

# Mitochondrial genome characterization of *Melipona bicolor*, an endemic bee from the Brazilian Atlantic Rain Forest



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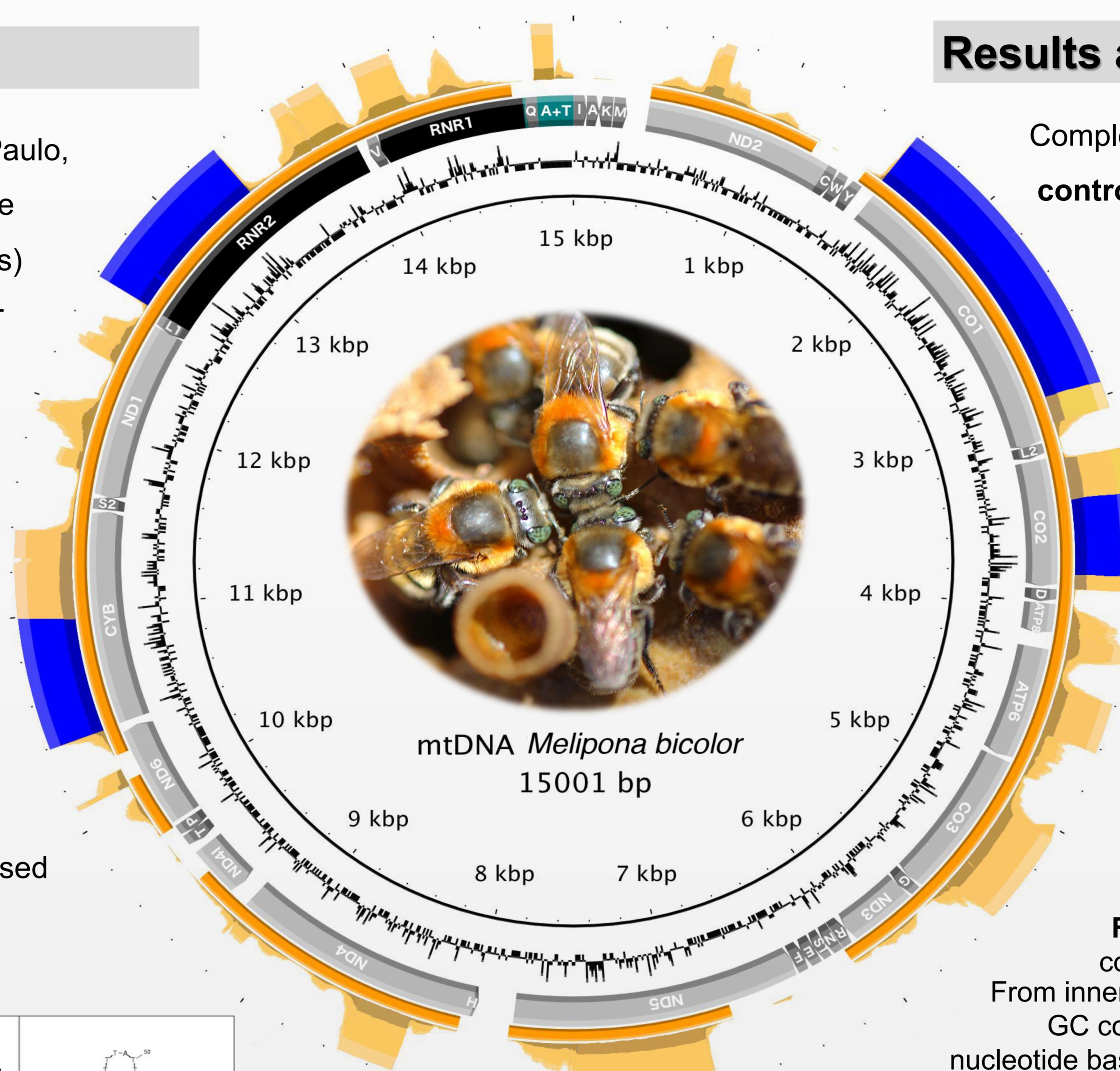
## Introduction

