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Characterizing the bacterioma of *Linognathus stenopsis* from Iberian Ibex. Preliminary results

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Introduction

The sucking lice *Linognathus stenopsis* (Burmeister, 1838) (Phthiraptera, Anoplura, Linognathidae) represents a permanent, strictly host specific ectoparasite of ibex, domestic goats and chamois (*Capra* sp. and *Rupicapra* sp.) around the world[1]. From a sanitary point of view, sucking lice can cause weight loss, skin damage, moderate to severe anaemia, hypoproteinaemia, insufficient absorption of food and reduced vitality in infected goats[2,3]. In addition, they can transmit viruses, bacteria, protozoa and fungi like *Rickettsia* spp. and *Anaplasma ovis*[4-6]. Recent technological developments in DNA sequencing, as New Generation Sequencing have enabled "the microbiome revolution"[8,9.] Within this context, we have developed a strategy aimed at revealing the complete microbiome (both superficial and internal) including "non-culturable" species of bacteria present in *L. stenopsis*.

Material and Methods

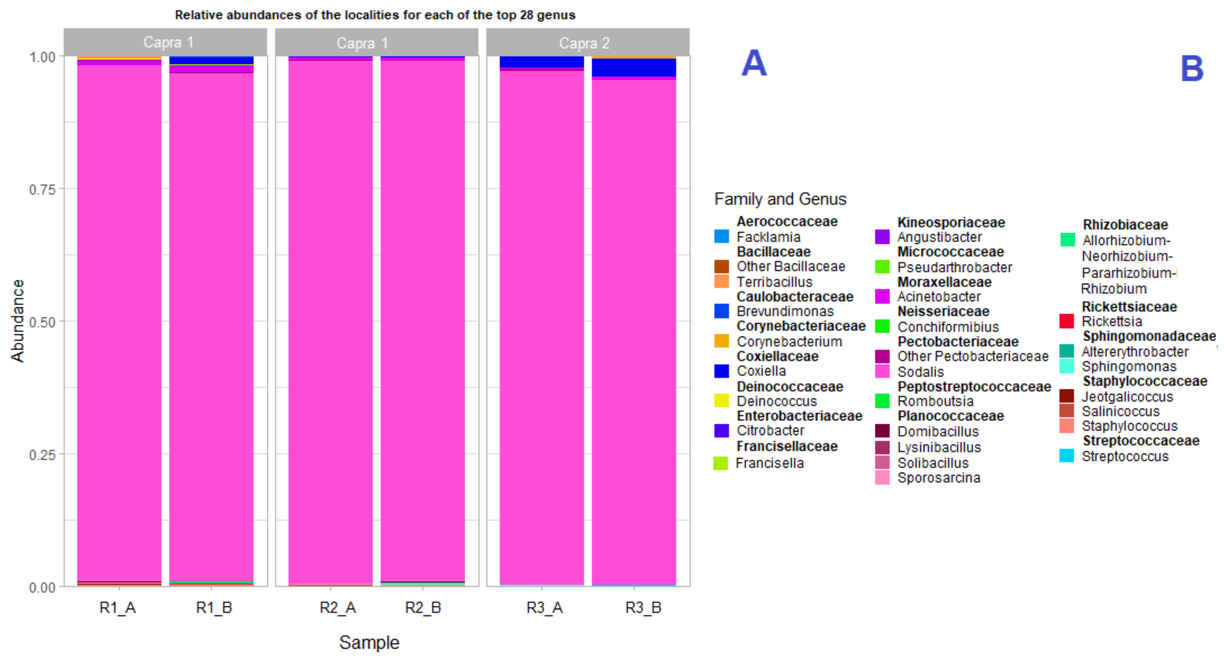
Linognathus stenopsis specimens were collected from two Iberian ibex (*Capra pyrenaica hispanica*) from Sierra Nevada Natural Space (southern Spain), preserved in 70° ethanol for laboratory identification, morphological observation, preparing photographic material and DNA extraction.
The study of bacterial profile of *L. stenopsis* began with the preparation of three batches of 12 adult lice. DNA extraction was made under appropriate sterile conditions using DNeasy Blood and Tissue kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. Library preparation and Illumina sequencing of V3V4 regions of 16S rRNA gene were carried out at the IPBLN Genomics Facility (CSIC, Granada, Spain). Amplicon libraries were generated by a two-steps PCR strategy. Sequencing was conducted on a MiSeq sequencer, according with Illumina MiSeq library preparation guide.
As a part of Bioinformatic study, Fastq files were pre-processed and analysed using specific analyisis of microbiome packages (DADA2 v.1.14.0[10] and Phyloseq[11]), complemented with other R packages (microbiome, microbiomeMarker, etc.) in the R 4.2.3 environment[12].

