Mitochondrial Genome Characterization of *Melipona bicolor*: Insights from the Control Region and Gene Expression Data

Natalia de Souza Araujo[1,2*] and Maria Cristina Arias[1]

[1] Universidade de São Paulo, Instituto de Biociências – Departamento de Genética e Biologia Evolutiva. Sala 320. Rua do Matão, 277 – Cidade Universitária – São Paulo – SP, Zip Code: 05508-900, Brazil.

[2] University of Liege, GIGA – Medical Genomics, Unit of Animal Genomics. Quartier Hopital, Avenue de I'Hopital, 11 – Liège, Zip Code: 4000, Belgium

* Corresponding authors: N.S. Araujo – na.araujo@usp.br

Mitochondrial Genome Characterization of *Melipona bicolor*: Insights from the Control Region and Gene Expression Data

Abstract: The stingless bee *Melipona bicolor* is the only bee in which true polygyny occurs. Its mitochondrial genome was first sequenced in 2008, but it was incomplete and no information about its transcription was known. We combined short and long reads of *M. bicolor* DNA with RNASeq data to obtain insights about mitochondrial evolution and gene expression in bees. The complete genome has 15,001bp, including a control region of 255bp that contains all conserved structures described in honeybees with the highest AT content reported so far for bees (98.1%). Displaying a compact and functional region. Gene expression control is similar to other insects however unusual patterns of expression suggest the existence of different isoforms for the 12S rRNA. Results reveal unique and shared features of the mitochondrial genome in terms of sequence evolution and gene expression making *M. bicolor* an interesting model to study mitochondrial genomic evolution.

Keywords: stingless bee, A+T rich region, Pacbio sequencing, NGS sequencing

1. Introduction

Bees are essential pollinators responsible directly or indirectly for about 75% of world's food production in commercial crops and also for most pollination in native plants (Ollerton et al. 2011; Potts et al. 2016). Specially in local ecosystems, native bee species, such as *Melipona bicolor*, play major roles in environmental maintenance (Garibaldi et al. 2014; Jaffé et al. 2016). However, these species are constantly threaten by a number of factors derived from human actions including habitat lost, inappropriate nest transportation practices and competition with introduced species (Jaffé et al. 2016; Dicks et al. 2016; Potts et al. 2016). The stingless bee *M. bicolor* is endemic of the Brazilian Atlantic Rain Forest (Silveira et al. 2002), and besides their ecological relevance in this endangered biome, this species is also

28 valuable for being the only bee species known to present true polygyny, a unique trait among
29 eussocial bees (Velthuis et al. 2001; Cepeda 2006).
30 The mitochondrial genome (mtDNA) sequence of *M. bicolor* was first published in
31 2008 however missing the control region sequence (Silvestre et al. 2008). By that time only
32 the honeybee mtDNA sequence was known, but since then many other bee species got their
33 mitochondrial genome published, including another Meliponini, *Melipona scutellaris*
34 (Silverio et al. 2014). While the gene sequences have been available for several species the
35 control region and other mitochondrial genomic features are usually lacking or not discussed.
36 The mtDNA control region of insects is also called A+T rich region due to its high content of
37 adenine (A) and thymine (T). For instance, in honeybees this region has up to 96% of A and T
38 (Gonçalves et al. 2015). The A+T rich region contains signals for the mtDNA transcription
39 and replication control and is organized in a series of T(n)A(n) motifs comprising a highly
40 polymorphic sequence, even within species (reviwed by Zhang and Hewitt 1997; Lessinger
41 and Azeredo-Espin 2000; Gonçalves et al. 2015).
42 Transcription data analyses of mitochondrial genes is also another frequently
43 overlooked feature in mitochondrial studies that may reveal important traits of mitochondrial
44 metabolism and evolution as discussed in (Smith 2016) and demonstrated in (Tian and Smith
45 2016; Gao et al. 2016; Gao et al. 2018). In the present study, *M. bicolor* DNA sequencing
46 from short and long reads where combined with RNASeq data to get insights about
47 mitochondrial evolution in bees through the mitochondrial genomic characterization of this
48 species control region and transcription pattern.

