

# Characterization of the footrot microbiome in Portuguese sheep breeds through metagenomics



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CONGRESSO  
LUSO-ESPANHOL  
DE **PECUÁRIA  
EXTENSIVA**  
E DESENVOLVIMENTO RURAL  
SUSTENTABILIDADE GARANTIDA

1 E 2 DE DEZEMBRO DE 2022 | CENTRO DE CONGRESSOS DE CÁCERES

# Introduction



Footrot is a costly disease in the sheep and goat industry, being the sheep the most affected, resulting in:

- Lameness
- reduced weight gain
- decreased milk and wool production
- decreased reproductive capabilities

These conditions result in production losses, treatment and prevention costs, premature culling, and reduced sale value of infected animals.

## Causes of Footrot:

- The main agent is the anaerobic bacterium *Dichelobacter nodosus*.
- *D. Nodosus* interacts with other species found in the microbiome, such as the *Fusobacterium necrophorum*.
- The microbiome is largely uncharacterized





# Materials & Methods



Within the scope of Gen-Res-Alentejo's project, clinical diagnosis of footrot were carried out on approximately **1,900 animals** across **17 farms**.

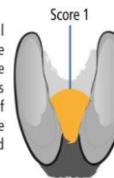


**Normal Foot**



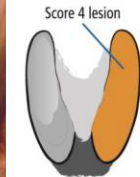
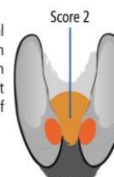
**Score 3b:**  
Separation of the horn extends up to halfway across the heel or sole.

**Score 1:**  
Limited mild interdigital dermatitis; slight to moderate inflammation confined to the interdigital skin and involves erosion of the surface layer of the skin. The skin between the toes is inflamed, red, moist and shedding hair.



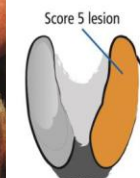
**Score 3c:**  
More extensive separation of the heel or sole but not extending to the outside edge of the sole of the foot.

**Score 2:**  
More extensive interdigital dermatitis; severe inflammation of the interdigital skin which involves part or all of the soft horn of the inside wall of the toe.



**Score 4:**  
As for Score 3 but with the separation extending to the walls and outside edge of the sole of the foot.

**Score 3a:**  
Separation at the skin horn junction, with under-running extending no more than 5 mm.



**Score 5:**  
Necrotising inflammation of the deeper tissue of the outside wall with consequent separation of hard horn of the foot.  
Horny layer detachment

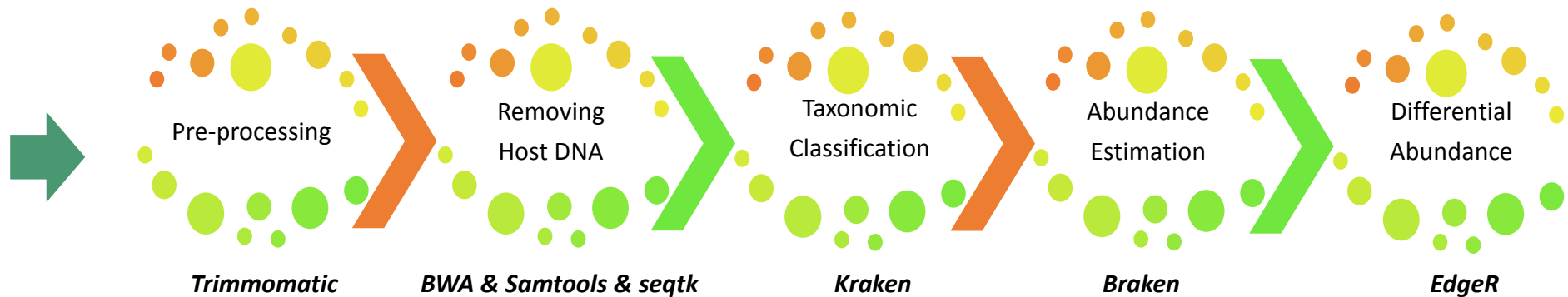
# Materials & Methods



- 261 interdigital tissue biopsy samples were collected from 210 animals for DNA extraction.
- After DNA extraction and quality validation, 210 samples were sequenced for metagenomic analysis.
- In these samples, an analysis was performed via PCR to identify the presence of the causal agent of the footrot, *D. nodosus*. In all samples except those belonging to farms D, I, J and Q, the presence of *D. nodosus* was confirmed.
- Samples from the farms with negative results for the presence of *D. nodosus* were designated as “outgroup”.

