

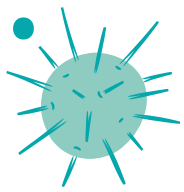
Behavior observations
Faecal samples
Storage

Microbiota analysis

DNA extraction
PCR amplification
Sequencing

Data Analysis

Bioinformatic analysis
Statistic analysis



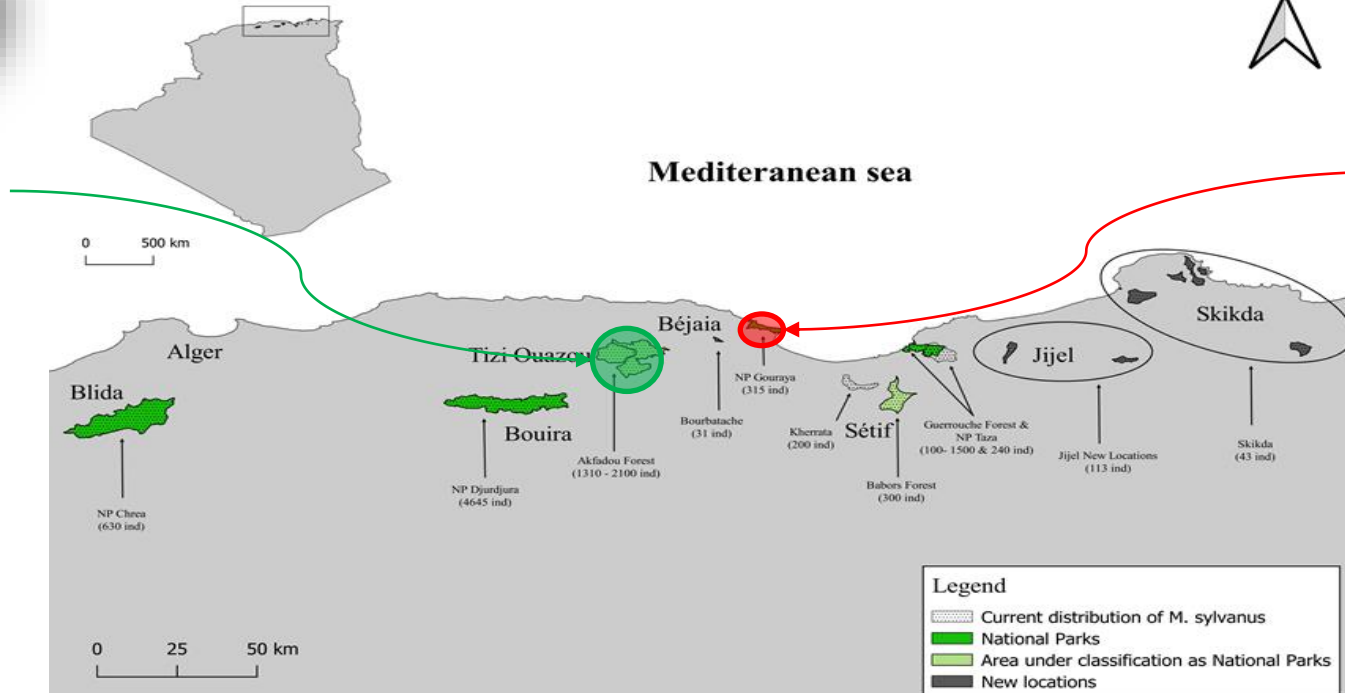
Study sites and groups



Wild- fed group



Tourist- provisionned group



Akfadou Forest

24 individuals

National park of Gouraya

37 individuals

The analysis of the UK dust indicated that the feeding type of anthropogenic resources was dominant

Samples collection

Algeria

Behaviour observations



12 individuals
- 6 males
- 6 females

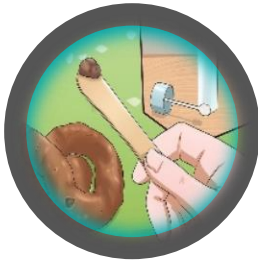


Method « focal sampling »
10 minutes

21 Days
307 focal sample,
3070 minutes

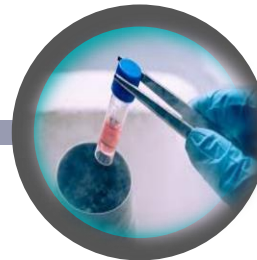


Data analysis
by Excel



Faecal sample collection

2 - 10 g feces (within 10 minutes of defecation)
12 samples (6 samples/ Group) adulte
Individuals



