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First identification of bacterial endosymbionts in three South-American spittlebug pests: *Notozulia entreriana*, *Deois mourei* and *Deois knoblauchii*

Alvaro Foieri[1,2], Cecilia Decker-Franco[1,2], Ana M. Marino de Remes Lenicov[3], Joel D. Arneodo[1,2]
[1]*Instituto de Agrobiotecnología y Biología Molecular-CONICET/INTA, Hurlingham, Argentina*
[2]*Instituto de Microbiología y Zoología Agrícola-INTA, Hurlingham, Argentina*
[3]*División Entomología, Facultad de Ciencias Naturales y Museo (FCNyM), Universidad Nacional de La Plata, Argentina*

Abstract

Spittlebugs cause major pasture damage in the Neotropics. As most xylem-feeders, they depend on microbial symbionts to supply essential amino acids to their diet. Here, the obligate nutritional endosymbiont '*Candidatus* Sulcia muelleri' (Bacteroidetes) was detected in three main cercopid pests of South America: *Notozulia entreriana* (Berg), *Deois* (*Deois*) *mourei* Cavichioli et Sakakibara and *Deois* (*Deois*) *knoblauchii* (Berg) (Cercopidae Ishnorhininae). In all insect species, bacteriomes were located laterally in the abdomen, and ultrathin sections of *N. entreriana* bacteriocytes showed typical sulcia-like bacteria. PCR and sequencing of a 914-bp fragment of the bacterial 16S rRNA gene revealed 100% nucleotide identity among sulcia strains obtained from the three host species. These sequences were also identical to those previously obtained from two other New World spittlebugs of the same subfamily, providing evidence for host/symbiont coevolution. Microscopic and molecular analyses suggested that *N. entreriana* lacked additional symbionts (*i. e.* '*Candidatus* Zinderia insecticola' or sodalis-like bacteria [Proteobacteria]) that often co-occur with sulcia within members of the superfamily Cercopoidea. Though amplicons were occasionally generated from *D. (D.) mourei* and *D. (D.) knoblauchii* with primers intended for zinderia, they failed to sequence. Further research is needed to elucidate the identity of bacteria other than sulcia in *Deois* spp.

Key words: Cercopidae, Bacteriome, '*Candidatus* Sulcia muelleri', Phylogeny, South America.

Introduction

Spittlebugs (Hemiptera Cercopoidea Cercopidae) represent a major biological constraint to the production and sustainability of forage crops in the Neotropics (Thompson, 2004). Many grasses are susceptible to the attack of these insects, which can reduce pasture yields up 21 to 70% in infested areas (Thompson, 2004; Leite *et al*., 2005). Other Poaceae, such as sugarcane (*Saccharum officinarum* L.) and rice (*Oryza sativa* L.) are also affected (Paladini *et al*., 2018). During xylem feeding, spittlebugs cause mechanical damage and induce adverse physiological effects by injecting toxic salivary secretions (Holmann and Peck, 2002). Additionally, Cercopidae have been identified as potential vectors of the bacterium *Xylella fastidiosa* Wells to grapevines in Brazil (Ringenberg *et al*., 2014) and to olive trees in Italy and the Mediterranean basin (Saponari *et al*., 2014; Panzavolta *et al*., 2019; Theodorou *et al*., 2021). In Argentina, 24 spittlebug species were identified. These include *Notozulia entreriana* (Berg), *Deois* (*Deois*) *mourei* Cavichioli et Sakakibara and *Deois* (*Deois*) *knoblauchii* (Berg), which are among the most important pests of grasses in several countries of South America, owing to their abundance and wide distribution from southern USA to central Argentina (Marino de Remes Lenicov *et al*., 2004; Foieri *et al*., 2018).