	Nº Samples
Score 0	52
Score 1	39
Score 2	35
Score 3	46
Score 4	16
Score 5	2
Outgroup	22

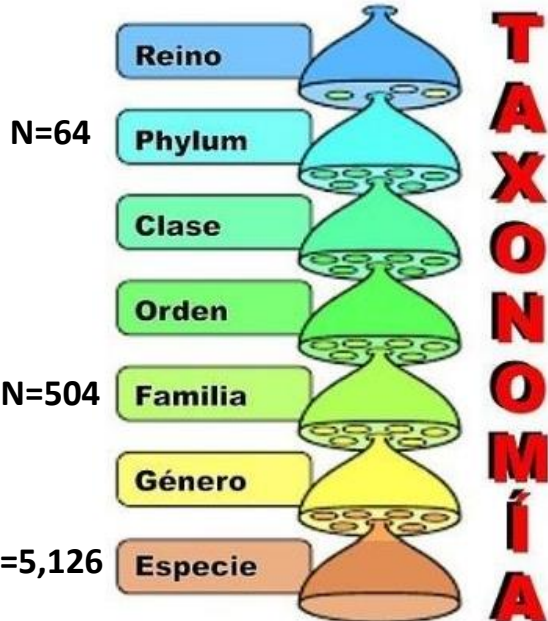
## Bioinformatics Pipeline