Storage

At - 196 in Liquid nitrogen



DNA Extraction

kit « GF-1 Bacterial DNA Extraction Kit »

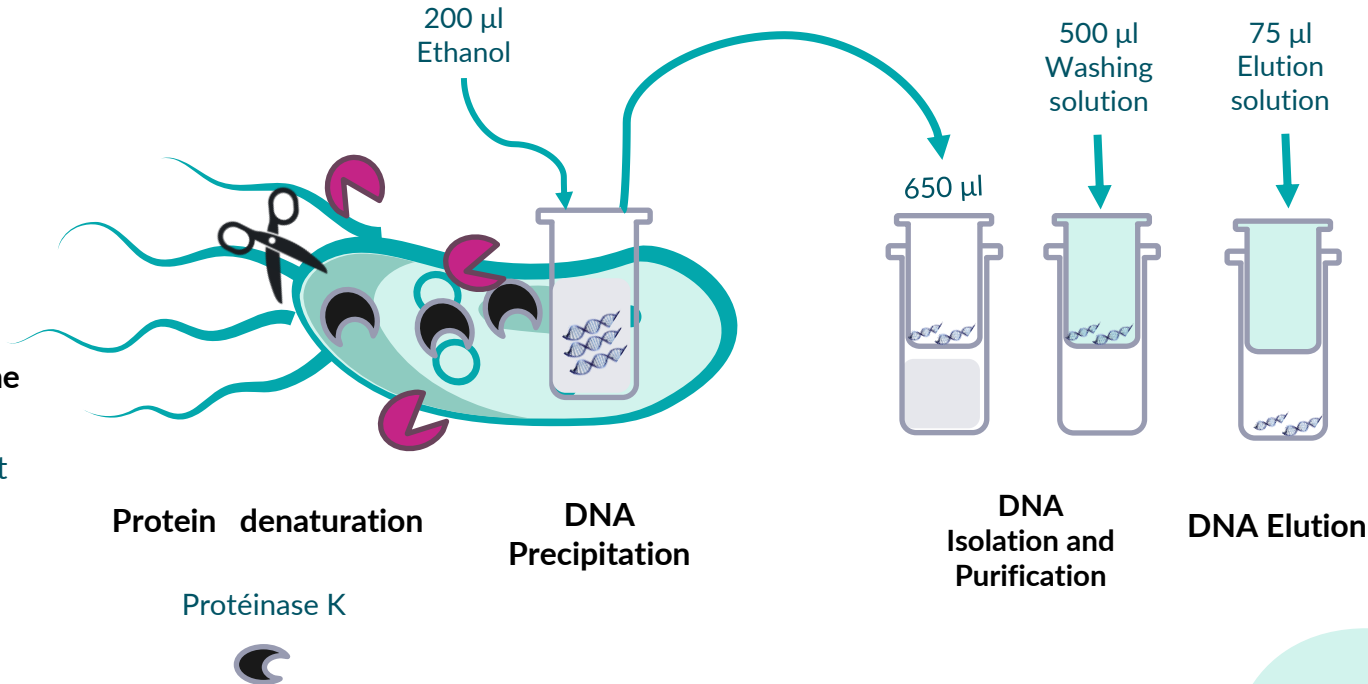
Algeria



Centrifugation
recovering the
supernatant

**Chemical and
enzymatic lysis of the
cell membrane**

Lysozyme treatment
incubate at 37°C

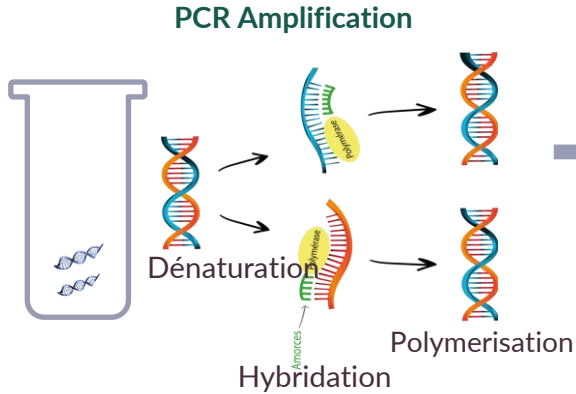


PCR amplification and Sequencing

Belgium

Library creation
By metagenomics

Bio-informatic analyses



Sequencing
2.616.654 raw sequences
(reads)

