



Received: 21 April 2019  
Accepted: 01 October 2019  
First Published: xx xxx xxxx

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Reviewing editor:  
Jurg Bahler, University College London, London, UK

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## GENETICS & GENOMICS | REVIEW ARTICLE

# Complete mitochondrial genome of the Malagasy poison frog *Mantella baroni* through RNAseq

Yasmireilda Richard<sup>\*1</sup>, David R. Vieites<sup>2</sup> and Fanomezana Mihaja Ratsoavina<sup>1</sup>

**Abstract:** Within the Malagasy endemic family of Mantellidae, the only completely sequenced mitochondrial genome (mitogenome) is that of *Mantella madagascariensis*. Yet, this genome has proven to be the largest among all vertebrates with 23 kbp in size, and shows a duplication of the tRNA methionine coding gene, a pseudogene of this same gene and a duplicated control region. In this study, we report the complete mitochondrial genome of *Mantella baroni*, the second mitogenome sequenced for the Mantellidae family. This genome sequence has been generated using next-generation sequencing technics performed on Illumina Hi-seq. The genome is 20,945 bp (21 kbp) in size with 13 protein-coding genes, 23 tRNA coding genes, 2 rRNA coding genes and 2 Control Regions (CR1 and CR2). This newly generated mitogenome shows duplication of the tRNA glycine coding gene (G1 and G2) and translocation of tRNA methionine coding gene M2 in the CR2. This gene organization is unique among anurans. Both *M. baroni* and *M. madagascariensis* mitogenomes are amongst the largest in vertebrates which might be related to their aposematism or their skin toxicity by alkaloid secretion. We also hypothesize that other *Mantella* species likely have large genomes, being not clear how the genome size and organization of mitochondria evolved in Malagasy frogs. Testing such a hypothesis require more mitogenome sequencing for *Mantella* and other representatives of the mantellid diversity. The mitogenome generated here will be

### ABOUT THE AUTHOR

We work in Ecology, Conservation and genetics. This work is a contribution in assessing Madagascar biodiversity by inventories, investigating taxonomy and phylogeny, evaluating habitat structure and characteristics and conservation genetics. To date many species belonging to different taxa in Madagascar are still being discovered and many are not yet known or have not been assessed. The mitochondrial genome of the poison frog *Mantella baroni*, first genome generated for this species and second genome sequenced in the Mantellidae family after *M. madagascariensis* will help bring more insight into the evolution and phylogeny of the *Mantella* frogs. Nonetheless, more genomic investigation of other *Mantella* as well as other species in the Mantellidae family is needed to explore phylogeography and population genetics for the conservation of the *Mantella* species.

### PUBLIC INTEREST STATEMENT

The poison frog genus *Mantella*, included in the Malagasy endemic family of Mantellidae, contains species such as *M. baroni* which is able to hybridize with other sympatric *Mantella* species. Besides that uncommon behavior, two *Mantella* species, *M. baroni* and *M. madagascariensis* are morphologically similar to the point that it is not evident to differentiate them. They mimic each other to display the toxic coloration that repel predators. Despite the small size of the *Mantella* frogs (~20–30 mm in snout-vent length), the only available mitochondrial genome (mitogenome) of *M. madagascariensis* for the Mantellidae has proven to be the largest mitogenome in all vertebrates with 23 kbp and very specific gene rearrangements. This study aims to generate the mitogenome of *M. baroni* and to compare it with *M. madagascariensis*. The objective is to explore the genomic size, pattern and feature characterizing the two aposematic and mimetic species of *M. baroni* and *M. madagascariensis*.

useful for comparative genomic studies but also to answer the question on how mitogenomes evolved in the Mantellidae family.

**Subjects:** Bioscience; Biology; Genetics

**Keywords:** RNA-seq; next generation sequencing; gene duplication; Mantella; anurans; Madagascar

### 1. Introduction

Metazoan mitochondrial genome (mitogenome) typically encodes genes for 22 transfer (t) RNAs, two ribosomal (r) RNAs, and 13 proteins. The mitogenome usually presents one long noncoding region (Control Region or D-loop) that contains signals for regulation and initiation of mitochondrial DNA (mtDNA) replication and transcription (Boore, 1999; Burger, Gray, & Lang, 2003; Wolstenholme, 1992). Vertebrate mtDNA is a relatively small-closed circular molecule with a genome size of 16 kbp. However, the genome size can range from 15 to 23 kbp (Kurabayashi et al., 2006). Gene contents and organization of mitogenome are highly conserved in vertebrates (Rokas, Ladoukakis, & Zouros, 2003) thus making it useful for testing hypothesis of microevolution, studying population structure, phylogeography, and phylogenetic relationships at various taxonomic levels (Avice, 2000; Kurabayashi et al., 2008; Saccone, De Giorgi, Gissi, Pesole, & Reyes, 1999; Zhang et al., 2005).

