Microbiome structure and diversity of *Hyalomma lusitanicum* in South Spain

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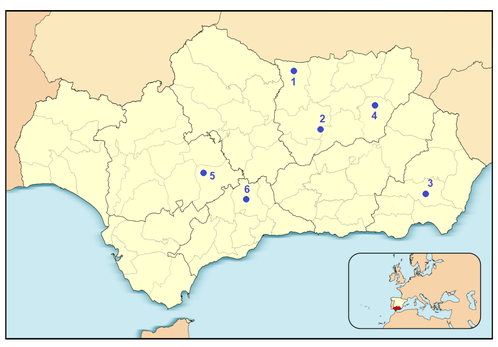
Introduction

Ticks (Acarina, Ixodidae) are proven to be vectors of different pathogens that affect both people and domestic and wild animals to different degrees. In the Iberian Peninsula, *Hyalomma lusitanicum* has been mentioned as a vector of several pathogenic bacteria [*Coxiella burnetii* (Q fever), *Anaplasma phagocytophilum* (Anaplasmosis), *Francisella tularensis* (Tularemia), *Rickettsia aeschlimannii*][1,2], and piroplasmida [*Theileria annulata* (agent of Mediterranean theileriosis), *T. equi and Babesia caballi*][2,3]. It has recently been implicated in the transmission of Crimea-Congo virus, which is recognized to have an increasing distribution associated with the two *Hyalomma* species (*H. marginatum* and *H. lusitanicum*)[4].

Material and Methods

In order to determine the structure and diversity of microbiome of *H. lusitanicum*, a total of 85 questing adults ticks (50 females and 35 males) were used for the study of their bacterial profile. Ticks had been obtained by flagging methods over vegetation in six localities from Andalusia, three in the province of Jaén (47 ticks) and one in the provinces of Almería (12), Málaga (7) and Seville (12), respectively (Fig. 1, Tab. 1).



Loc. code	Locality	Province	Samples	Female	Male
1	Andújar	Jaén	20	8	12
2	Mancha Real	Jaén	19	9	10
3	Tabernas	Almería	12	9	3
4	La Iruela	Jaén	8	7	1
5	Lantejuela	Seville	19	10	9
6	Antequera	Malaga	7	7	0

Fig. 1. Localities of procedence of *Hyalomma (H.) lusitanicum.*

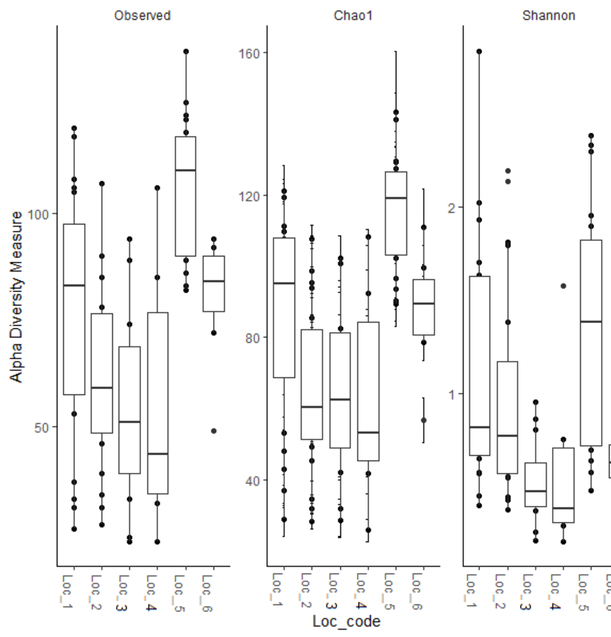
DNA extraction were made under appropriate sterile conditions. Sterile single-use instruments were used whenever possible. Ticks were transferred to sterile petri dishes and longitudinally cut into two halves. The DNA was extracted using DNeasy Blood and Tissue kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. Negative controls of extraction corresponding to extraction tubes without tick samples were included in parallel. Library preparation and Illumina sequencing were carried out at the IPBLN Genomics Facility (CSIC, Granada, Spain). Amplicon libraries targeting the 16S rRNA gene, were generated by a two-steps PCR strategy. Gene-specific amplification was performed in triplicate with 15 ng of gDNA in a final volume of 10 µl. Gene specific primers V3V4 regions of 16S rRNA were used with Nextera overhang adapters. Amplicon libraries were generated by a two-steps PCR strategy. Sequencing was conducted on a MiSeq sequencer, according with Illumina MiSeq library preparation guide. Library preparation and Illumina sequencing of gene were carried out at the IPBLN Genomics Facility (CSIC, Granada, Spain).