49 **2. Material and Methods**

50 **2.1. Sampling and sequencing**

51 Two males, collected from one *M. bicolor* monogyne colony, were individually used for a

phenol-chloroform total DNA extraction (Chomczynski and Sacchi 1987). One sample was sequenced in the Illumina® Hiseq4000 platform (Truseq 350pb library, 100pb paired-end reads) and another in the Pacbio® Sequel for long reads. Total RNA was obtained from three females workers, while nursing (collection time between 10 am and 12 pm), using the RNeasy Mini Kit (Qiagen®). RNA quality was measure using Agilent 2100 Bioanalyzer® and all three RNA samples were pooled for sequencing. Library was prepared using the TruSeq RNA Sample Preparation Kit and sequencing was performed in the Illumina® Hiseq2000, with 100bp paired-end reads. All samples were obtained from the same *M. bicolor* colony kept at São Paulo, Brazil (23°33'S). Library preparation and sequencing were performed by Macrogen (South Korea).

2.2. Genome assembly

First, we extended the mitochondrial genome using the previous published assembly (Silvestre et al. 2008) as reference for the alignment of the short reads with MITObim 1.9 (Hahn et al. 2013). To keep a maximum of 50 times coverage, as required by MITObim, cleaned reads were digitally normalized before assembly following the protocol from Brown et al. (2012) and paired reads were interleaved using khmer 2.0 (Crusoe et al. 2015). The resulting sequence was then used as reference to align the long reads using blasR 5.3.2 (Chaisson and Tesler 2012). From this alignment, a consensus sequence was obtained using pbdagcon (Chin et al. 2013). To improve the assembly of the A+T rich region, we manually circularized the molecule and afterwards linearizing it at 1,000 nt before the A+T region, in the 3' direction (end of the mitochondrially encoded 16S RNA). Then new assemblies of the short and long reads were performed, using this sequence as a reference. Resulting assemblies, independently obtained from short and long reads, were aligned and compared through their dotplot graph using Geneious 9.1.6 (https://www.geneious.com), which allowed genome circularization. The ultimate confirmation of assembly continuity was verified

77 through the alignment of short reads to the A+T rich region and adjacent areas using Bowtie2
78 (Langmead and Salzberg 2012) end to end alignment mode. Potential secondary structures in
79 the A+T rich region were detected using the MFOLD program (Zuker 2003) and MITOS was
80 used for genome annotation (Bernt et al. 2013). Annotation of gene start and end positions
81 were manually curated to match methionine (start), stop codons (end) and transcription data,
82 when possible.

83 **2.3. Transcripts assembly**

84 Mitochondrial transcripts were obtained performing a reference assembly using the generated
85 mitochondrial genome with HISAT 2.0.5 (Kim et al. 2015) for paired read alignment and
86 StringTie 1.2.2 (Pertea et al. 2015) for transcript assembly.
87

88 **3. Results**

89 **3.1. Completion of the mitochondrial genome sequence**

90 The final genome assembly of *M. bicolor* (Figure 1) is 15,001bp long with 13 coding genes, 2
91 rRNAs, 22 tRNAs and a control region of 255bp [Gene bank accession number – to be
92 included]. Gene order is identical to the previous published assembly (Silvestre et al. 2008).
93 However, in this latter the A+T rich region, the final portion of the mitochondrially encoded
94 12S RNA (MT-RNR1) and two tRNAs genes (MT-TS1 and MT-TQ) are missing. As
95 previously stated in (Silverio et al. 2014), the MT-TS1 (tRNA *Serine 1*) was indeed already
96 present in the previous assembly of *M. bicolor* mtDNA, but it had not been annotated. Its
97 position is the same as found in *M. scutellaris* mtDNA (Silverio et al. 2014). The other
98 previously missing tRNA gene, MT-TQ (Glutamine), is located after the MT-RNR1. This
99 tRNA gene was also not annotated in *M. scutellaris* mtDNA sequenced portion (Silverio et al.
100 2014). Nonetheless its location in *M. bicolor* is the same as found in *Bombus ignitus*

mitochondrial genome (Cha et al. 2007).