Results

The results gathered from our study showed that the bacterial composition of *L. stenopsis*, considering surface and internal microbiome, has a high biodiversity that include several hundred of taxa (Fig. 1A and 1B). Microorganisms taxonomic composition of each replica show a high predominance of a sequence related with the order Enterobacterales, which we notated as "Linognathus stenopsis endosymbiont" (LSE), initially identified by the system as belonging to the genus *Sodalis* (Fig 2.). In order to obtain more information about the possible identity of this taxa and the phylogenetic relationships of the LSE sequences, we performed a phylogenetic analysis on the V3-V4 region of the 16S rRNA sequences, using Blast to search for the highest sequence identity in GenBank. Blast revealed that the two selected 429 bp sequences of LSE have a maximum percentage identity (id) ranging from 94.61% to 93.01% with the Enterobacterales, mainly Pectobacteriacea of the genus *Sodalis*[13-15], Yersiniaceae of the genus *Serratia*, or several uncultured endosymbion as "candidatus Steffania", among others.
Of relevant sanitary importance should be considered the presence of *Rickettsia* sp. (100% id with a large range of species), *Coxiella* sp. (100% to 96% id with *Coxiella*-like endosymbiont of the tick *Haemaphysalis punctata* (GenBank accesion MT313147), *Francisella* sp. (100% id with several *Francisella* like endosymbions of ticks of genus *Dermacentor, Hyalomma* and *Haemaphysalis*) and *Acinetobacter* sp. (100% id with *Acinetobacter johnsonii*, GenBank accesion MN826149)[5,16].

Discussion

We consider highly probable that LSE may correspond to a primary endosymbiont (P endosymbiont) of this sucking lice, taking into account that it is a key factor in the evolutionary success of approximately 15% of insect species. P-endosymbionts are obligate and mutualistic bacterial endosymbionts vertically inherited, implicated in strict cospeciation processes[17-20]. A particularly relevant aspect is determined by the presence of at least four genera with health concern such as *Rickettsia, Coxiella, Francisella* and *Acinetobacter*[5].



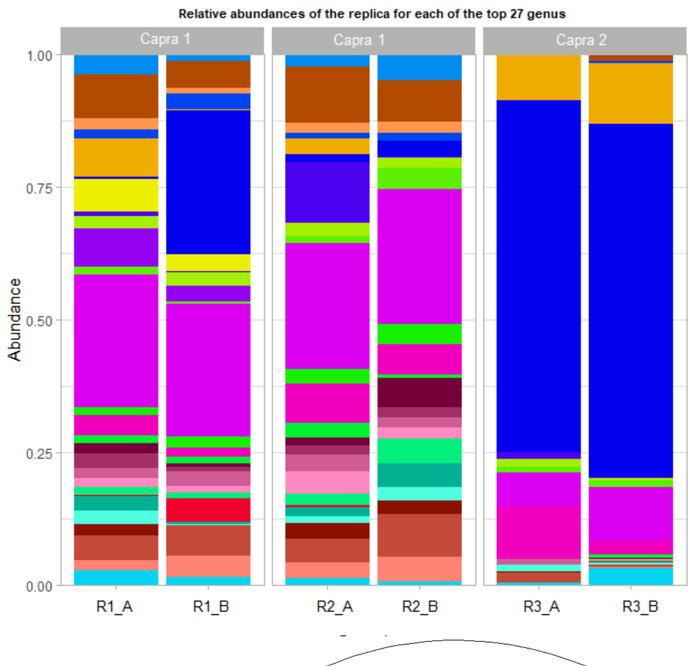


Fig. 1.A. Taxonomical composition of *L. stenopsis* microbiota by family and genus. **1.B.** Taxonomical composition of *L. stenopsis* microbiota by family and genus, excluding LSE cf *Sodalis* genus.

