



Complete Mitochondrial Genomes and Bacterial Metagenomic Data From Two Species of Parasitic Avian Nasal-Mites (Rhinonyssidae: Mesostigmata)

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Keywords: gene order, genome assembly, genome-resolved metagenomics, mitochondria, parasites

OPEN ACCESS

Edited by:

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Specialty section:

This article was submitted to
Phylogenetics, Phylogenomics, and
Systematics,
a section of the journal
Frontiers in Ecology and Evolution

Received: 23 October 2019

Accepted: 24 April 2020

Published: 19 May 2020

Citation:

Osuna-Mascaró C, Doña J,
Johnson KP, Esteban R and de
Rojas M (2020) Complete

Mitochondrial Genomes and Bacterial
Metagenomic Data From Two Species
of Parasitic Avian Nasal-Mites
(Rhinonyssidae: Mesostigmata).

Front. Ecol. Evol. 8:142.
doi: 10.3389/fevo.2020.00142

INTRODUCTION

Rhinonyssidae (Mesostigmata) is a family of mites with more than 500 described species worldwide (George, 1961; Fain, 1994; Dimov and de Rojas, 2012). Most of these mites are hematophagous endoparasites that inhabit the nasal cavity of passerine birds (Vitzthum, 1935). Specifically, their feeding activity may damage the nasal cavities of birds and may even lead to the death of the hosts (Rhinonyssidosis avium disease) (Dimov, 2011). Also, though yet not investigated, these mites are expected to be reservoirs or vectors of infections such as West Nile fever, Q fever, avian influenza, and Lyme disease (Dimov, 2012; Arabkhazaeli et al., 2016).

However, mainly due to sampling difficulties (e.g., these mites usually can only be collected from dead birds), this host-parasite system is very poorly understood. Almost all species of birds are inhabited by rhinonyssid mites (Walter and Proctor, 2013). Despite their broad distribution, there is almost no genomic information available for mites of this family (De Rojas et al., 2001, 2002; Dimov and de Rojas, 2012), thus hampering studies on host-parasite system.

In this data report, with the aim of obtaining mitochondrial and bacterial information, we performed shotgun whole genome sequencing of two species of Rhinonyssidae (*Tinaminyssus melloi* and *Ptilonyssus chloris*) that inhabit the nasal cavity of *Columba livia* and *Chloris chloris*, respectively. For both species, we assembled and functionally annotated their mitochondrial genomes. We also carried out a metagenomic analysis (metagenomic classification and genome-resolved metagenomics) of these two mite species to provide bacterial information for these mite taxa for the first time (though note that this study should not be taken as a pathogen survey). Our study presents highly valuable mitogenomic and metagenomic resources for this host-parasite system, with implications for further eco-evolutionary studies.

SAMPLING, DNA EXTRACTION, AND SEQUENCING

We collected individual mites of *Tinaminyssus melloi* and *Ptilonyssus chloris* from freshly dead host individuals by dissecting the nasal cavities of birds under a stereomicroscope. We conserved the mites at -20°C in tubes with 100% ethanol. Then, mites were washed in ethanol (Andrews, 2013) and shipped to AllGenetics & Biology SL for DNA isolation, amplification, and library preparation. Given the low amount of DNA obtained from DNA extractions of single individuals,

individual mites of both species were pooled (6 mites for *Ptilonyssus chloris* and 10 mites for *Tinaminyssus melloi*) following previous studies on mites' high-throughput sequencing and mitogenome assembly (Esteban et al., 2018; Vizcaíno et al., 2018). The DNA was extracted from each pooled sample (i.e., not from individual mites) using the manufacturer's protocols of Quick-DNA MicroPrep Plus kit (Zymo). The libraries were constructed for each sample using the NextFlex DNA Sample Prep kit (Illumina) strictly following the manufacturer's protocols. The DNA was sheared and tagged at both 5' and 3' ends, and samples were dual-indexed. The constructed libraries were quantified using the Qubit dsDNA HT Assay kit (Thermo Scientific) and quality-checked in an Agilent 2100 Bioanalyzer (Agilent Technologies). Lastly, the libraries were pooled in equimolar amounts according to the Qubit results and sequenced in a fraction of an Illumina MiSeq 300PE (Novogen, China).