*Melipona bicolor* is a stingless bee, native from Atlantic Rain Forests in Brazil, known for being the only bee species in which true polygyny (i.e. multiple fertile queens in the same colony) occurs. Its mitochondrial genome (mt genome) was one of the first sequenced in bees, but as for many other genomes available, the sequence was incomplete and no information about its mitochondrial gene expression pattern was known. Due to its repetitive and polymorphic nature, the A+T rich region in insects is challenging to sequence and study. Therefore, the use of long read sequencing approaches may be very useful to provide insights about this genomic region. We combined long Pacbio and short Illumina reads of *M. bicolor* DNA with RNASeq data to obtain insights about mitochondrial evolution and gene expression in bees through the genomic characterization of this species control region and transcription pattern.

## Material and Methods

Bees were from the same colony kept at São Paulo, Brazil (23°33'S). Total DNA from two males; one sequenced with Pacbio® Sequel (for long reads) and one with Illumina® Hiseq4000 platform (for short reads), and RNASeq data from a pool of females workers, were used.

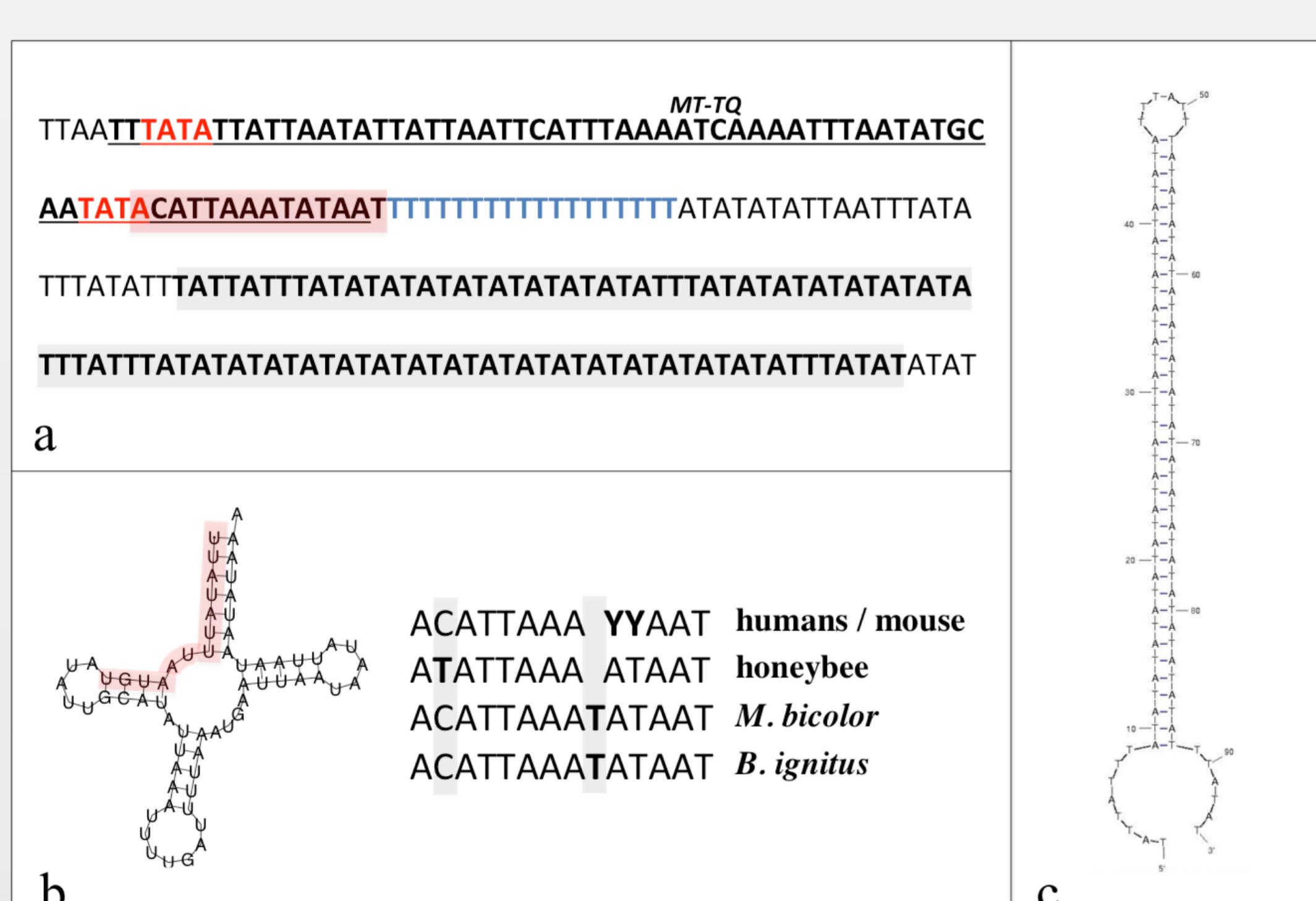
Genome assembly used previous incomplete mt genome as reference and combined assemblies from long (using **pbdagcon**) and short reads (using **MITObim 1.9**) with dotplot. To complete the A+T rich region, we manually circularized the molecule and afterwards linearized it at 1,000 nt before the A+T region start, in the 3' direction. Transcripts assembly used **HISAT 2.0.5** and **StringTie 1.2.2**.