Fastq files were pre-processed and analysed in the R 4.2.3 enviroment[5] using the microbiome program DADA2 v.1.14.0[6], applied to raw sequence data from each sample with standard parameters defined in the pipeline tutorial. Chimeras were identified among inexact matches using a combination of program-specific chimera finding software (removeBimeraDenovo), followed by FindChimeras from the DECIPHER package[7]. In order to reduce the number of taxa included in the analysis, most part of analysis has been made at family-genus level. In the next step, an environment object of the phyloseq package[8] is generated.

Results and Discussion

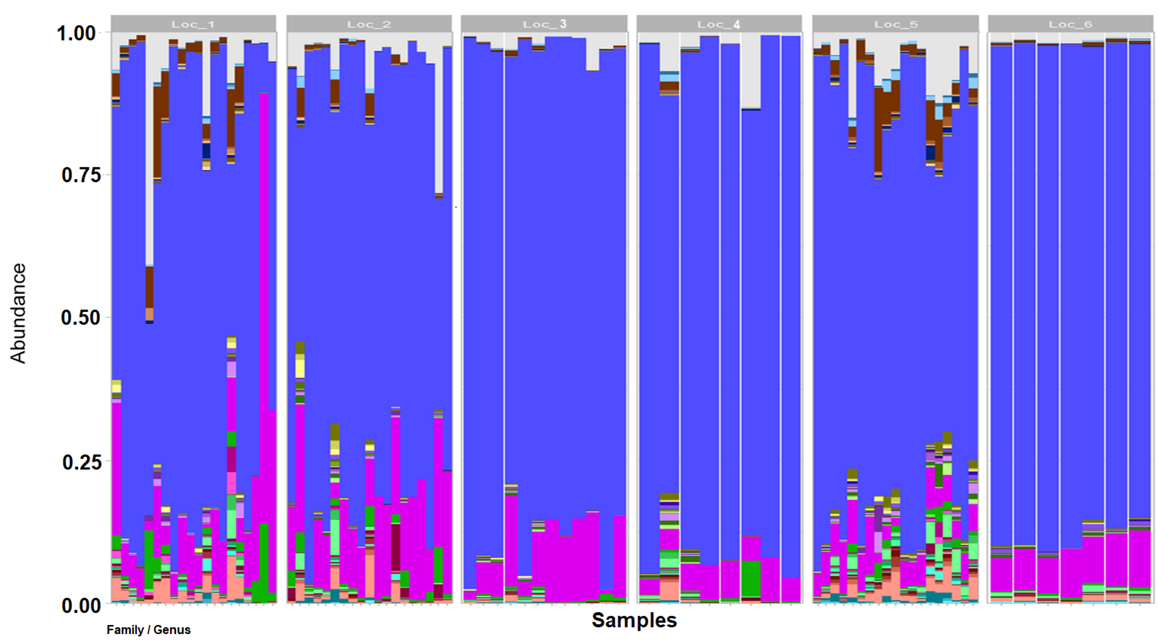
The initial resulting Amplicon Sequence Variants ASV table consisted of 21,638 ASVs. After taxa assignation[9,10], we retain 174 taxa features containing 11 phylum, 44 orders, 73 families and 174 genera. Six group samples were defined, according to the geographical origin of ticks (Fig. 1). The alpha diversity analysis revealed no significant differences in microbiome diversity (Fig. 2). Overall, *H. lusitanicum* it is enriched by the phylum Proteobacteriae, Actinobacteriota, followed by Bacteroidota and Firmicutes and dominated by *Francisella* spp and candidatus Midichloria (Fig. 3 and 4)[11]. Results of the principal coordinate analysis (PCoA) showing differences in beta diversity between localities. PCoA plots using Bray-Curtis metric. The x-axis explains 49.8% variation of the data. The y-axis explains 29.6% variation of the data (Fig. 5).

It is known that non-pathogenic microorganisms may be involved in the transmission of tick-borne pathogens, which affects the health of both wild and domestic populations of various hosts as well as humans[12,13]. On the other hand, when studying the intragenus phylogeny of tick-transmissible pathogens, it has been recognized that within these lineages there are some mutualistic symbionts of the tick itself with defined metabolic functions. In this circumstance, within the bacteriome of the *H. lusitanicum* populations studied, the case of *Francisella*[11,14] and candidatus Midichloria[15,16] would stand out. A portion of the detected bacteria could be interpreted as non-pathogenic microorganisms that exhibit alternative lifestyles as mutualistic tick symbionts[17,18].



Dorsal view of a female of *Hyalomma (H.) lusitanicum*

Fig. 2. Box plots showing differences in alpha diversity between samples ordered by localities. The box-and-whisker plot describes the distribution of data points, including minimum, maximum, median, first quantile and third quantile.