[Insert Figure 1 around here]

3.2. Control region

The base composition of *M. bicolor* control region (255 bp long) is A – 42.0%; T – 56.1%; C – 1.6%; and G – 0.4%, i.e. 98,1% AT content. All conserved structures reported in the A+T rich region of the honeybee mtDNA (Gonçalves et al. 2015) could also be found in *M. bicolor* (Figure 2): **[1]** TATA box 1 and 2; **[2]** putative stop signal; **[3]** T(n), or poliT stretch; and **[4]** stem-loop. These structures are conserved among different species and putatively of functional relevance for the control region. Nevertheless, in *M. bicolor* and *B. ignitus* these structures can only be identified if the last tRNA (MT-TQ) is considered also as part integrant of the A+T rich region (Figure 2a). This suggests that this tRNA has a dual function in the mtDNA of these bees, being additionally a functional part of the A+T rich region. Differently from what is observed in the honeybee that likewise differs from these species by lacking a thymine in the putative stop signal (Figure 2b).

[Insert Figure 2 around here]

3.3. Transcripts assembly and analyses

From all reads generated by RNASeq for *M. bicolor* nurses, 16.03% aligned to the mtDNA. RNASeq coverage and transcript assembly of the aligned reads (Figure 1) indicate that the expression control in this genome follows the tRNA punctuation model predictions (Ojala et al. 1981), with transcripts fragmentation and coverage reduction in tRNAs areas. Highest coverage regions, or regions with higher levels of gene expression, are the MT-CO1, MT-CO2, MT-CYB and MT-RNR2 (coverage > 5,000 times). A marked drop in coverage can be observed in the 5' region of the MT-RNR1 (Figure 3). Transcription data may also be useful

to correct start/ end position of genes (Stewart and Beckenbach 2009), in *M. bicolor* mtDNA we used this information to correct the annotation of methionine start sites for the genes MT-ND5 and MT-ND2. These corrections reduced the non-coding space between MT-TM and MT-ND2 (from 173bp to 105bp), but increased the non-coding region size between MT-TH and MT-ND5 (from 0bp to 189bp).

[Insert Figure 3 around here]

4. Discussion

Due to its repetitive and polymorphic nature, the A+T rich region in insects is challenging to sequence and study (Zhang and Hewitt 1997; Lessinger and Azeredo-Espin 2000; Gonçalves et al. 2015). Therefore the use of long read sequencing approaches may be very useful to provide insights about this genomic region (Gao et al. 2016; Gao et al. 2018). In bees, much of the information obtained about the A+T region comes from *Apis mellifera*, in which this region is known to be a mosaic, presenting conversed sequences embedded within polymorphic areas (Gonçalves et al. 2015). Herein, combining long and short reads, we were able to complete the genome sequencing of *M. bicolor* obtaining the control region sequence, which for the first time allowed comparisons of the A+T region among eussocial corbiculate bees. We verified that among the three eussocial lineages there is a great difference in A+T rich region size, in *Apis mellifera* it ranges from 897bp to 984bp (Gonçalves et al. 2015), in *Bombus ignitus* reference it is 859bp long (Cha et al. 2007) and in *Melipona bicolor* it has 255bp, the shortest so far reported for bees. Previous estimations of mitochondrial genomic size in *M. bicolor* by RFLP suggested a much larger A+T rich region of around 3,300 bp (Weinlich et al. 2004; Silvestre et al. 2008). Observed size differences are most likely due to the use of distinct technical approaches.

Despite of the size variation found among different bee lineages, conserved structures

148 previously observed in the honeybee mtDNA are also present in the other two eussocial
149 corbiculate bees (Figure 2). Evidencing the closer phylogenetic relation between Bombini and
150 Meliponini, in these two tribes the putative TATA box 1 and 2 and the stop signal commonly
151 overlaps the MT-TQ sequence, indicating that this tRNA is a functional part of the control
152 region in these bees. Conversely, in honeybee this transfer RNA is located in a tRNA cluster
153 on the opposite side of the A+T rich region. Similarly, the Bombini and Meliponini stop
154 signals are identical and differ in two nucleotides from the Apini sequence (Figure 2b).
155 Interestingly, the A+T content in the control region of *M. bicolor* is the highest among these
156 bees (98% against ≅ 96% in *Apis* and *Bombus*). All together these results show that the
157 control region in *M. bicolor* comprises all the standard functional elements but in a more
158 compact arrangement in comparison to the other bee species, a trend already observed for the
159 entire mitochondrial genome of this bee as discussed in (Silvestre et al. 2008). Moreover, the
160 identification of these conserved structures in the control region of bees may be useful for
161 population and phylogenetic studies (Zhang and Hewitt 1997).
162 Transcriptome analyses also revealed some relevant aspects of mitochondrial
163 biogenesis in this stingless bee. First, the tRNA punctuation model (Ojala et al. 1981) in RNA
164 processing is corroborated by the observation of at least six polycistronic transcripts that
165 received poly adenilation tails (known because library preparation of the transcripts relied on
166 poly adenilation selection). Second, the high expression of MT-CO1, MT-CO2, MT-CYB and
167 MT-RNR2 genes suggest that these regions are either expressed or processed differently from
168 other genes in *M. bicolor*, at least during the nursing life stage. These findings have been
169 observed in different organisms (Torres et al. 2009; Neira-Oviedo et al. 2011; Gao et al.
170 2016), but the number of polycistronic transcripts generated and the regions with different
171 levels of expression observed differ among species, highlighting the functional relevance of