MITOCHONDRIAL GENOME ASSEMBLY AND ANNOTATION

We assembled the mitochondrial genomes using the MitoZ v2.4 pipeline (Meng et al., 2019). We used the "all" module with default parameters. In brief, MitoZ first filters out raw reads as follows: reads with many ambiguities (>10 N's), low-quality reads ($Q \leq 17$), and PCR duplicates. Then, MitoZ *de-novo* assembles the mitogenome based on a modified version of SOAPdenovo-Trans. We used the quick mode assembly (default), and if any gene was missing after the assembly (see results section "Mitochondrial genome assembly and annotation" of *Ptilonyssus chloris*), we used the multi-kmer mode for recovering the missing genes that failed in the previous step, as recommended by the developers. Finally, MitoZ filters out the candidate mitogenome sequences using a confidence score and annotates the protein-coding genes, tRNA, and rRNA. Additionally, we used the MITOS Web Server (Bernt et al., 2013) to compare the annotations and to ensure the correct annotation of all the genes. Then, the mitogenomes were plotted with OGDRAW v.1.3.1 (Greiner et al., 2019). Lastly, we evaluated the potential incidence of index swapping (i.e., a recently described sequencing artifact that results in 1 to 10% of reads misassigned to the wrong sample; Esling et al., 2015; Sinha et al., 2017; Owens et al., 2018). Specifically, we mapped all raw reads from *Tinaminyssus melloi* to the *Ptilonyssus chloris* mitochondrial genome assembly and, vice versa (i.e., all raw reads from *Ptilonyssus chloris* to the *Tinaminyssus melloi* mitochondrial genome assembly). For the read mapping analyses, we used Geneious R.11 mapper (Kearse et al., 2012) with high sensitivity and default parameters.

GENE REARRANGEMENT ANALYSES

We evaluated the mitochondrial gene order in these taxa, because mitochondrial genome rearrangements have been reported in some mesostigmatid mites species (Li et al., 2019). We used CREx (Bernt et al., 2007), to determine the gene arrangement in the two assembled mite mitochondrial genomes. In particular, we

constructed a gene order distance matrix using common intervals for *Tinaminyssus melloi* and *Ptilonyssus chloris* and compared both of them (1) with each other, (2) with the mitochondrial gene order of *Varroa destructor*, an obligate parasite of *Apis mellifera* (Navajas et al., 2002), and (3) with the established ancestral gene order of the arthropods (Staton et al., 1997).