Sap-feeding insects harbour obligate bacterial endosymbionts, which are generally housed in specialized organs called bacteriomes and contribute nutrients to their highly specific diet (Koga *et al*., 2013). The Bacteroidetes '*Candidatus* Sulcia muelleri' (hereafter sulcia) established a symbiotic relationship with the common ancestor of the Auchenorrhyncha about 270-300 million years ago (Bennett and Mao, 2018). This association persists in many members of the main lineages of the suborder, including the superfamilies Fulgoroidea, Membracoidea and Cercopoidea. Sulcia supplies their hosts with most of the 10 essential amino acids (EAAs) (Koga and Moran, 2014). A few complete sulcia genomes (McCutcheon and Moran, 2007; Bennett *et al*., 2013; 2016; Mao *et al*., 2017) and several 16S rDNA partial sequences (Moran *et al*., 2005; Takiya *et al*., 2006; Urban and Cryan, 2012; Ishii *et al*., 2013; Koga *et al*., 2013; Szklarzewicz *et al*., 2016; Kobiałka *et al*., 2018a; 2018b; 2018c) have been obtained from different species of Auchenorrhyncha. Often, additional lineage-specific symbionts contribute to the biosynthesis of EAAs (Koga and Moran, 2014). Some species of the cercopoid families Cercopidae, Clastopteridae, Machaerotidae and Aphrophoridae harbour, together with sulcia, the coresident symbiont '*Candidatus* Zinderia insecticola' (hereafter zinderia), for which a complete genome and a number of partial sequences are available (McCutcheon and Moran, 2010; Koga *et al*., 2013). Furthermore, a replacement of zinderia by a symbiont related to *Sodalis glossinidius* (hereafter sodalis) has been reported within the tribe Philaenini in the Aphrophoridae (Koga *et al*., 2013). Regarding Neotropical spittlebugs, two previous works have reported partial 16S rDNA sequences of sulcia and zinderia from a number of host species (Moran *et al*., 2005; Koga *et al*., 2013). However, the cercopids distributed in the southern half of South America remain unexplored.

This work presents a brief description of the bacteriomes of the main South American spittlebug pests *N. entreriana*, *D.* (*D*) *mourei* and *D.* (*D*) *knoblauchii*, including electron microscopy examinations for *N. entreriana*.

Following DNA extraction from both excised bacteriomes and whole insect bodies, PCR assays for sulcia, zinderia and sodalis-like bacteria detection was conducted with specific primers targeting the 16S rRNA gene.

Materials and methods

For molecular analyses, specimens of *N. entreriana* and *D.* (*D*) *mourei* were sampled in Río Cuarto (Córdoba province, Argentina) in March 2016, while *D.* (*D*) *knoblauchii* individuals were captured in June 2014 in Alberdi (Salta province, Argentina). Insects were collected from grasses and conserved in 70% ethanol until processing. Bacteriomes of adults of each species were excised under binocular microscope and pooled in groups of ten; total DNA was extracted following a CTAB protocol (Doyle and Doyle, 1987). Based on sulcia sequences available at GenBank (https://www.ncbi.nlm.nih.gov/genbank/), a new set of primers was designed with Vector NTI (Invitrogen, Carlsbad, CA) to specifically amplify a 914-bp fragment of the 16S rRNA gene. Non-specific primer annealing against other bacterial symbionts in the Auchenorrhyncha was excluded by in silico analysis. Primers SulciaJFw (5' CCTTATGCTGAGGGATAGCC 3') and SulciaJRv (5' GCAGCACCTTGTACTCCGTC 3') were used in 35 cycles of PCR at 95 °C, 52 °C and 72 °C for 1 minute each followed by an elongation at 72 °C for 5 minutes, in an IVEMA T18 thermocycler (IVEMA Desarrollos, Llavallol, Argentina). PCR mix (20 µl/tube) consisted of 2 µl reaction buffer 10X, 1.2 µl MgCl2 (25 mM), 0.5 µl dNTPs 5mM, 1 µl (each) forward and reverse primers, 0.1 µl DNA polymerase free from bacterial DNA contamination (5 U/µl), 1 µl template (<500 ng DNA) and 13.2 µl molecular biology grade water (INBIO, Tandil, Argentina). Amplicons were resolved by agarose (1%) gel electrophoresis. PCR products corresponding to each spittlebug species were purified (ADN PuriPrep-GP kit, INBIO, Tandil, Argentina) and sequenced bidirectionally (using the same primers above) in an ABI PRISM 3500 XL genetic analyser (Applied Biosystems, Foster City, California, USA) at the Instituto de Biotecnología-INTA (Hurlingham, Argentina). These and previously reported sequences of sulcia strains from hosts in the superfamilies Fulgoroidea, Membracoidea and Cercopoidea were trimmed to the same length and aligned with MUSCLE. A Maximum-Likelihood (ML) phylogenetic tree was then constructed with PhyML vers. 3.0 (Guindon *et al*., 2010) under the GTR nucleotide substitution model. A consensus tree was generated using the SPR algorithm, and topological robustness was assessed by 100 bootstrap replicates. The Bacteroidetes *F. frigidarium* served as outgroup.