mitochondrial expression control in distinct lineages (Taanman 1999; Stewart and Beckenbach 2009; Tian and Smith 2016; Gao et al. 2016; Gao et al. 2018).

More unusual patterns of gene expression were observed in the A+T rich region (poliT stretch and stem loop regions) and in the MT-RNR1 (5' portion). Low levels of gene expression in the mtDNA control region was associated in mammals to initiation of transcription or replication (Taanman 1999) and to the existence of long non coding RNAs in the mitochondria (Gao et al. 2018). In the present study some RNA-Seq reads similarly aligned to the control region of *M. bicolor* (Figure 1), indicating a functionally relevant expression pattern in this area. However, it is also possible that reads aligned inaccurately to these regions due to its repetitive profile. Regarding the mitochondrially encoded MT-RNR1, the sudden drop in expression observed in its 5' portion (Figure 3) may suggest the presence of an alternative isoform for this gene. The existence of alternative forms for this rRNA was first proposed based on transcription data from long reads of *Erthesina fullo* (Gao et al. 2016), but differently from what was observed in this insect, in *M. bicolor* MT-RNR1 change in transcription is observed in the 5' and not in the 3' region.

Present findings, demonstrated unique and shared features of the mitochondrial genome in terms of sequence evolution and gene expression control. More specifically in *M. bicolor* the mtDNA as a whole, but specially the control region, is very compact when compared to other bee species but still maintain functional relevant structures. Which makes this bee an interesting model to study mitochondrial genomic evolution. Additionally, gene expression control in *M. bicolor* resembles patterns observed in other insect species and supports the existence of gene expression in the A+T rich region and of different isoforms for the mitochondrially encoded 12S RNA in bees.

5. Acknowledgments

Authors would like to thank Dr. Isabel Alves-dos-Santos, Dr. Sheina Koffler and Dr. Sergio

Dias Hilário from the Laboratório de Abelhas (University of Sao Paulo) for the support during *M. bicolor* sampling and to Susy Coelho for technical assistance.

6. Funding details

This study was financed by FAPESP - São Paulo Research Foundation [Processes number 2013/12530-4 and 2016/24669-5]; CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil [Finance Code 001]; and CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico [research sponsorship to MCA, Process number 306932/2016-4].

7. Declaration of interest

Authors declare to have no conflict of interests.

8. References

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69:313–319.

Brown CT, Howe A, Zhang Q, Pyrkosz AB, Brom TH. 2012. A reference-free algorithm for computational normalization of shotgun sequencing data. Genome Announc. 2:1–18.

Cepeda OI. 2006. Division of labor during brood production in stingless bees with special reference to individual participation. Apidologie. 37:175–190.

Cha SY, Yoon HJ, Lee EM, Yoon MH, Hwang JS, Jin BR, Han YS, Kim I. 2007. The complete nucleotide sequence and gene organization of the mitochondrial genome of the bumblebee, *Bombus ignitus* (Hymenoptera: Apidae). Gene. 392:206–220.

Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using basic local alignment with successive refinement (BLASR): application and theory. BMC Bioinformatics. 13:238.

Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, et al. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods. 10:563–569.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 162:156–159.

Crusoe MR, Alameldin HF, Awad S, Boucher E, Caldwell A, Cartwright R, Charbonneau A, Constantinides B, Edvenson G, Fay S, et al. 2015. The khmer software package: enabling efficient nucleotide sequence analysis. F1000Research. 4:900.