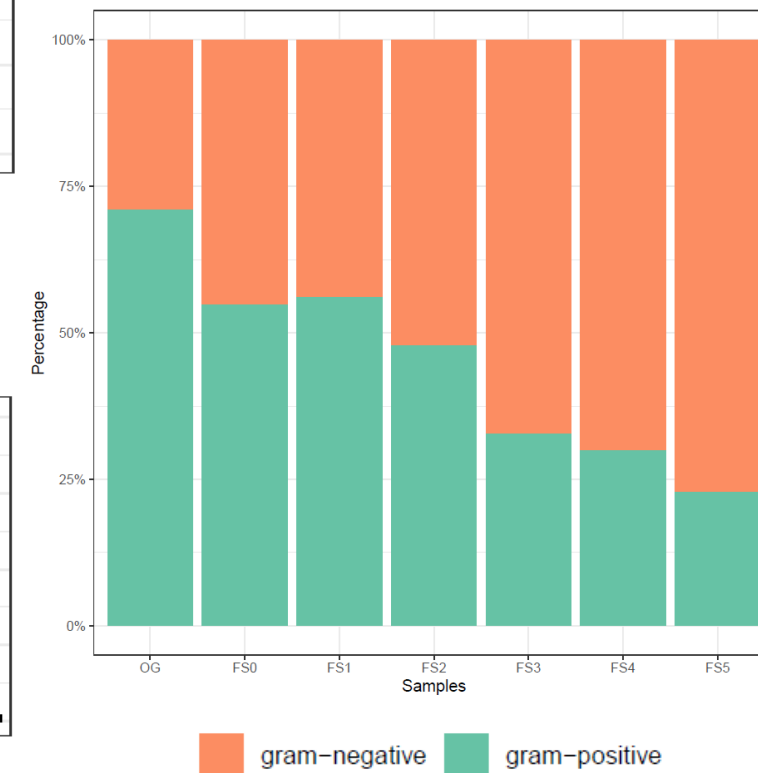
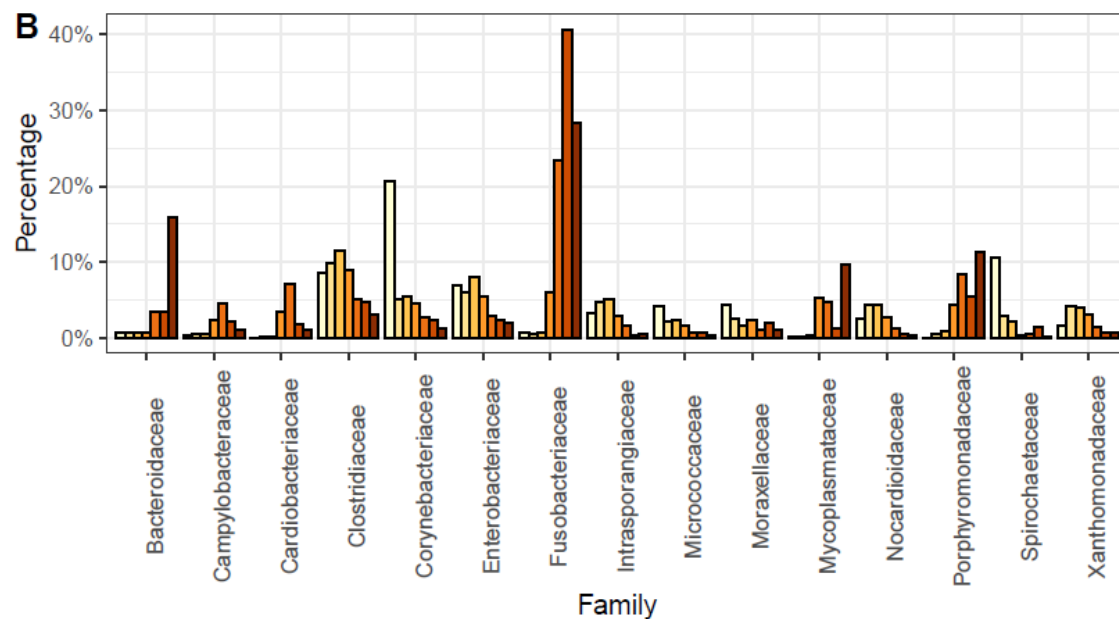
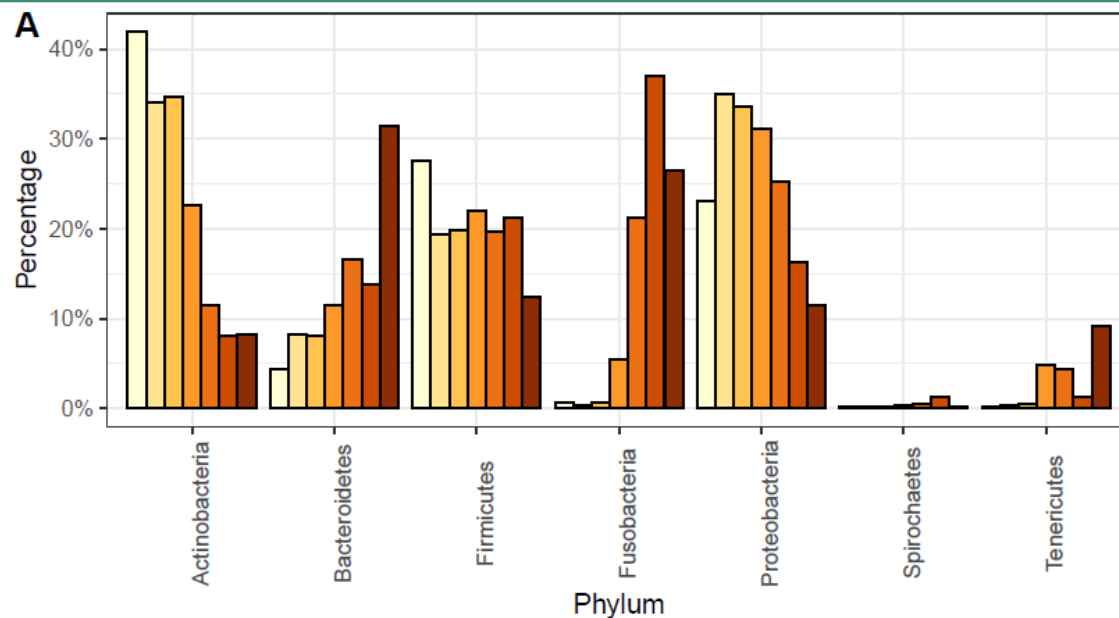
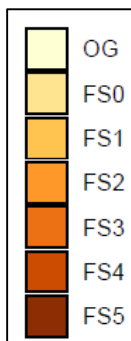
# Results