The same DNA templates were used in a preliminary PCR survey for zinderia and sodalis with primers Bet937F/Zin1288R (amplicon size: ~390 bp) and SpSod506F/Sod1248R (~770 bp), respectively (Koga *et al*., 2013). Further *N. entreriana* and *Deois* spp. were sampled during 2020 in La Plata (Buenos Aires province, Argentina) and Tucumán (Tucumán province, Argentina), respectively. DNA was extracted from whole insect bodies, including cercopoid species known to respectively harbour zinderia and sodalis: *Clastoptera* sp. (Cercopoidea Clastopteridae) from La Plata (Argentina) and *Philaenus spumarius* L. (Cercopoidea Aphrophoridae) from Italy. PCRs were run again with the above-mentioned sulcia, zinderia and sodalis primers and 10F/Zin1288R, which yield a longer (~1300 bp) fragment of the zinderia 16S rRNA gene (Koga *et al*., 2013). Amplicons were eventually sequenced as explained before.

For electron microscopy observations, *N. entreriana* adults were collected in pastures at the experimental field of INTA (Hurlingham, Buenos Aires province, Argentina) in March 2017. Freshly dissected bacteriomes were fixed in 2.5% glutaraldehyde, post-fixed in osmium tetroxide and embedded in epoxy resin (Arneodo *et al*., 2008). Ultrathin sections of circa 100 nm were stained with uranyl acetate and lead citrate, and examined under a JEOL 1200 EX II microscope (JEOL Ltd., Tokyo, Japan) at a voltage of 80 kV.

Results and discussion

Bacteriomes of *N. entreriana*, *D.* (*D*) *mourei* and *D.* (*D*) *knoblauchii* consisted of grouped globular structures of reddish or yellowish colour, and were located laterally in each side of the abdominal body cavity (figure 1). Transmission electron microscopy observations of sectioned bacteriomes of *N. entreriana* revealed the presence of large, pleomorphic bacteria occupying most of the cytoplasm of the bacteriocytes (i.e., insect cells that constitute the bacteriome) (figure 1a). Their location and overall appearance were similar to sulcia symbionts reported in other auchenorrhynchan hosts (Bressan *et al*., 2009; Brentassi *et al*., 2017).

The new primer pair SulciaJFw/SulciaJRv yielded a single strong band of the expected size in all bacteriome samples, thus indicating the association of sulcia with the three spittlebug species surveyed. Partial 16S rDNA sequences were obtained using the same primers in the sequencing reactions (GenBank accession numbers MK908986, MK908987 and MK908988 for *N. entreriana*, *D.* (*D*) *mourei* and *D.* (*D*) *knoblauchii*, respectively). The three sequences were identical to one another and to two sulcia sequences from the cercopids *Aeneolamia contigua* (Walker) and *Huaina inca* (Guerin-Meneville) collected in Central America (Koga *et al*., 2013), as identified by BLAST searches. In the phylogenetic analysis, the newly obtained and GenBank-retrieved sulcia 16S rDNA sequences formed three clades (bootstrap support >89%) that correlated with the host superfamily (Fulgoroidea, Membracoidea, Cercopoidea). In this ML tree, the novel bacterial sequences clearly grouped with those of the other Cercopoidea-associated sulcia strains (figure 2).

Sequence analyses conducted to date have shown that, in spite of a long co-evolutionary process, the ubiquitous sulcia exhibits a rather conserved genome across different auchenorrhynchan hosts (Bennett and Mao, 2018). The results obtained here are in line with these observations, and also revealed that sulcia strains associated to Argentinean spittlebugs are most closely related among