## Results and Conclusions

Complete genome (Figure 1) has **15,001bp**, including a **control region of 255bp**, with the **highest AT content** reported so far for bees (98.1%). Interestingly, **conserved structures** were identified for the first time in the control region of all eusocial corbiculate bees sequenced so far (Figure 2). Gene expression control in *M. bicolor* is similar to other insect species however unusual patterns of **expression occur in the A+T rich region** (Figure 1) and at the mitochondrially encoded 12S rRNA (Figure 3), supporting the existence of **different isoforms** for this rRNA in bees. Results suggest *M. bicolor* is an interesting **model** to study mitochondrial genomic evolution.

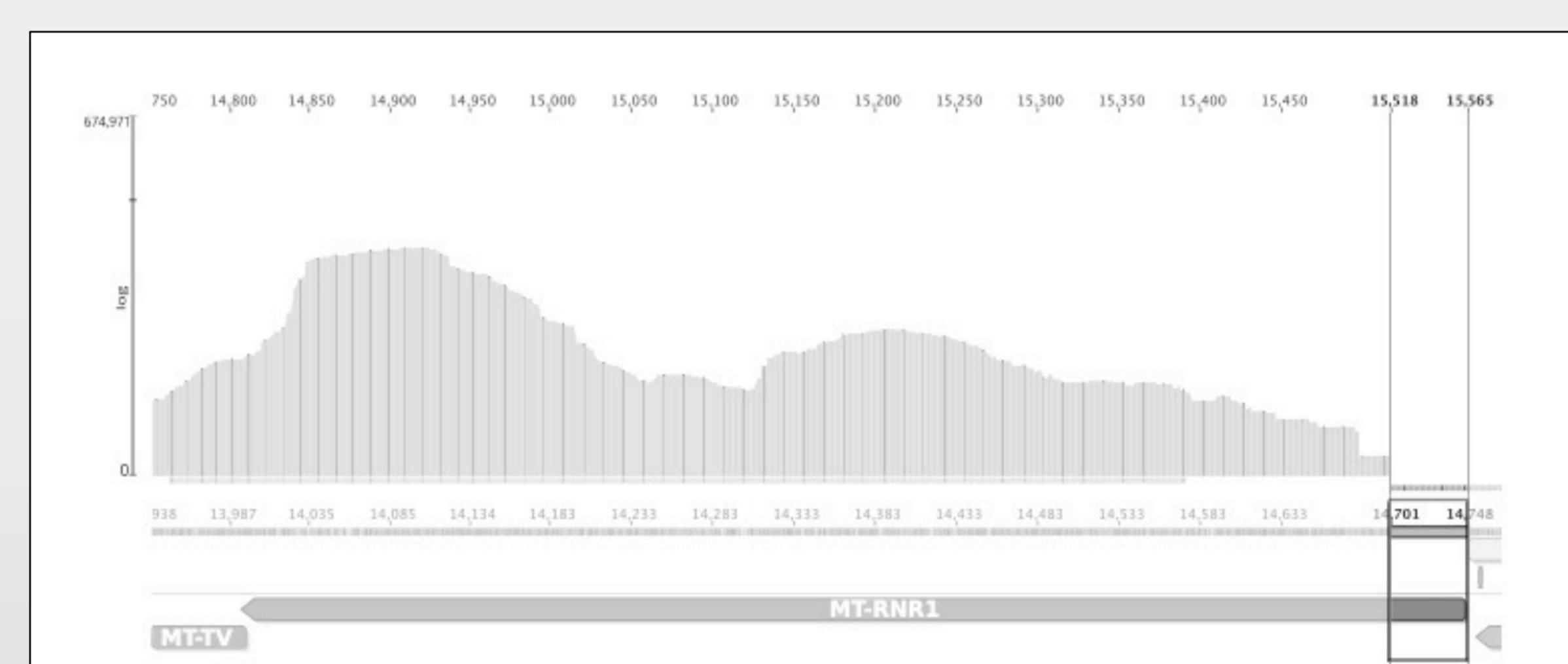
**Figure 1.** Schematic representation of *Melipona bicolor* complete mitochondrial genome sequence and features. From inner to outer circles: [1] Genomic scale representation; [2] GC content graph, outer peaks meaning guanine or cytosine nucleotide bases; [3] Genome annotation, blank spaces – intergenic regions, light grey – protein genes, dark grey – tRNA genes, black – rRNA genes and ciano – A+T control region; [4] Mitochondrial transcripts (orange bars); and [5] Expression coverage, in which blue areas represent coverage greater than 5,000 (y scale from 0 to 300 times coverage).



**Figure 2.