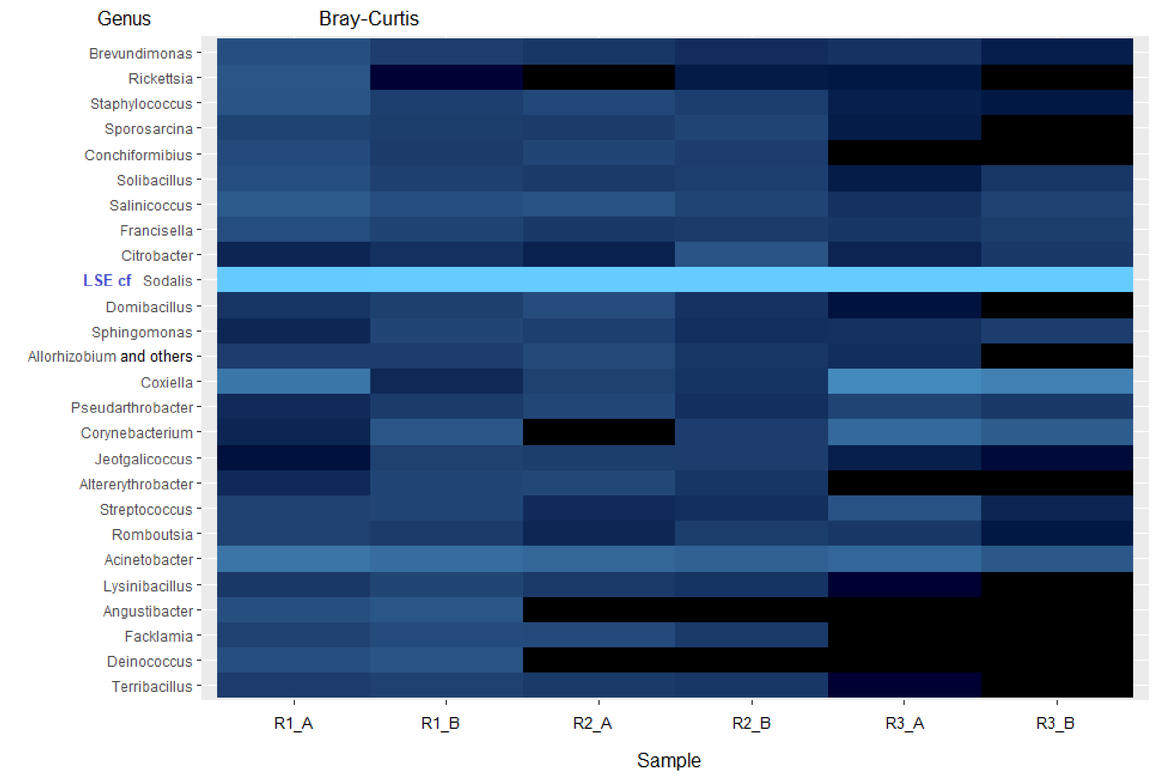


Fig. 2. Abundance percentage for the bacteria genera detected in this study.

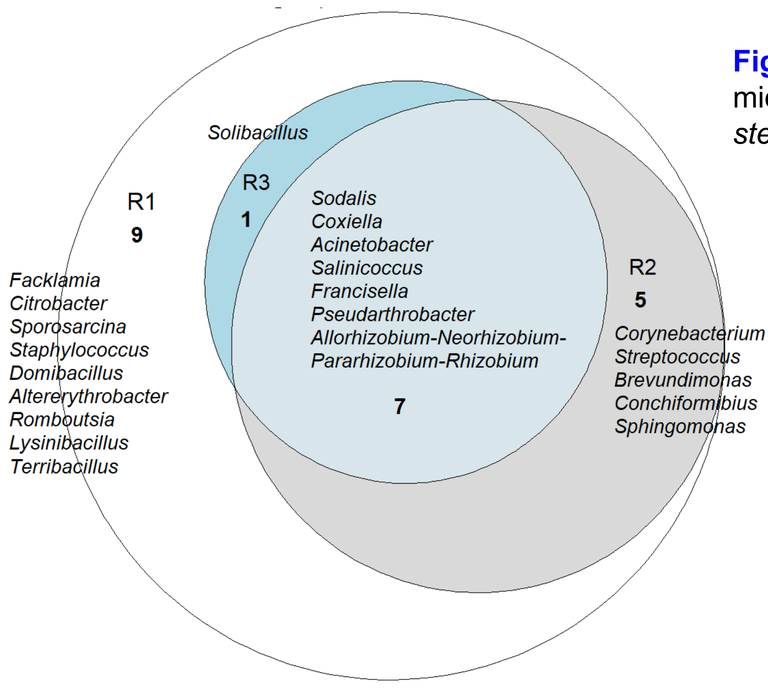


Fig. 3. Core genera of microbiome detected in *L. stenopsis.*

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