MOTHUR v1.41
Alignment of the raw sequences obtained on
known bacterial 16s rDNAs present in the
SILVA database
similarity threshold of 97%.

silva
high quality ribosomal RNA databases

V1-V3 hypervariable region
of the 16S rDNA

Primers with Illumina adapters

Forward (5'-GAGAGTTTGATYMTGGGCTCAG-3')

Reverse (5'-ACCGCGGCTGCTGGCAC-3')

**Building the Operational Taxonomic
Units (OTUs) table**

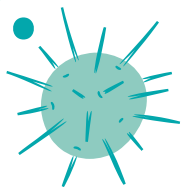
A table containing the number of
sequences by OTU and by sample.

Statistical analyses

	Paramètre	Test	Logiciel
Abondance des genres		Test T	
Diversité Alpha	Biodiversité microbienne: indice de Simpson réciproque	Test T	Mothur
	Estimation de la richesse: Estimateur Chao1 ,	Test T	Mothur
	Régularité de la population dérivé de l'indice de Simpson	Test T	Mothur
La diversité bêta	Composition de la communauté bactérienne	test AMOVA	Mothur
	bêta-dispersion	Test HOMOVA	Mothur
Visualisation de la biodiversité entre les deux groupes	Analyse d'échelle multidimensionnelle non métrique (NMDS), basée sur la matrice de dissimilitude de Bray-Curtis,		Vegan, Vegan3D, R

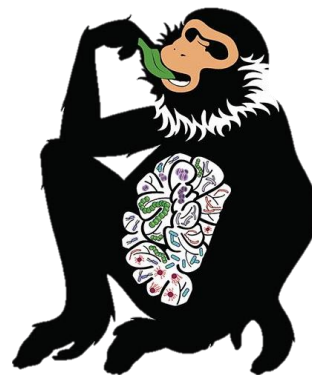
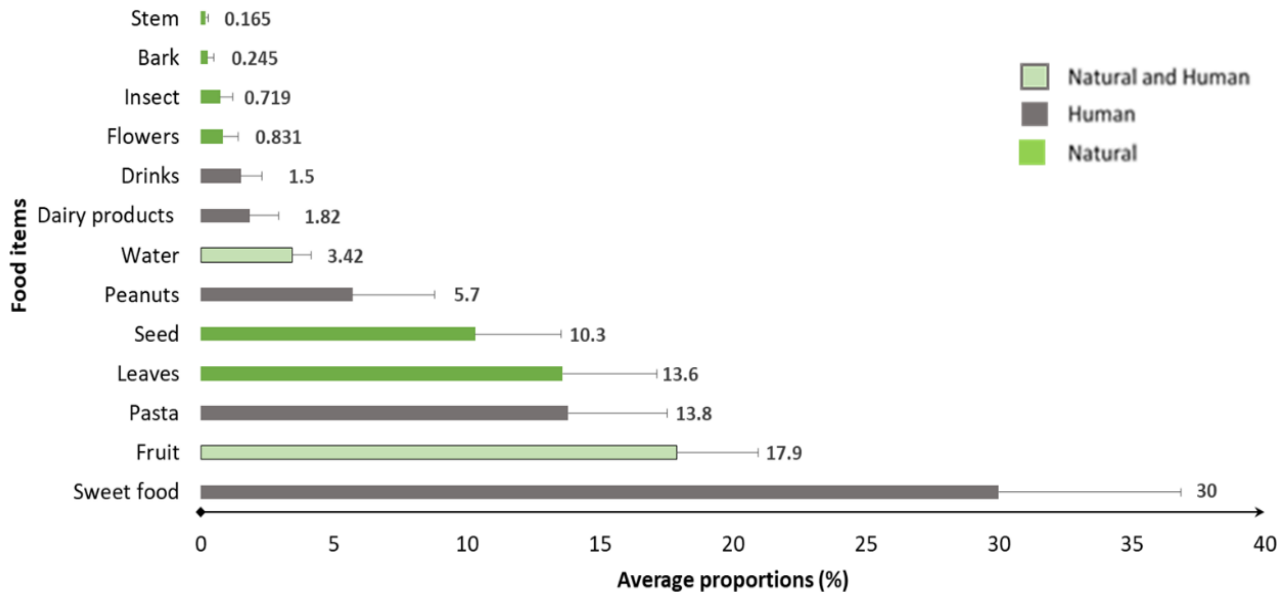
A pair of hands wearing blue nitrile gloves is shown from a top-down perspective, holding a clear petri dish. Inside the dish is a green, spherical model of a virus with several sharp, pointed spikes protruding from its surface. The background is a solid light pink color. In the bottom left corner, there is a white circular graphic containing the word "Results" in a bold, black, sans-serif font. There are also several small, dark purple dots scattered around the white circle and on the right side of the image.