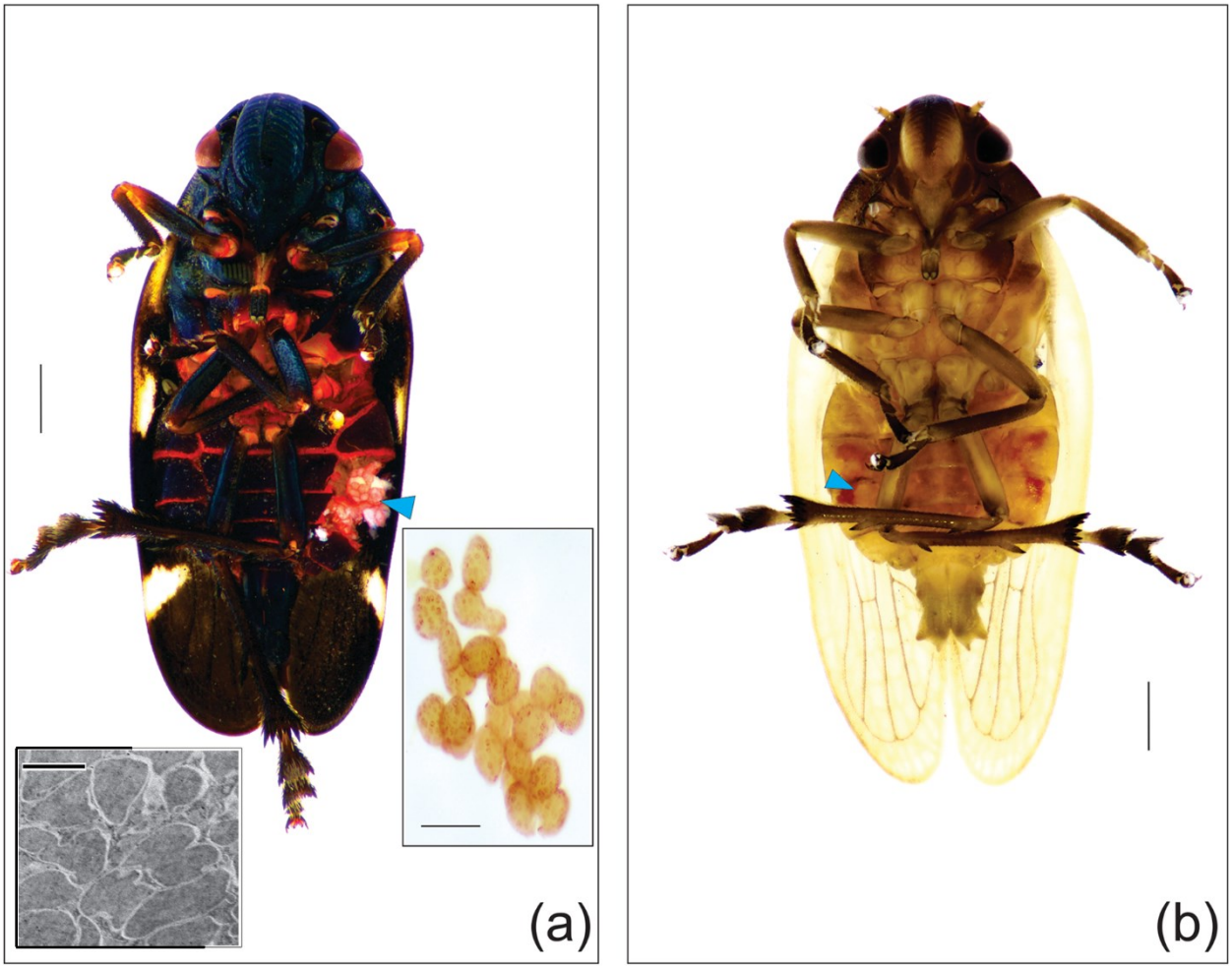

Figure 1. (a) Ventral view of a *N. entreriana* adult female. A piece of the insect cuticle was removed to expose the bacteriomes (arrowhead) (scale bar = 1 mm). An enlarged image of dissected bacteriome tissue is shown at the right (scale bar = 0.5 mm). At the bottom-left, ultrathin section of a bacteriocyte showing typical pleomorphic sulcia-like symbionts occupying the cell cytoplasm (scale bar = 2 µm). **(b)** Ventral view of a *D.* (*D.*) *mourei* adult female; bacteriomes can be observed directly through the cuticle (arrowhead) (scale bar= 1 mm).

themselves and to those from other Neotropical Cercopidae. *N. entreriana*, *D.* (*D*) *mourei*, *D.* (*D*) *knoblauchii*, *A. contigua* and *H. inca* (which shared 100% identity in their sulcia 16S rDNA) belong all to the monophyletic New World subfamily Ishnorhininae, within the Cercopidae (Paladini *et al*., 2015). Thus, congruence was found between insect and symbiont phylogeny at this host taxonomic level.

Neither zinderia nor sodalis were detected by PCR with Koga *et al*. (2013) diagnostic primers in the bacteriome-extracted DNA of the three species. To discard false negatives owing to DNA degradation, new tests were performed on freshly captured specimens (whole-body DNA), including the positive controls (insect species harbouring sulcia, zinderia and sodalis). Again, only sulcia was amplified from *N. entreriana*. However, bands of the expected size for zinderia were occasionally amplified from *D.* (*D*) *mourei* and *D.* (*D*) *knoblauchii* using Bet937F/Zin1288R, though of lower intensity compared to the zinderia positive controls (*Clastoptera* sp.). Sodalis-specific primers SpSod506F/Sod1248R amplified only the positive controls, *P. spumarius*. PCRs on the same samples with sulcia primers yielded the expected 914-bp fragment (figure 3). When using 10F/Zin1288R primers, the predicted zinderia product (~1300 bp) was obtained from *Clastoptera* sp. Instead, shorter (~1000 bp), weaker and less sharp bands were obtained from *Deois* spp. and no signal was observed in *N. entreriana* (data not shown). To verify specificity, the Bet937F/Zin1288R and 10F/Zin1288R primed products of *D. mourei* and the ~1300 bp product of *Clastoptera* sp. were directly sequenced. The partial sequence obtained from *Clastoptera* sp. (GenBank accession number MW802594) exhibited 99% nucleotide identity with zinderia strains from three North American *Clastoptera* spp. (McCutcheon and Moran, 2010; Koga *et al*., 2013). In contrast, *D.* (*D*) *mourei* samples yielded no sequence data (Bet937F/Zin1288R amplicons) or chromatograms showing multiple peaks (10F/Zin1288R), possibly due to non-specific primer binding and/or the spurious co-amplification of other bacterial DNA present in the samples.