## Taxonomic Classification



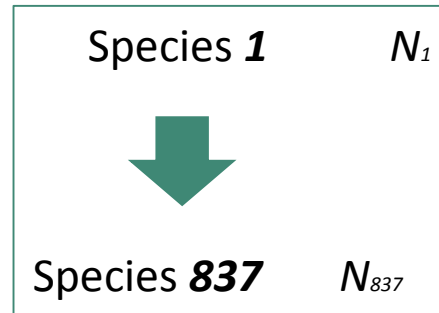
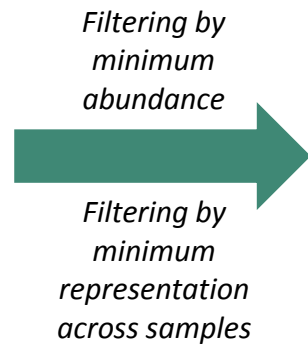
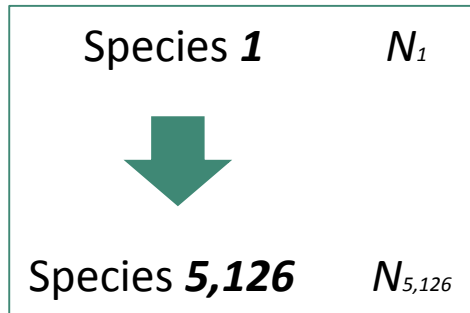
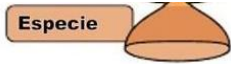
OG – Outgroup  
FS – Score Peira