Results



Diet Analysis

Diet Composition of the Tourist-Provisioned Group



The analysis of the TPG diet indicated that the feeding time of anthropogenic resources was dominant during the study period (61.69% of the feeding time)

Diet Analysis

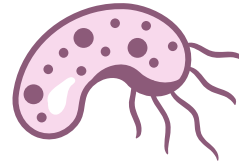


Source FD: BOUMENIR Mourad

Microbiota Analysis

209

bacterial genera

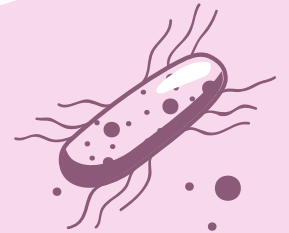
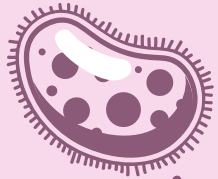


17

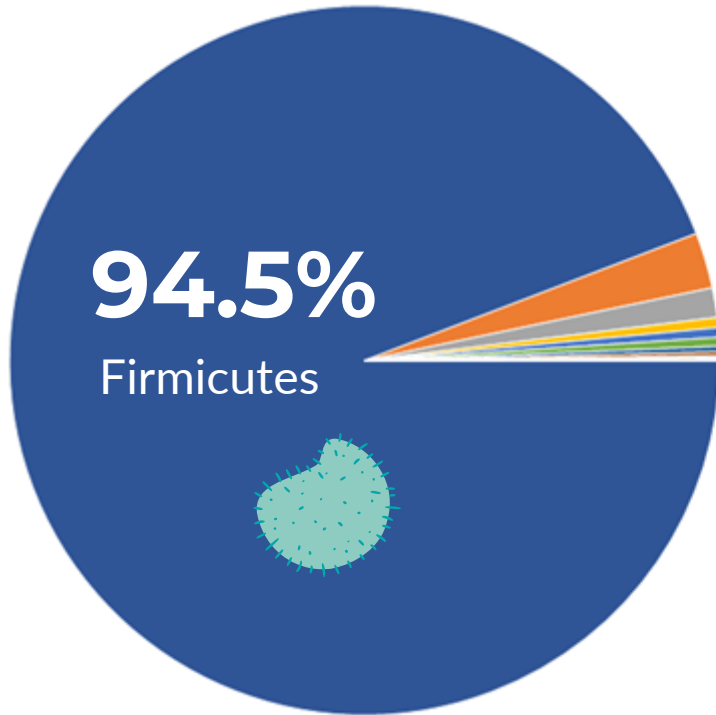
Phyla

1731

Operational taxonomic units (OTUs)