METAGENOMIC ANALYSES: METAGENOMIC CLASSIFICATION AND GENOME-RESOLVED METAGENOMICS

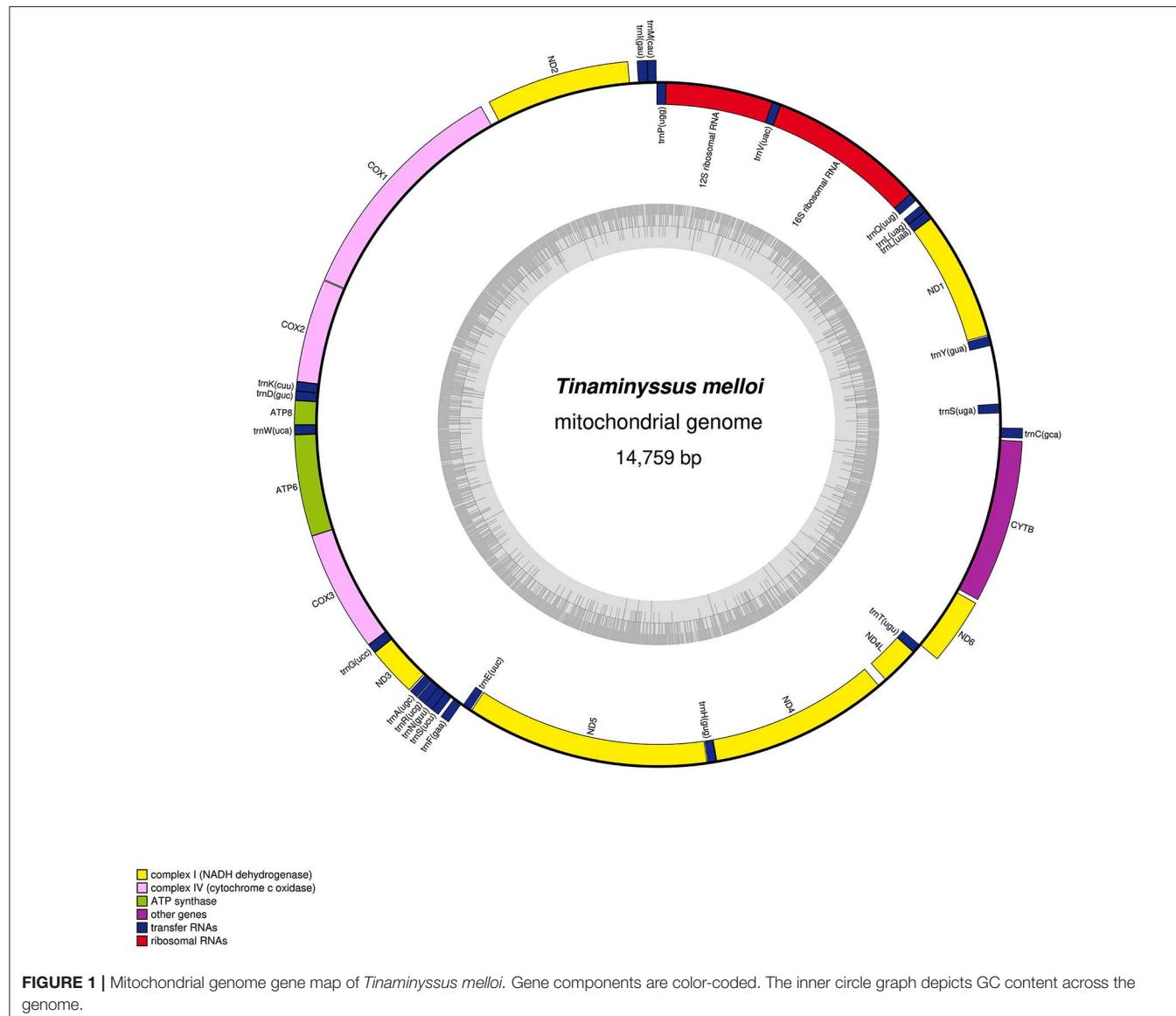
To characterize the overall taxonomic content of the metagenomes, we used the metagenomic classifier Kaiju (Menzel et al., 2016) with the following parameters: Reference database: nr +euk; Database date: 2017-05-16; SEG low complexity filter: yes; Run mode: greedy; Minimum match length: 11; Minimum match score: 75; Allowed mismatches: 5.

Then, we processed raw reads with the complete metaWRAP v1.1.5 pipeline (Uritskiy et al., 2018) using the recommended databases. These analyses were carried out on a 4 AMD Opteron with 16 2.4 GHz processors and 64 CPU cores and 504 GB RAM, maintained by the UIUC Life Sciences Computing Services (University of Illinois, Urbana, IL, USA). (1) We used the metaWRAP Read_qc module (default parameters) to quality-trim the reads of each sample. (2) Then, as recommended for assembling and binning low-abundance organisms, we co-assembled reads from the two samples with the metaWRAP Assembly module (-use-metaspades option) (Nurk et al., 2017). (3) After the assembly, we binned reads with the metaWRAP Binning module (-maxbin2 -concoct -metabat2 options). (4) We consolidated the resulting bins into a final bin set with both metaWRAP's Bin_refinement module (-c 1 -x 1 options) and the Reassemble_bins module. (5) We quantified the bins (i.e., resulting from the Bin_refinement module) with Salmon (Patro et al., 2017) using the Quant_bins module (default parameters). (6) Finally, we classified bins using the Classify_bins module. This module uses Taxator-tk, which gives highly accurate but conservative classifications (Dröge et al., 2014). Accordingly, we uploaded our final bacterial bin to MiGA for a complementary analysis to determine the most likely taxonomic classification and novelty rank of the bins (Rodríguez et al., 2018). We used the NCBI Genome database (Prokaryotes) for this analysis.