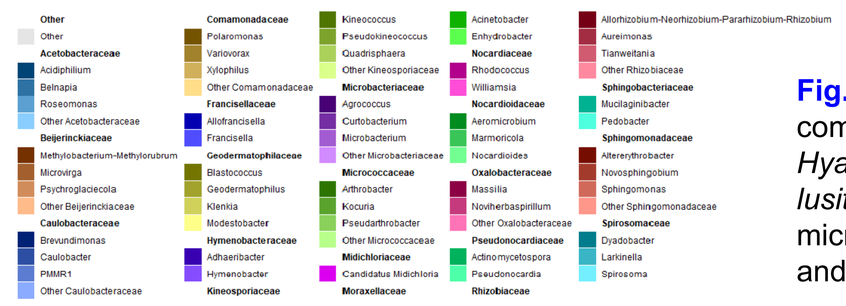


Fig. 3. Taxonomical composition of *Hyalomma lusitanicum* microbiota by family and genus.

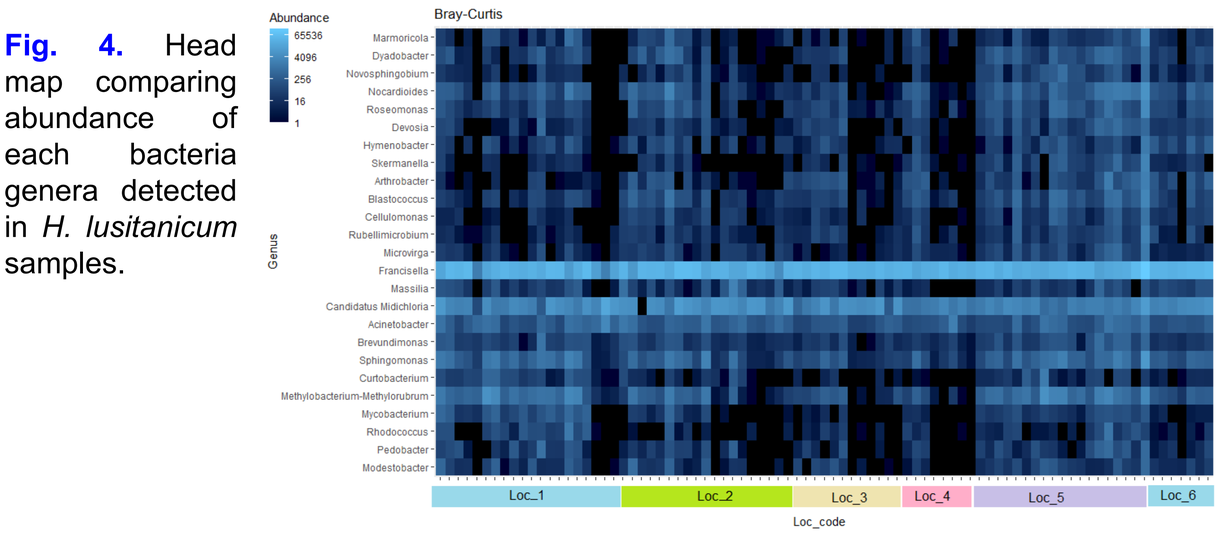


Fig. 4. Head map comparing abundance of each bacteria genera detected in *H. lusitanicum* samples.

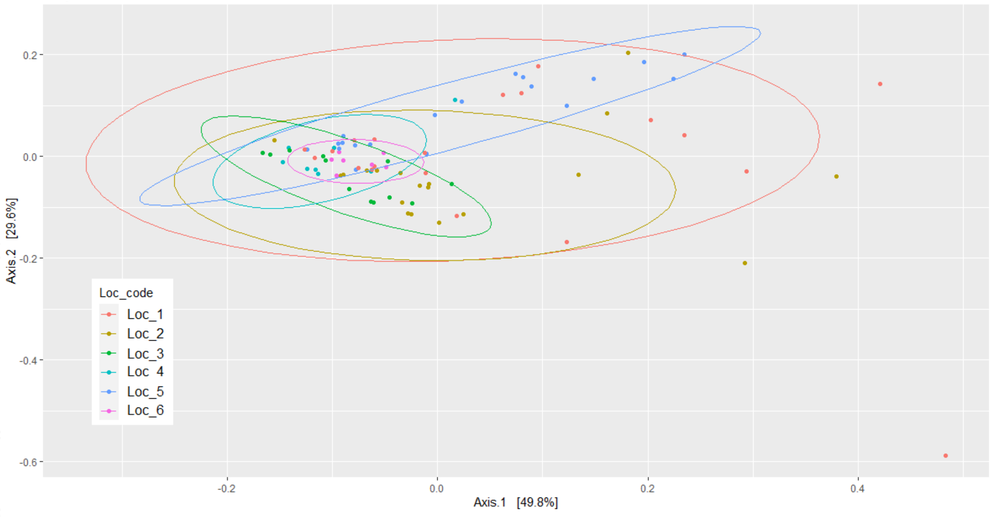


Fig. 5. Results of principal coordinate analysis (PCoA) showing differences in beta diversity between localities (Bray Curtis distance).

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