Dicks L V., Viana B, Bommarco R, Brosi B, Arizmendi M del C, Cunningham SA, Galetto L, Hill R, Lopes A V., Pires C, et al. 2016. Ten policies for pollinators. Science. 354:975–976.

Gao S, Ren Y, Sun Y, Wu Z, Ruan J, He B, Zhang T, Yu X, Tian X, Bu W. 2016. PacBio full-length transcriptome profiling of insect mitochondrial gene expression. RNA Biol. 13:820–825.

Gao S, Tian X, Chang H, Sun Y, Wu Z, Cheng Z, Dong P, Zhao Q, Ruan J, Bu W. 2018. Two novel lncRNAs discovered in human mitochondrial DNA using PacBio full-length transcriptome data. Mitochondrion. 38:41–47.

Garibaldi LA, Carvalheiro LG, Leonhardt SD, Aizen MA, Blaauw BR, Isaacs R, Kuhlmann M, Kleijn D, Klein AM, Kremen C, et al. 2014. From research to action: enhancing crop yield through wild pollinators. Front Ecol Environ. 12:439–447.

Gonçalves R, Freitas AI, Jesus J, De la Rúa P, Brehm A. 2015. Structure and genetic variation of the mitochondrial control region in the honey bee *Apis mellifera*. Apidologie. 46:515–526.

Hahn C, Bachmann L, Chevreux B. 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads - A baiting and iterative mapping approach. Nucleic Acids Res. 41:e129.

Jaffé R, Pope N, Acosta AL, Alves DA, Arias MC, De la Rúa P, Francisco FO, Giannini TC, González-Chaves A, Imperatriz-Fonseca VL, et al. 2016. Beekeeping practices and geographic distance, not land use, drive gene flow across tropical bees. Mol Ecol. 25:5345–5358.

Kim D, Langmead B, Salzberg SL. 2015. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 12:357–360.

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods. 9:357–359.

Lessinger AC, Azeredo-Espin AML. 2000. Evolution and structural organisation of mitochondrial DNA control region of myiasis-causing flies. Med Vet Entomol. 14:71–80.

Neira-Oviedo M, Tsyganov-Bodounov A, Lycett GJ, Kokoza V, Raikhel AS, Krzywinski J. 2011. The RNA-Seq approach to studying the expression of mosquito mitochondrial genes. Insect Mol Biol. 20:141–152.

Ojala D, Montoya J, Attardi G. 1981. tRNA punctuation model of RNA processing in human mitochondria. Nature. 290:470–474.

Ollerton J, Winfree R, Tarrant S. 2011. How many flowering plants are pollinated by animals? Oikos. 120:321–326.

Pertea M, Pertea GM, Antonescu CM, Chang T-C, Mendell JT, Salzberg SL. 2015. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol. 33:290–295.

Potts SG, Imperatriz-Fonseca V, Ngo HT, Aizen MA, Biesmeijer JC, Breeze TD, Dicks L V., Garibaldi LA, Hill R, Settele J, Vanbergen AJ. 2016. Safeguarding pollinators and their values to human well-being. Nature. 540:220–229.

Silveira FA, Melo GAR, Almeida EAB. 2002. Abelhas brasileiras: Sistemática e identificação. First edit. Belo Horizonte.

Silverio MS, Rodovalho VDR, Bonetti AM, de Oliveira GC, Cuadros-Orellana S, Ueira-Vieira C, Rodrigues dos Santos A. 2014. Preliminary characterization of mitochondrial genome of *Melipona scutellaris*, a Brazilian stingless bee. Biomed Res Int. 2014:1–6.

Silvestre D, Dowton M, Arias M. 2008. The mitochondrial genome of the stingless bee *Melipona bicolor*