GENOME ORGANIZATION, STRUCTURE, AND COMPOSITION

For *Tinaminyssus melloi*, we obtained a total of 3,600,154 raw reads. The assembled mitogenome was circular and 14,759 bp in length (mean coverage: 99.3X). The base composition was 39.9% A, 18.9% C, 8.8% of G, 33.0% of T. The GC content was 27.7%. We recovered a total of 37 genes: 13 protein-coding genes (PCGs), 22 tRNA, and 2 rRNA (Figure 1).

For *Ptilonyssus chloris*, we obtained a total of 1,554,790 raw reads and assembled a complete circular mitogenome with 14,433 bp in length (mean coverage: 197X). The base composition was 40.4% of A, 6.1% of C, 10.1% of G, and 43.4% of T. The



GC content was 16.2%. Using the MitoZ annotation module, we recovered a total of 34 genes, containing 12 protein-coding genes (PCGs), 21 tRNA, and 1 rRNA. However, ND6, trnS, and the 1-rRNA genes were missing. These genes were still missing after using the multi-kmer mode recommended for recovering genes missed during the assembly. Nonetheless, using the MITOS annotation Web Server, we found 37 genes in the assembly, including the three genes missed by the MitoZ pipeline (Figure S2).

A very low incidence of index-swapping was found in both assemblies. In particular, only 10 out of 1,800,077 raw reads (<0.001%) mapped to the wrong sample in *Ptilonyssus chloris* analysis and 13 out of 777,395 raw reads (0.001%) did so in the *Tinaminyssus melloi* analysis. Therefore, index swapping should not have impacted our assemblies.

The mitochondrial gene order was the same for both mite species (**Figure S1**). We found that they did not retain the established ancestral mitochondrial organization of arthropods (Staton et al., 1997). In addition, the mitochondrial gene order was also different from that of *Varroa destructor* (Mesostigmata) (**Figure S1**). Specifically, compared to *Varroa*, these mitogenomes contain transpositions in *trnY*, *trnM*, *trnP*, and *rrnS*, and inversions in *trnC*, *trnY-trnQ-nad1*, *trnF-trnE*, and *trnT*. Mitochondrial genomes rearranged or highly rearranged in gene order have been previously described and studied in mesostigmatid mite species (Li et al., 2019). Interestingly, to our knowledge, the rhynonissid mitochondrial genome organization plan found here has not been found in other mesostigmatid families.