% Bacterial phyla

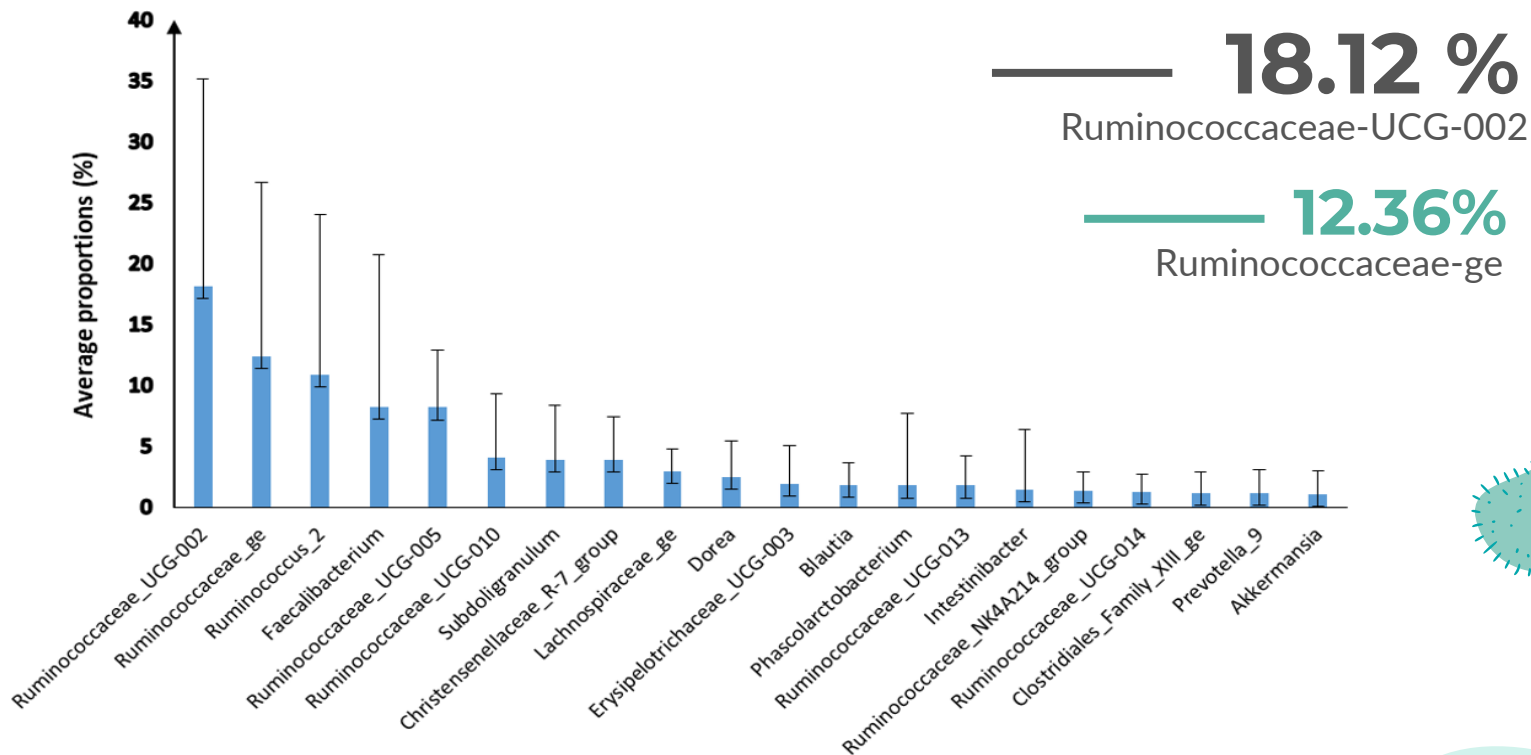


2.6%

- Firmicutes
- Bacteroidetes
- Verrucomicrobia
- Spirochaetes
- Tenericutes
- Actinobacteria
- Proteobacteria
- Bacteria_ph
- Cyanobacteria
- Epsilonbacteraeota
- WPS-2
- Fusobacteria
- Patescibacteria
- Lentisphaerae
- Chloroflexi
- Deinococcus-Thermus
- Gemmatimonadetes

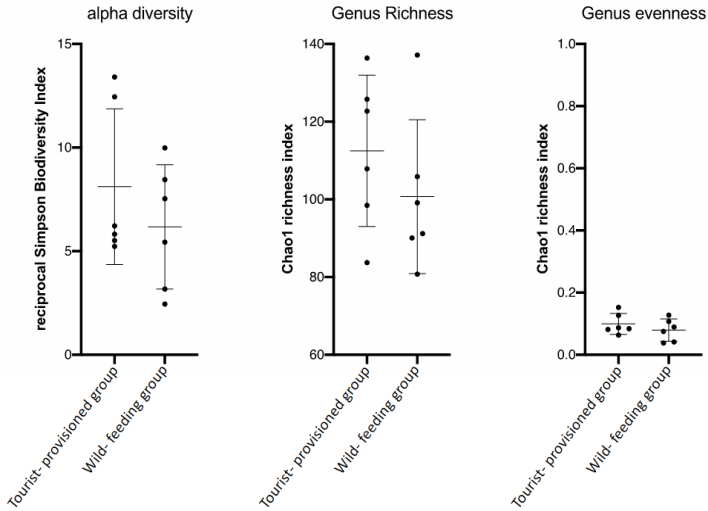
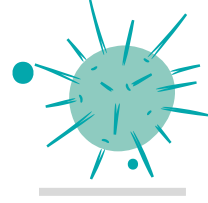
The most abundant bacterial phyla were Firmicutes (94.475%), Bacteroidetes (2.655%); and Verrucomicrobia (1.084%).