** Main features of *Melipona bicolor* mitochondrial A+T rich region. **a** – The initial 213 bp of the A+T rich DNA sequence harboring the conserved regions as reported in *Apis mellifera*: [1] TATA box 1 and 2 – bases in red, [2] putative stop signal – shaded in red, [3] poliT stretch – bases in blue and [4] stem-loop – shaded in grey; and MT-TQ (tRNA Glutamine) imbed (underlined sequence); **b** – Comparisons of the putative stop codon sequence in different organisms (divergences are highlighted) and its position in *M. bicolor* MT-TQ structure (shaded in red); **c** – stem loop structure of *M. bicolor*, structure refers to the 94 bp region shaded in grey in **a**.

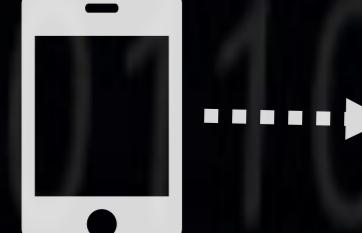
## Highlights

- Most compact mt genome reported in bees so far
- Conserved structures in the A+T rich control region can be found
- Gene expression may occur in the mt control region
- Possible 12s rRNA new isoform



**Figure 3.** Drop in coverage in the 5'portion of the mitochondrially encoded 12S RNA (MT- RNR1) in nurses of *M. bicolor*. Highlighted region of the MT-RNR1 has no coverage in RNASeq data. The arrow indicates 5' to 3' direction. Coverage is represented in log scale above the sequence annotation. Exact genomic position is numerically indicated above gene annotation.

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Acknowledgments:



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