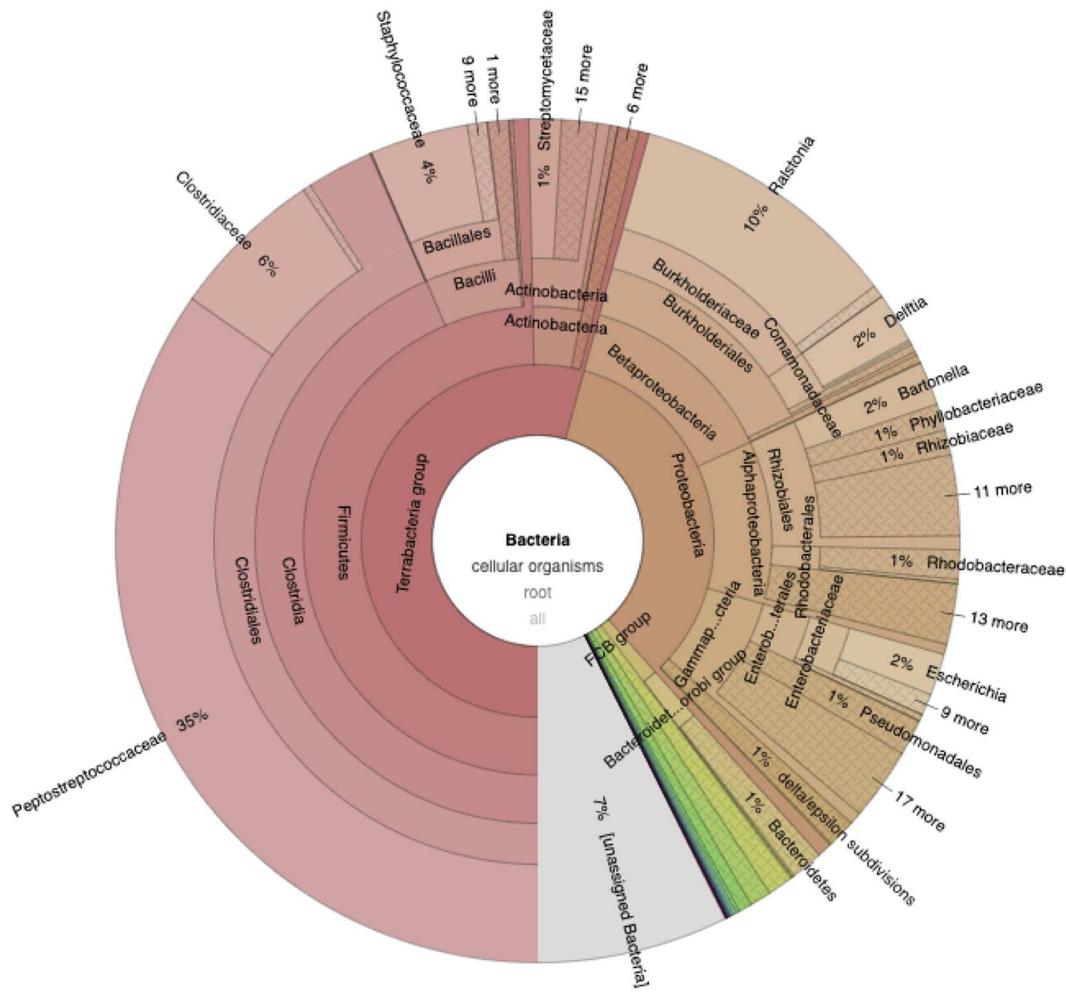


FIGURE 2 | Krona plot showing the relative distribution of Bacteria in *Tinaminyssus melloi*. Note that while this chart depicts Kaiju results, the taxonomic level displayed differs from that of **Tables S1, S2** (see full interactive krona plots for comparisons at the same taxonomic level; Links to the Deposited Data section).

METAGENOMIC COMPOSITION AND METAGENOME-ASSEMBLED GENOMES (MAGS)

For *Tinaminyssus melloi*, the top three bacterial species in terms of the number of assigned reads were (Figure 2; Tables S1, S2): (1) *Clostridioides difficile* [n (i.e., number of assigned reads) = 15,019], (2) *Ralstonia solanacearum* (n = 4,518), and (3) Kingdom Bacteria (n = 3,194). Note that some hits could not be identified at the species level. For *Ptilonyssus chloris*, the top three bacterial species were: (1) *Ralstonia solanacearum* (n = 1,609), (2) *Clostridioides difficile* (n = 1,341), and (3) *Delftia* (n = 990). Some of these bacteria may be pathogenic [e.g., *Clostridioides difficile*, which may cause diarrhea and mortality in humans, and also may cause clinical disease in livestock and birds (Lawson et al., 2016; Moono et al., 2016)] but also may have an environmental origin (Zhu et al., 2018). Further studies are needed on the potential role of rhinonyssid mites as reservoirs of pathogenic bacteria.