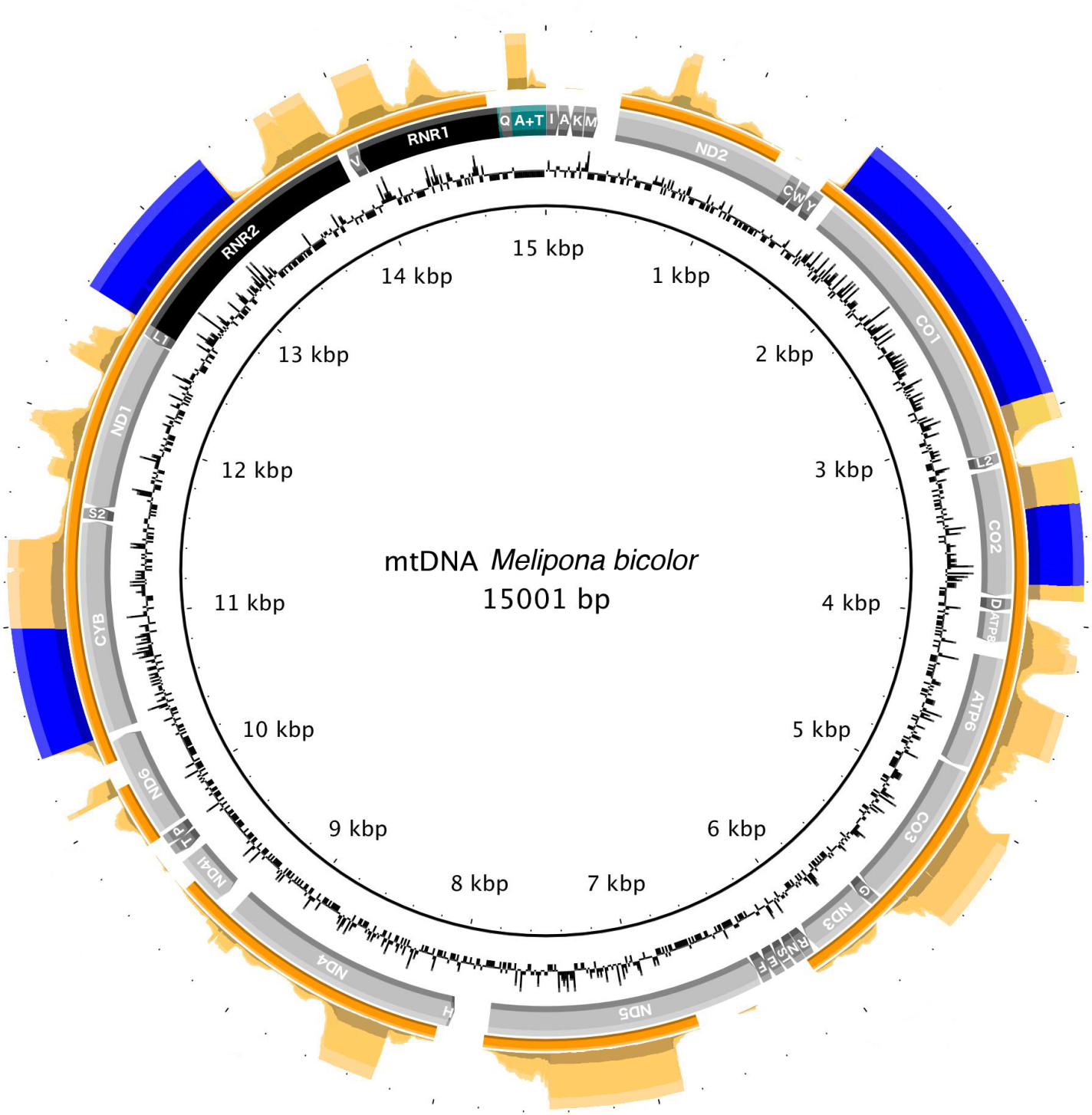
(Hymenoptera, Apidae, Meliponini): Sequence, gene organization and a unique tRNA. Genet Mol Biol. 460:451–460.
Smith DR. 2016. The past, present and future of mitochondrial genomics: Have we sequenced enough mtDNAs? Brief Funct Genomics. 15:47–54.
Stewart JB, Beckenbach AT. 2009. Characterization of mature mitochondrial transcripts in *Drosophila*, and the implications for the tRNA punctuation model in arthropods. Gene. 445:49–57.
Taanman J. 1999. The mitochondrial genome: structure, transcription, translation and replication. Biochim Biophys Acta - Bioenerg. 1410:103–123.
Tian Y, Smith DR. 2016. Recovering complete mitochondrial genome sequences from RNA-Seq: A case study of *Polytomella* non-photosynthetic green algae. Mol Phylogenet Evol [Internet]. 98:57–62.
Torres TT, Dolezal M, Schlötterer C, Ottenwälder B. 2009. Expression profiling of *Drosophila* mitochondrial genes via deep mRNA sequencing. Nucleic Acids Res. [cited 2017 Oct 6]; 37:7509–18.
Velthuis HHW, Roeling A, Imperatriz-Fonseca VL. 2001. Repartition of reproduction among queens in the polygynous stingless bee *Melipona bicolor*. In: Proc Sect Exp Appl Entomol Netherlands Entomol Soc. Vol. 12. p. 45–49.
Weinlich R, de Oliveira Francisco F, Arias MC. 2004. Mitochondrial DNA restriction and genomic maps of seven species of *Melipona* (Apidae: Meliponini). Apidologie. 35:365–370.
Zhang D-X, Hewitt GM. 1997. Insect mitochondrial control region: A review of its structure, evolution and usefulness in evolutionary studies. Biochem Syst Ecol. 25:99–120.
Zuker M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 31(13): 3406-3415.