# Results



## Abundance Estimation & Differential Abundance



**Nº Species 5,126**

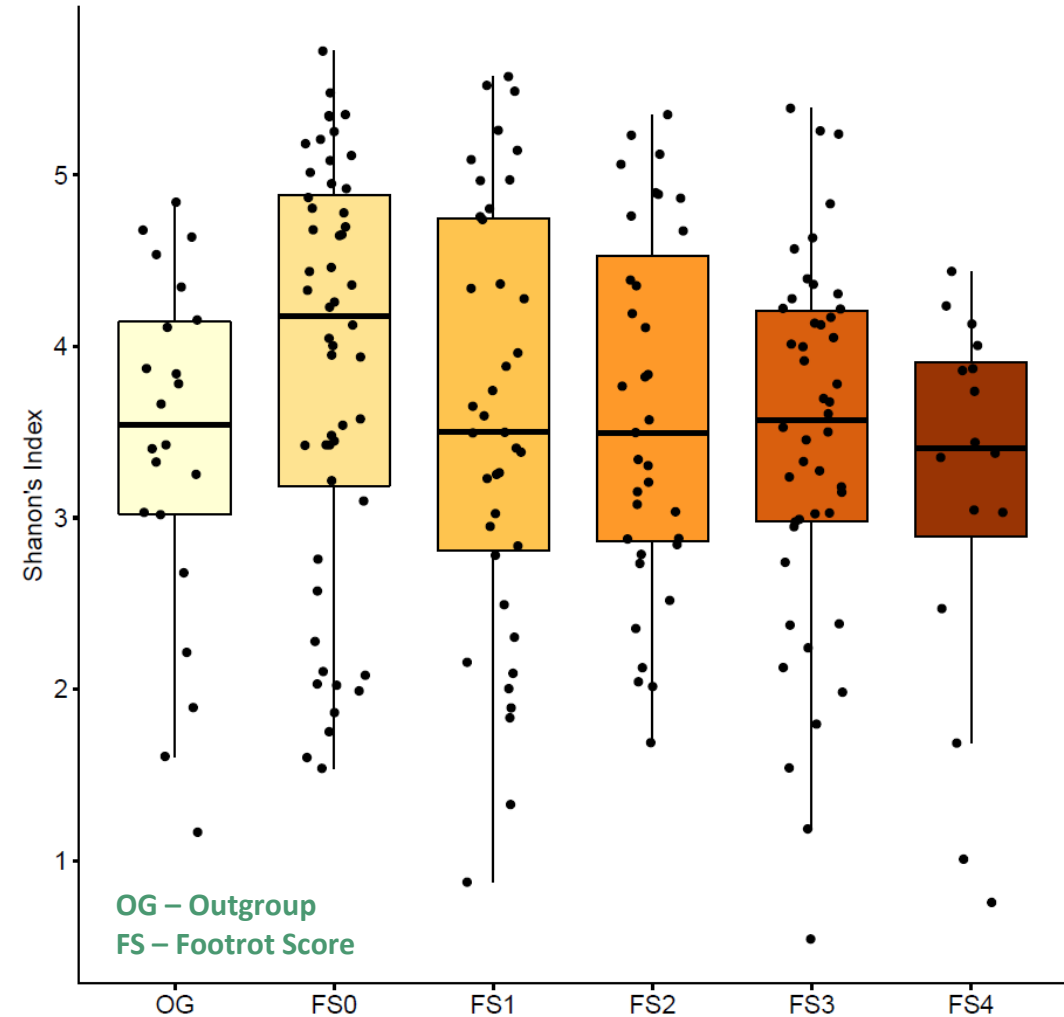
**Nº Species 837**

	Nº Samples
Score 0	52
Score 1	39
Score 2	35
Score 3	46
Score 4	16
Score 5	2
Outgroup	22

**Excluded due to low representation of samples**

**"Noise" in the statistical analyses**

## Shannon's index





# Results



## Differential Abundance

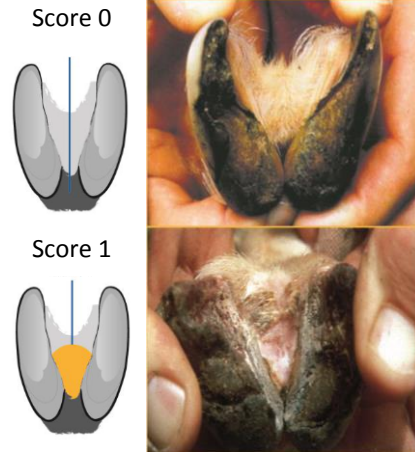
### Outgroup - OG

**NÃO** *D. nodosus*



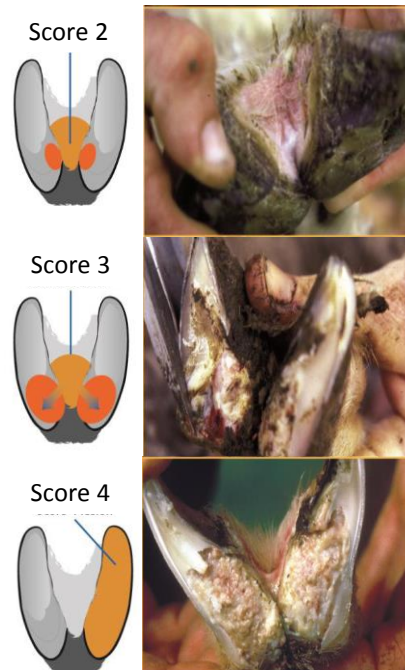
### NFIS

Samples with mild footrot signs



### FIS

Samples with severe footrot signs



Pairwise comparisons **Strategy 1**:

- OG vs **NFIS** ← 133/175
- OG vs **FIS** ← 172/219
- NFIS vs **FIS** ← 105/128
- OG vs. **(NFIS + FIS)** ← 146/186