The microscopic visualization of a single bacterial type (sulcia-like) and the lack of amplification with zinderia and sodalis primers, suggest that sulcia might be the sole

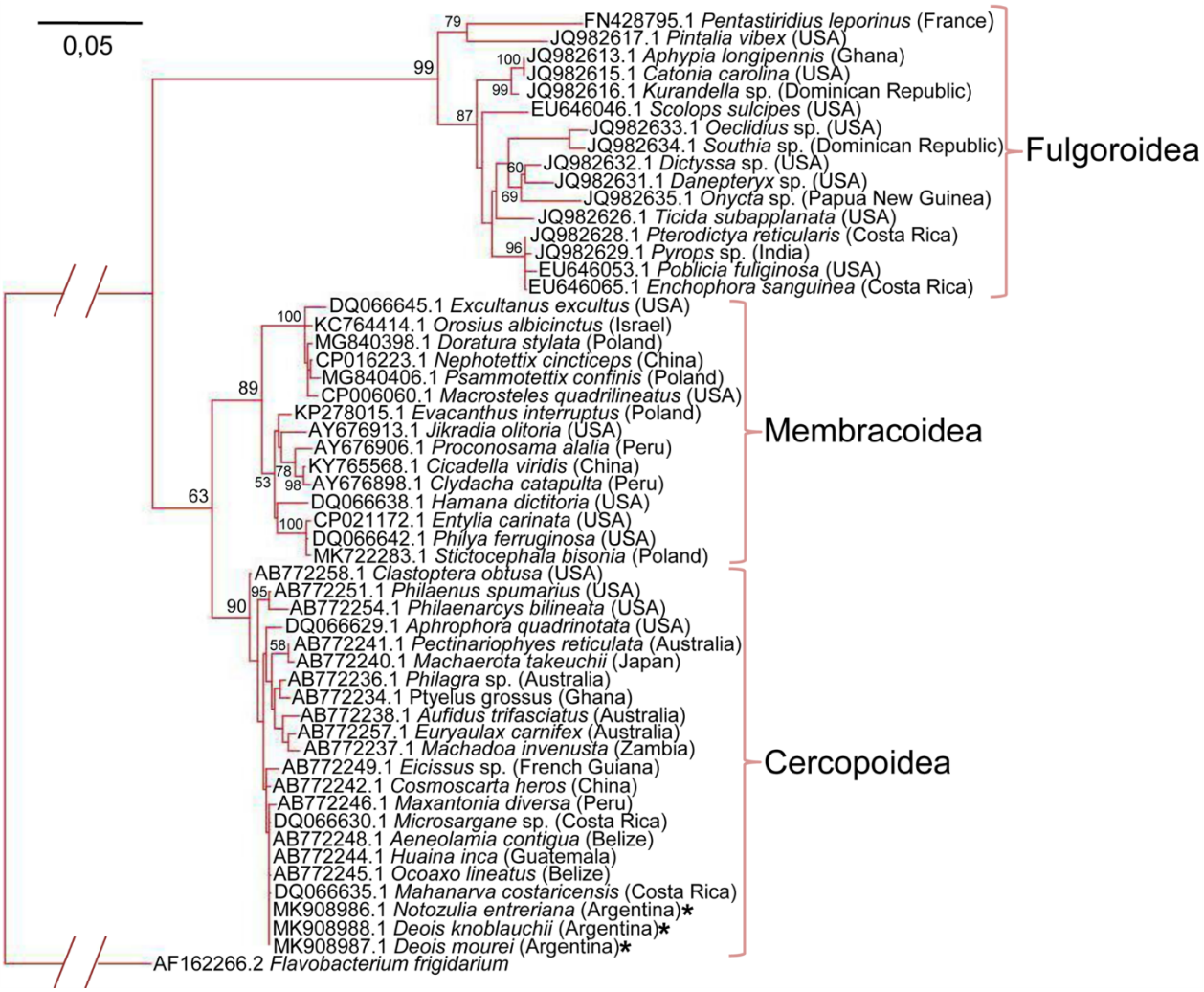

Figure 2. ML phylogenetic tree (100 bootstrap replicates) of partial 16S rRNA gene sequences of '*Candidatus* Sulcia muelleri' obtained in this work (highlighted by an asterisk) and previously reported in GenBank, with the Bacteroidetes *F. frigidarium* as outgroup. Accession numbers, host identifications and geographic origins are provided at the branch tips. Bootstrap percentages (>50%) are shown at nodes. The scale bar refers to the number of substitutions per site.