From the genome-resolved metagenomic approach, 1,517,456 and 3,549,318 high-quality reads were used after running the read_qc module of metaWRAP for *Tinaminyssus melloi* and *Ptilonyssus chloris*, respectively. We retrieved a single bacterial metagenome-assembled genome (MAG) from the co-assembly. According to CheckM, the MAG was 26% complete (Salmon contig coverage: 26X), with 0.46% contamination, GC = 0.32, N50 = 1,539, and the total length was 382,098 bp. The metaWRAP Classify_bins module classified the MAG as belonging to the family Bartonellaceae. A further taxonomic classification analysis in MiGA revealed highly congruent results. Specifically, the closest related species found was *Bartonella bacilliformis* KC583 (NC008783; 52.27% AAI, i.e., maximum average amino acid identity). In addition, the MAG was classified as possibly belonging to the family Bartonellaceae (p-value: 0.37). Interestingly, Bartonellaceae taxa have been found consistently associated with mites, though the particular role of these bacteria is still unknown (Kim et al., 2005; Hubert et al., 2017; Doña

et al., 2019). We also found the MAG most likely belongs to a species not represented in the MiGA database (*p*-value: 0.0015) and probably belongs to a genus not represented in the database (*p*-value: 0.08). Lastly, the MAG was more abundant in *Tinaminyssus melloi* (68.06 genome copies per million reads) than in *Ptilonyssus chloris* (1.75 genome copies per million reads), in which this very low abundance require further study to rule out methodological issues (e.g., index-swapping) (Esling et al., 2015; Sinha et al., 2017; Owens et al., 2018). Future studies are needed to fully assemble the genome of this bacterial taxon, ideally with higher coverage to ultimately uncover its potential role in these mites.

Discrepancies between the genome-resolved metagenomic and Kaiju results (e.g., in which a relatively small number of reads was classified as *Bartonella*) may reflect the already known differences between these two approaches (i.e., assembly-based and read-based analyses), in which many variables affect (e.g., database completeness; Quince et al., 2017). In addition, given that our coverage for the metagenomic assemblies was not very high, many bins belonging to taxa with a higher number of reads in Kaiju may have been discarded throughout the metaWRAP pipeline because of high levels of heterogeneity in the assembled bin/MAGs. Future metagenomic studies with higher coverage are encouraged to provide genomic resources of the whole community of microbial taxa associated with these mites.

Overall, the resources presented here contained useful information for future studies on rhinonyssid mites, a largely neglected group of parasites. In particular, the data provided here can be useful for phylogenomic studies, studies on mitochondrial genome evolution, studies of the microbiomes of parasitic mites, and for studies of disease dynamics.

LINKS TO THE DEPOSITED DATA

The mitogenome sequences of *Tinaminyssus melloi* and *Ptilonyssus chloris* are available at NCBI genome database with the BioProject number PRJNA575688. The high-throughput

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sequencing data of each sample is available at the Sequence Read Archive SRA under the accession numbers SRR10228588 and SRR10228589. The first draft mitochondrial genome sequences for both species with all genes annotated has been submitted to GenBank under the accessions numbers: MN557820 and MN557819. An interactive version of Krona plots can be found at: <https://figshare.com/s/8f6df0887c7ca918813a> (private link for review). The metagenome-assembled genome is available at NCBI (submission ID: SUB6386351).

DATA AVAILABILITY STATEMENT

The names of the repository/repositories and accession number(s) can be found above in the Links to the deposited data section.

AUTHOR CONTRIBUTIONS

CO-M, JD, and MR conceived this study. CO-M and JD analyzed the data. KJ supervised the research and provided logistical support. RE managed and uploaded data to specific repositories. CO-M wrote the first draft, which was revised by all other authors.

FUNDING

This work was supported by a V plan propio de investigación de la Universidad de Sevilla, Spain grant to MR, and the NSF DEB-1239788, DEB-1342604, and DEB-1855812 to KJ.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2020.00142/full#supplementary-material>

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Conflict of Interest: RE was employed by the company AllGenetics & Biology SL.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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