*Dichelobacter nodosus*  
*Fusobacterium necrophorum*  
*Treponema spp.*

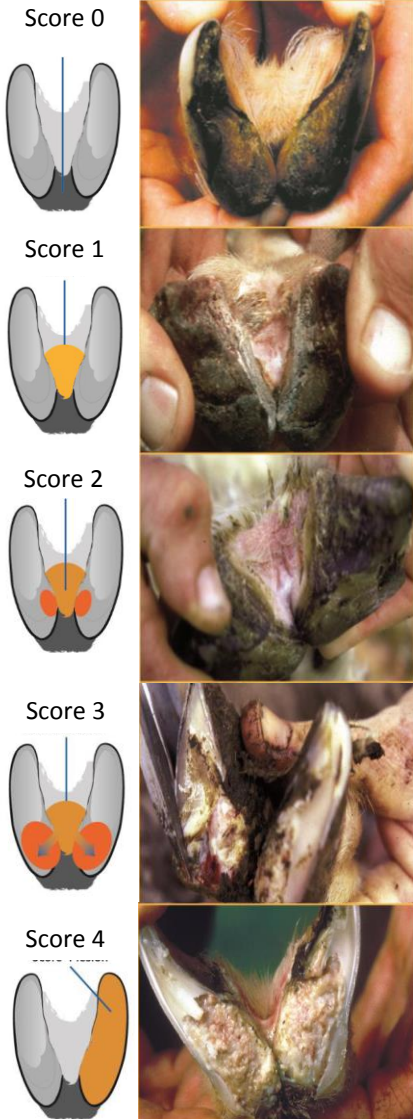
...