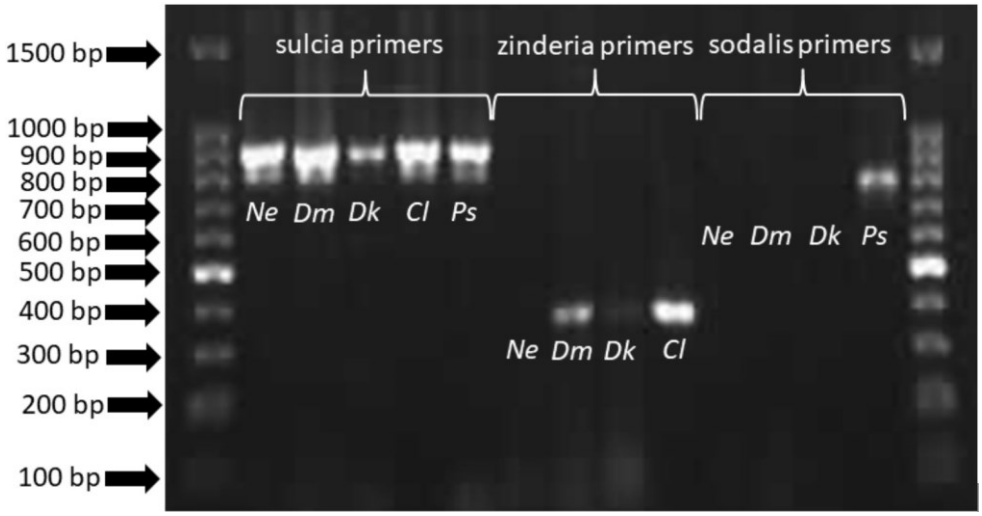

Figure 3. Agarose gel electrophoresis of PCR products using sulcia (SulciaJFw/SulciaJRv), zinderia (Bet937F/Zin1288R) and sodalis (SpSod506F/Sod1248R) specific primers. *Ne*: *Notozulia entreriana*, *Dm*: *Deois* (*Deois*) *mourei*, *Dk*: *Deois* (*Deois*) *knoblauchii*, *Cl*: *Clastoptera* sp. (zinderia positive control); *Ps*: *Philaenus spumarius* (sodalis positive control).

obligate bacterial symbiont of *N. entreriana*, as it happens in the closely related *A. contigua* (Koga *et al*., 2013). Regarding *Deois* spp., further research is needed to clarify the possible occurrence of additional bacteria and the nature of these associations.

Conclusions

This study reports for the first time the presence of the primary endosymbiont '*Ca*. Sulcia muelleri' in the bacteriomes of the Neotropical spittlebugs *N. entreriana*, *D.* (*D*) *mourei* and *D.* (*D*) *knoblauchii*. The complete nucleotide similarity among the sulcia partial 16S rDNA sequences obtained from these and other cercopids of the subfamily Ishnorhininae may be the result of host-symbiont coevolution. Morover, phylogenetic analysis of new and previously reported sulcia partial 16S rDNA sequences obtained from Auchenorrhyncha grouped them according to host superfamily. Longer sulcia sequence data, and information on the molecular phylogeny of their insect hosts, should shed more light on the evolutionary history of these associations in South-American (and world) spittlebugs. Finally, preliminary results suggesting the putative occurrence of bacteria other than sulcia in *Deois* spp. prompt further research on their associated microbiota.

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References

ARNEODO J. D., BRESSAN A., LHERMINIER J., MICHEL J., BOUDON-PADIEU E., 2008.- Ultrastructural detection of an unusual intranuclear bacterium in *Pentastiridius leporinus* (Hemiptera: Cixiidae).- *Journal of Invertebrate Pathology*, 97: 310-313.

BENNETT G. M., MAO M., 2018.- Comparative genomics of a quadripartite symbiosis in a planthopper host reveals the origins and rearranged nutritional responsibilities of anciently diverged bacterial lineages.- *Environmental Microbiology*, 20: 4461-4472.

BENNETT G. M., MORAN N. A., 2013.- Small, smaller, smallest: the origins and evolution of ancient dual symbioses in a Phloem-feeding insect.- *Genome Biology and Evolution*, 5: 1675-1688.