% Bacterial genera

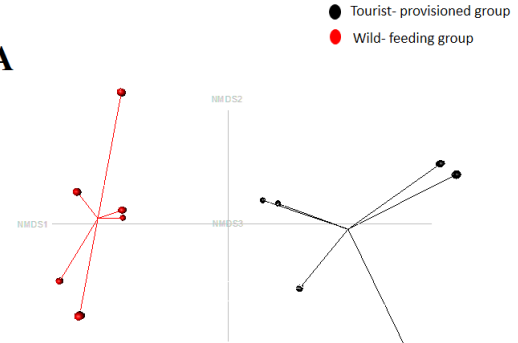


- The most abundant bacterial genera were Ruminococcaceae-UCG-002 (18.12%), Ruminococcaceae-ge (12.36%), Ruminococcus-2 (10.86%) and Faecalibacterium (8.21%).

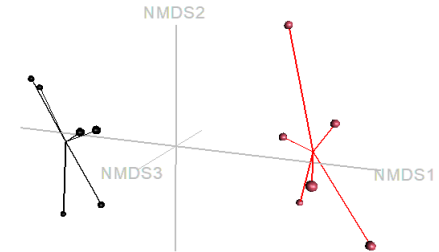
Evaluation of the Impact of Food Provisioning on the Faecal Microbiota of Barbary Macaques



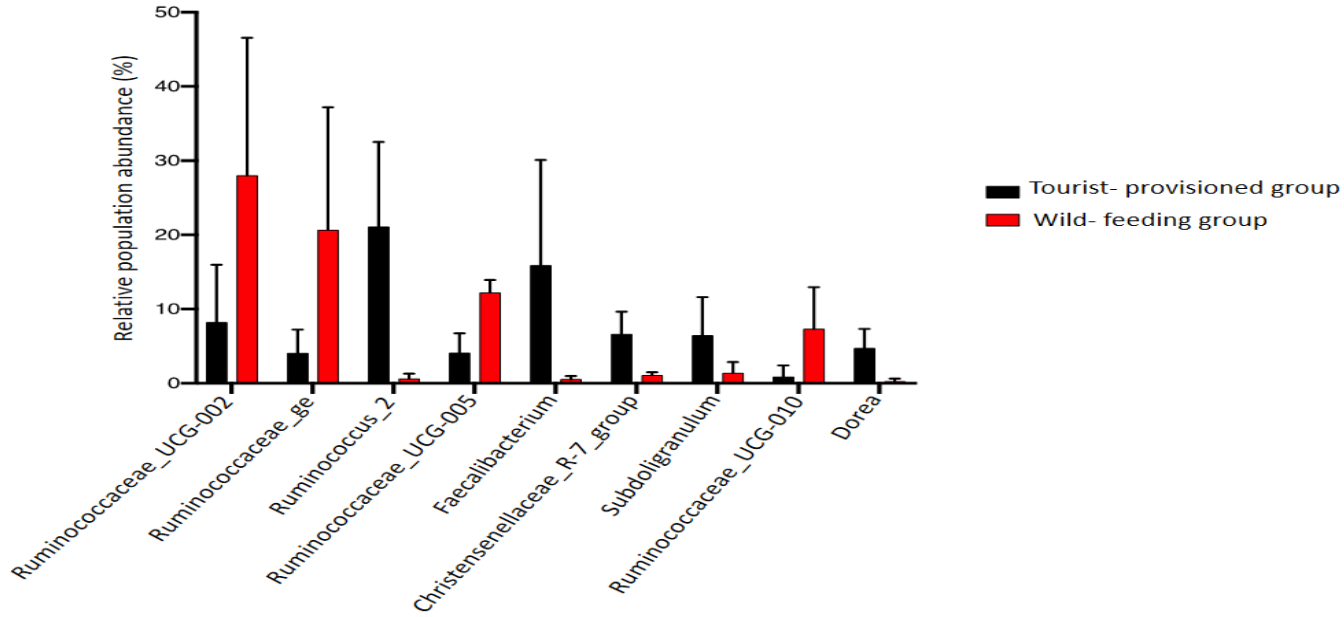
A



B



Evaluation of the Impact of Food Provisioning on the Faecal Microbiota of Barbary Macaques

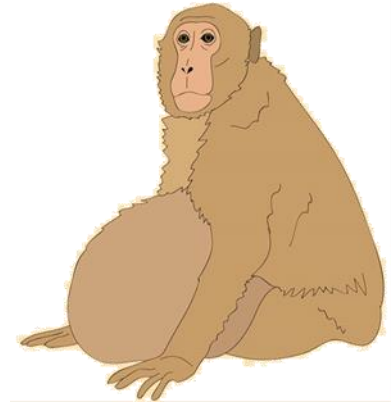


Microbiota and diseases

Firmicutes/Bacteroidetes

- Diabetes ! → No consensus.
- Obesity

Small sample size !