290 **Figure captions**

291 **Figure 1**. Schematic representation of *Melipona bicolor* complete mitochondrial genome
292 sequence and features. From inner to outer circles: [1] Genomic positions representation; [2]
293 GC content graph, outer peaks meaning guanine or cytosine nucleotide bases; [3] Genome
294 annotation, blank spaces – intergenic regions, light grey – protein genes, dark grey – tRNA
295 genes, black – rRNA genes and ciano – A+T control region; [4] Mitochondrial transcripts
296 (orange bars); and [5] Expression coverage, in which blue areas represent coverage greater
297 than 5,000 (y scale from 0 to 300 times coverage).
298

299 **Figure 2.** Main features of the *Melipona bicolor* mitochondrial A+T rich region. **a** – The
300 initial 213 bp of the A+T rich DNA sequence harboring the conserved regions as reported in
301 *Apis mellifera*: [1] TATA box 1 and 2 – bases in red, [2] putative stop signal – shaded in red,
302 [3] poliT stretch – bases in blue and [4] stem-loop – shaded in grey; and MT-TQ ($tRNA^{Gln}$)
303 imbed (underlined sequence); **b** – Comparisons of the putative stop codon sequence in
304 different organisms (divergences are highlighted) and its position in *M. bicolor* MT-TQ
305 structure (shaded in red); **c** – stem loop structure of *M. bicolor*, this structure refers to the 94
306 bp region shaded in grey in **a.**
307

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


mtDNA *Melipona bicolor*
15001 bp
15 kbp
1 kbp
2 kbp
3 kbp
4 kbp
5 kbp
6 kbp
7 kbp
8 kbp
9 kbp
10 kbp
11 kbp
12 kbp
13 kbp
14 kbp
RNR1
RNR2
Q
A+T
I
A
K
M
N
ND2
C
W
Y
CO1
L2
CO2
D
ATP8
ATP6
CO3
G
ND3
R
N
S1
E
F
ND5
H
ND4
ND4L
T
P
ND6
CYB
S2
ND1
L1