BENNETT G. M., ABBA S., KUBE M., MARZACHI C., 2016.- Complete genome sequences of the obligate symbionts '*Candidatus* Sulcia muelleri' and '*Ca*. Nasuia deltocephalinicola' from the pestiferous leafhopper *Macrosteles quadripunctulatus* (Hemiptera: Cicadellidae).- *Genome Announcements*, 4 (1): e01604-15.

BRENTASSI M. E., FRANCO E., BALATTI P., MEDINA R., BERNABEI F., MARINO DE REMES LENICOV A. M., 2017.- Bacteriomes of the corn leafhopper, *Dalbulus maidis* (DeLong et Wolcott, 1923) (Insecta, Hemiptera, Cicadellidae: Deltocephalinae) harbor *Sulcia* symbiont: molecular characterization, ultrastructure, and transovarial transmission.- *Protoplasma*, 254: 1421-1429.

BRESSAN A., ARNEODO J., SIMONATO M., HAINES W. P., BOUDON-PADIEU E., 2009.- Characterization and evolution of two bacteriome-inhabiting symbionts in Cixiid planthoppers (Hemiptera: Fulgoromorpha: Pentastirini).- *Environmental Microbiology*, 11: 3265-3279.

DOYLE J. J., DOYLE J. L., 1987.- A rapid DNA isolation procedure for small quantities of fresh leaf tissue.- *Phytochemical Bulletin*, 19: 11-15.

FOIERI A., PEDRINI N., TOLEDO A., 2018.- Natural occurrence of the entomopathogenic genus *Pandora* on spittlebug pests of crops and pastures in Argentina.- *Journal of Applied Entomology*, 142: 363-370.

GUINDON S., DUFAYARD J. F., LEFORT V., ANISIMOVA M., HORDIJK W., GASCUEL O., 2010.- New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0.- *Systematic Biology*, 59: 307-321.

HOLMANN F., PECK D. C., 2002.- Economic damage caused by spittlebugs (Homoptera: Cercopidae) in Colombia: a first approximation of impact on animal production in *Brachiaria decumbens* pastures.- *Neotropical Entomology*, 31: 275-284.

ISHII Y., MATSUURA Y., KAKIZAWA S., NIKOH N., FUKATSU T., 2013.- Diversity of bacterial endosymbionts associated with *Macrosteles* leafhoppers vectoring phytopathogenic phytoplasmas.- *Applied and Environmental Microbiology*, 79: 5013-5022.

KOGA R., MORAN N. A., 2014.- Swapping symbionts in spittlebugs: Evolutionary replacement of a reduced genome symbiont.- *ISME Journal*, 8: 1237-1246.

KOGA R., BENNETT G. M., CRYAN J. R., MORAN N. A., 2013.- Evolutionary replacement of obligate symbionts in an ancient and diverse insect lineage.- *Environmental Microbiology*, 15: 2073-2081.

KOBIAŁKA M., MICHALIK A., SZKLARZEWICZ T, 2018a.- An unusual symbiotic system in *Elymana kozhevnikovi* (Zachvatkin, 1938) and *Elymana sulphurella* (Zetterstedt, 1828) (Insecta, Hemiptera, Cicadellidae: Deltocephalinae).- *Folia Biologica*, 66: 13-24.

KOBIAŁKA M., MICHALIK A., SZWEDO J., SZKLARZEWICZ T., 2018b.- Diversity of symbiotic microbiota in Deltocephalinae leafhoppers (Insecta, Hemiptera, Cicadellidae).- *Arthropod Structure and Development*, 47: 268-78.

KOBIAŁKA M., MICHALIK A., WALCZAK M., SZKLARZEWICZ T., 2018c.- Dual "Bacterial-fungal" symbiosis in Deltocephalinae leafhoppers (Insecta, Hemiptera, Cicadomorpha: Cicadellidae).- *Microbial Ecology*, 75: 771-782.

LEITE L. G., MACHADO-LAERETE A., GOULART R. M., TAVARES F. M., FILHO B. A., 2005.- Screening of *Heterorhabditis* sp. against the sugarcane root spittlebug *Mahanarva fimbriolata* (Fabr.) (Hemiptera: Cercopidae).- *Neotropical Entomology*, 34: 785-790.

MAO M., YANG X., POFF K., BENNETT G., 2017.- Comparative genomics of the dual-obligate symbionts from the treehopper, *Entylia carinata* (Hemiptera: Membracidae), provide insight into the origins and evolution of an ancient symbiosis.- *Genome Biology and Evolution*, 9: 1803-1815.