**Know species associated to  
different ovine's foot diseases**

# Results



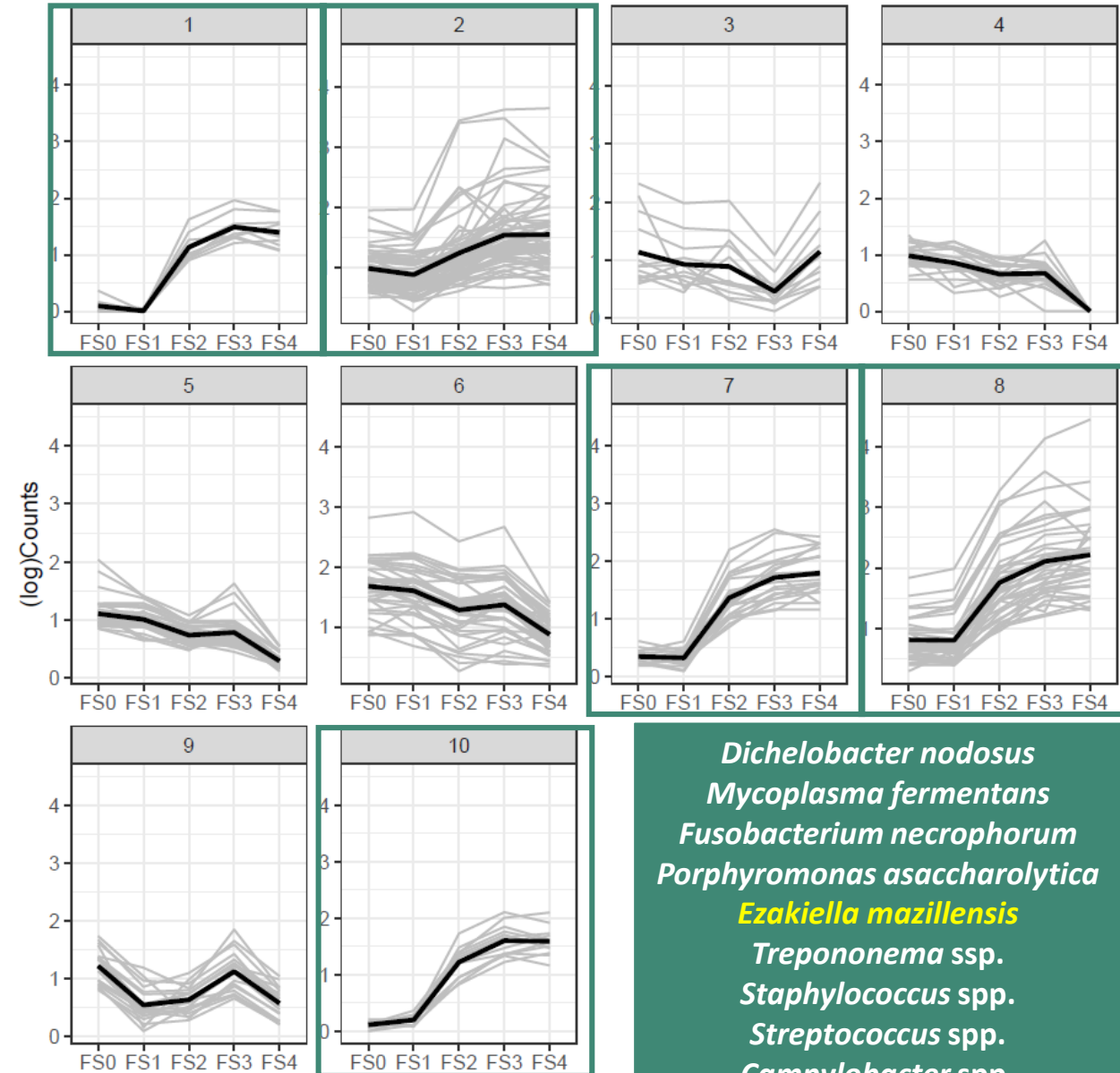
## Differential Abundance



## Pairwise comparisons **Strategy 2:**

- FS0 vs. FS1 **24**
- FS0 vs. FS2 **99**
- FS0 vs. FS3 **126**
- FS0 vs. FS4 **218**
- FS1 vs. FS2 **100**
- FS1 vs. FS3 **152**
- FS1 vs. FS4 **185**
- FS2 vs. FS3 **13**
- FS2 vs. FS4 **34**
- FS3 vs. FS4 **37**

**Total number of species: 281**



*Dichelobacter nodosus*  
*Mycoplasma fermentans*  
*Fusobacterium necrophorum*  
*Porphyromonas asaccharolytica*  
*Ezakiella mazillensis*  
*Trepononema* ssp.  
*Staphylococcus* spp.  
*Streptococcus* spp.  
*Campylobacter* spp.



# Conclusions



- ✓ This is the first study of the ovine footrot whole metagenome sequencing-based microbiome.
- ✓ Clear differences regarding the microbiome composition were identified in different diseases states.
- ✓ A set of species were identified that proliferates as the diseases aggravates being key species that differentiated mild and severe footrot infection stages.
- ✓ Further analysis on the individual species of *Campylobacter* and *E. massilensis*, as well as species with similar abundance profiles, are necessary to further understand their roles in footrot.

# Acknowledgments



**Ana Usié**

Ref. ALT20-05-3559-FSE-000076

Ref. CEECINST/00100/2021/CP2774/CT0001

Célia Leão  
Daniel Gaspar  
Helena Monteiro  
Lino Tábuas  
Sandra Branco  
Elisa Bettencourt

Pedro Caetano  
Ludovina Padre  
Nuno Carolino  
Marcos Ramos  
Claudino Matos

**GEN-RES  
ALENTEJO**

**Gen-Res-Alentejo – Utilização de Genómica na  
Seleção de ovinos resistentes a Parasitas e Peeira  
no Alentejo. Ref. ALT20-03-0145-FEDER-000037**



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de Desenvolvimento Regional

**RHAQ - Contratação de Recursos Humanos Altamente Qualificados  
(PME ou CoLAB) Ref. ALT20-05-3559-FSE-000076**



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UNIVERSIDADE  
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Instituto Nacional de  
Investigação Agrária e  
Veterinária, I.P.



DRAP Alentejo  
Direção Regional de Agricultura e Pescas do Alentejo

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Fundação  
para a Ciência  
e a Tecnologia

Ref. UIDB/05183/2020

Ref. CEECINST/00100/2021/CP2774/CT0001