MT-TQ

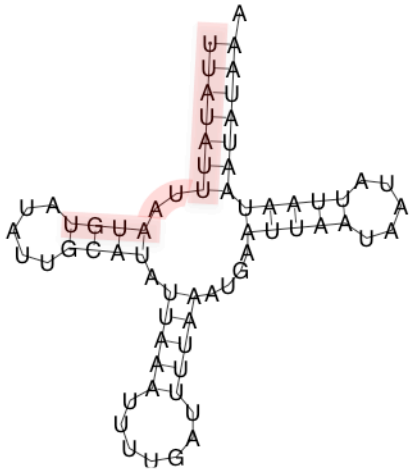
TTAATTTATATTATTAATATTATTAATTCATTTAAAATCAAAATTTAATATGC

AATATACATTAAATATAATTTTTTTTTTTTTTTTTATATATATTAATTTATA

TTTATATTTATTATTTATATATATATATATATATATATTTATATATATATATATA

TTTATTTATATATATATATATATATATATATATATATATATATATATTTATATATAT

a



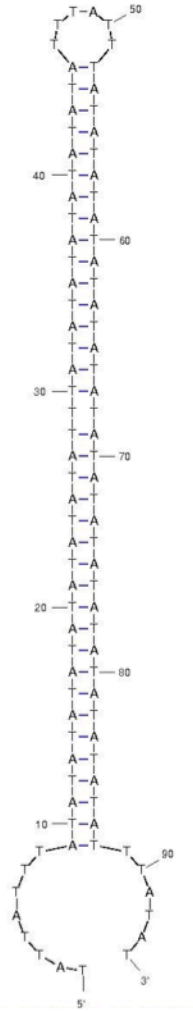
ACATTAAA **YY**AAT **humans / mouse**

A**T**ATTAAA ATAAT **honeybee**

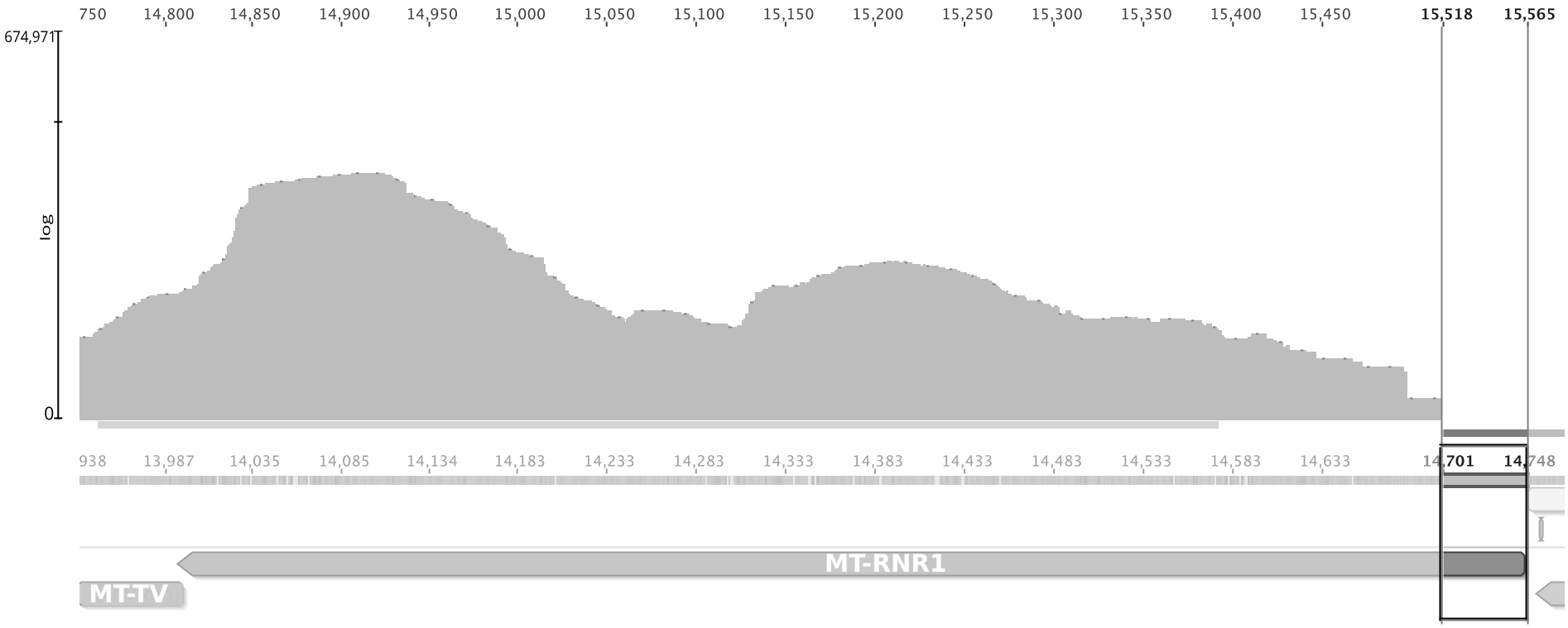
ACATTAAA**T**ATAAT ***M. bicolor***

ACATTAAA**T**ATAAT ***B. ignitus***

b



c


750
14,800
14,850
14,900
14,950
15,000
15,050
15,100
15,150
15,200
15,250
15,300
15,350
15,400
15,450
15,518
15,565
674,971
log
0
938
13,987
14,035
14,085
14,134
14,183
14,233
14,283
14,333
14,383
14,433
14,483
14,533
14,583
14,633
14,701
14,748
MT-RNR1
MT-TV