MARINO DE REMES LENICOV A. M., PARADELL S., VIRLA E., 2004.- Homoptera, Auchenorrhyncha, pp. 330-342. In: *Catálogo de los insectos fitófagos de la Argentina y sus plantas asociadas* (CORDO H., LOGARZO G., BRAUN K., DIORIO O., Eds).- SEA ediciones, Buenos Aires, Argentina.

MCCUTCHEON J. P., MORAN N. A., 2007.- Parallel genomic evolution and metabolic interdependence in an ancient symbiosis.- *Proceedings of the National Academy of Sciences USA*, 104: 19392-19397.

MCCUTCHEON J. P., MORAN N. A., 2010.- Functional convergence in reduced genomes of bacterial symbionts spanning 200 My of evolution.- *Genome Biology and Evolution*, 2: 708-718.

MORAN N. A., TRAN P., GERARDO N. M., 2005.- Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes.- *Applied and Environmental Microbiology*, 71: 8802-8810.

PALADINI A., TAKIYA D. M., CAVICHIOLI R. R., CARVALHO G. S., 2015.- Phylogeny and biogeography of Neotropical spittlebugs (Hemiptera: Cercopidae: Ishnorhininae): revised tribal classification based on morphological data.- *Systematic Entomology*, 40: 82-108.

PALADINI A., DOMAHOVSKI A. C., KRINSKI D., FOERSTER L. A., 2018.- Reports of new wing color polymorphism and taxonomic information to cercopids (Auchenorrhyncha: Cercopidae) from upland rice crop, Pará state, Brazil.- *Brazilian Journal of Biology*, 78: 728-735.

PANZAVOLTA T., BRACALINI M., CROCI F., GHELARDINI L., LUTI S., CAMPIGLI S., GOTI E., MARCHI R., TIBERI R, MARCHI G, 2019.- *Philaenus italosignus* a potential vector of *Xylella fastidiosa*: occurrence of the spittlebug on olive trees in Tuscany (Italy).- *Bulletin of Insectology*, 72: 317-320.

RINGENBERG R., SPOTTI LOPES J. R., MÜLLER C., SAMPAIO DE AZEVEDO-FILHO W., AGUIAR JORDÃO PARANHOS B., BOTTON M., 2014.- Survey of potential sharpshooter and spittlebug vectors of *Xylella fastidiosa* to grapevines at the São Francisco River Valley, Brazil.- *Revista Brasileira de Entomologia*, 58: 212-218.

SAPONARI M., LOCONSOLE G., CORNARA D., YOKOMI R. K., DE STRADIS A., BOSCIA D., PORCELLI F., 2014.- Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy.- *Journal of Economic Entomology*, 107: 1-4.

SZKLARZEWICZ T., GRZYWACZ B., SZWEDO J., MICHALIK A., 2016.- Bacterial symbionts of the leafhopper *Evacanthus interruptus* (Linnaeus, 1758) (Insecta, Hemiptera, Cicadellidae: Evacanthinae).- *Protoplasma*, 253: 379-391.

TAKIYA D. M., TRAN P. L., DIETRICH C. H., MORAN N. A., 2006.- Co-cladogenesis spanning three phyla: leafhoppers (Insecta: Hemiptera: Cicadellidae) and their dual bacterial symbionts.- *Molecular Ecology*, 15: 4175-4191.

THEODOROU D., KOUFAKIS I., THANOU Z., KALAITZAKI A., CHALDEOU E., AFENTOULIS D., TSAGKARAKIS A., 2021.- Management system affects the occurrence, diversity and seasonal fluctuation of Auchenorrhyncha, potential vectors of *Xylella fastidiosa*, in the olive agroecosystem.- *Bulletin of Insectology*, 74: 27-40.

THOMPSON V., 2004.- Associative nitrogen fixation, C4 photosynthesis, and the evolution of spittlebugs (Hemiptera: Cercopidae) as major pest of neotropical sugarcane and forage grasses.- *Bulletin of Entomological Research*, 94: 189-200.

URBAN J. M., CRYAN J. R., 2012.- Two ancient bacterial endosymbionts have coevolved with the planthoppers (Insecta: Hemiptera: Fulgoroidea).- *BMC Evolutionary Biology*, 12 (1): 87.

Authors' addresses: Joel D. ARNEODO (corresponding author: arneodo.joel@inta.gob.ar), Alvaro FOIERI, Cecilia DECKER-FRANCO, Instituto de Microbiología y Zoología Agrícola (IMyZA) - Instituto Nacional de Tecnología Agropecuaria (INTA), Nicolás Repetto y de los Reseros, 1686 Hurlingham, Argentina; Ana M. MARINO DE REMES LENICOV, División Entomología, Facultad de Ciencias Naturales y Museo (FCNyM), Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900 La Plata, Argentina.

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