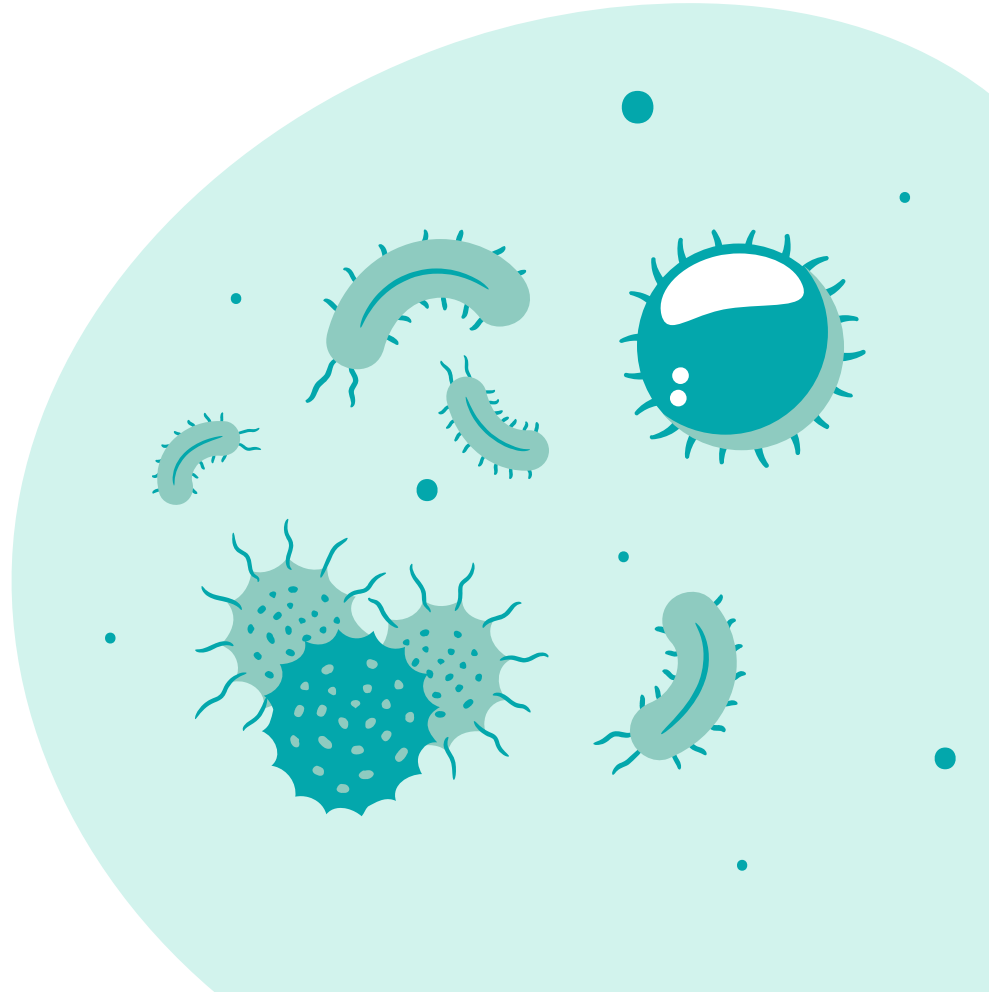


Conclusion

To conclude, the present study shows that tourism activity was associated with significant differences in the faecal microbiota of a tourist-provisioned group of Barbary macaques when compared to a wild-feeding group.

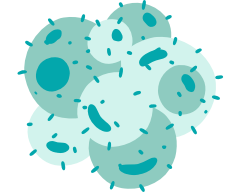
The consumption of anthropogenic foods—potentially poor in fibre and rich in simple sugars and fats—may have modulated the genera abundances and beta diversity of the faecal microbiota in the tourist-provisioned group with negative repercussions on the health status.

As a result, it is recommended for the Algerian authorities to implement special management measures to reduce the food-provisioning rate in the touristic areas.





Thanks!



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