Among Anurans, taxa belonging to the Archeobatrachian group have the gene arrangement identical and typical for all vertebrates (Roe, Ma, Wilson, & Wong, 1985; San Mauro, Garcia-Paris, & Zardoya, 2004; Kurabayashi et al., 2006, 2010). The Archeobatrachian group is considered to gather basal anuran families in contrast to the Neobatrachian group which is composed of more recently diverged anurans. Gene rearrangements, pseudogenization, and gene duplications have resulted in a deviation of the gene organization of Neobatrachian mitogenomes from that of typical vertebrate mitogenomes. The anuran family Mantellidae, exclusively endemic to Madagascar (Vieites et al., 2009), is placed within the Neobatrachians. In contrast to the gene organization of Archeobatrachians, the Mantellid mitogenomes show translocation of the Nicotinamide Dehydrogenase sub-unit 5 (ND5) gene after the Control Region (CR) and the tRNA gene cluster Leucine-Threonine-Proline-Phenylalanine (L2TPF) after ND5.

The genus *Mantella* (Mantellidae, sub-family of Mantelinae) comprises 16 species of, small (~20-30 mm in snout-vent length), brightly colored diurnal frogs known as the poison frogs of Madagascar (Glaw & Vences, 2007). All species show bright aposematic colorations and skin alkaloids secretion (Daly, Andriamaharavo, Andriantsiferana, & Meyers, 1996). The genetic relationships between them are only partially resolved (Chiari et al., 2004; Vences, Glaw, & Böhme, 1999), and the evolution of their aposematic colorations, including Müllerian mimicry events, is still subject of debate (Chiari et al., 2004). Two species within this genus, *Mantella baroni* and *M. madagascariensis*, are morphologically similar because of their colorations. Only the limit of the black extent to the hips on the ventral side and the existence of horseshoe marking on the chin differentiate *M. madagascariensis* from *M. baroni* in coloration. Those two cryptic species use Müllerian mimicry as a defensive strategy (Glaw & Vences, 2000; Schaefer, Vences, & Veith, 2002). Another intriguing fact about *M. baroni* is its syntopic distribution with *M. cowani* and *M. nigricans*. These species can occur syntopically and can also hybridize, making the ambiguous species definition more complex (Chiari, Andreone, Vences, & Meyer, 2005; Rabemananjara, Chiari, Ramilijaona, & Vences, 2007). Thus, for species such as *M. baroni*, genomic data are needed to understand the mitogenomic evolutionary pattern within *Mantella* species and all the species in the Mantellidae family.

To date, there have been few efforts in sequencing portions of mitogenomes [from the Cytochrome b (Cytb) to the Nicotinamide Dehydrogenase sub-unit 2 (ND2) protein-coding genes] for Mantellidae species. In this family, 17 species have partial mitogenomes available. Only two

species of *Mantella* (*M. baroni* and *M. bernhardi*) have a portion of their mitogenome sequenced, and only the complete mitogenome of one species of *Mantella*, *M. madagascariensis* has been sequenced so far. This genome has proven to be the largest within all vertebrates, with 23 kbp in length and has uncommon genomic feature. *M. madagascariensis* genome is characterized by two tRNA Methionine coding genes (M1 and M2), one pseudogene tRNA Methionine (M $\bar{S}$ ) and two CRs. This genome characteristic is only shared with the *Mantella* species based on the comparison of *M. madagascariensis* full genome and *M. baroni* and *M. bernhardi* partial genomes (Kurabayashi et al., 2008). Whether the long mitogenome size found in *M. madagascariensis* is a genomic feature shared with another *Mantella* species is not yet known. Considering the high frequency of gene reorganization of the mitogenome in anurans (Kurabayashi et al., 2008, 2006), it is important to gather other genomes as reference for future studies especially in the case of two cryptic Müllerian mimetic species such as *M. baroni* and *M. madagascariensis*.

This study aims to investigate the genomic pattern, feature and size of *M. baroni* mitogenome using RNAseq. RNAseq or transcriptomes sequencing is becoming a common technique for gathering mitogenomes using the Next-Generation Sequencing (NGS) (Choo et al., 2016; De Wit et al., 2012; Li et al., 2013; Neira-Oviedo et al., 2010; Peso- Fernández et al., 2016; Tian & Smith, 2016; Zhang, Wang, Zhang, Liu, & Zhang, 2017). The use of RNAseq to generate genomic sequences came from the knowledge of the phenomenon called pervasive transcription which stipulates that most of the DNA in the eukaryote genome is transcribed (Sanitá Lima & Smith, 2017). As the second mitogenome known within the Mantellidae family, *M. baroni* genome will serve as a baseline in future studies such as phylogeography and population genetics for the conservation of the *Mantella* species. With this study, we want to verify if the long size and the genomic feature characterizing *Mantella madagascariensis* are shared within other species of *Mantella* such as *M. baroni*. The mitogenome resulting from this study constitutes the second complete mitogenome within the Mantellids that can be used to answer evolutionary questions related to the evolution of mitogenomes in this family and their potential relationships with aposematism and alkaloid secretion.

## 2. Materials and methods

### 2.1. Sampling

*Mantella baroni* specimens were collected in Bekalalao forest (18°50'59.7"S, 48°22'17.7"E), Andasibe, Central east of Madagascar, in February 2016. We actively searched for the specimens in the forest during the day and caught it by hand.

The identification of *M. baroni* was made by trained taxonomists. To distinguish *M. baroni* from *M. madagascariensis* on the field the pattern on the ventral side were investigated (Glaw & Vences, 2007). *M. baroni* has a dot or a solid black on the throat and small markings on the forelimbs, while *M. madagascariensis* has a horseshoe-shaped marking on the throat and may or may not have markings on the forelimbs. The hind limbs of *M. baroni* are solid-red part way up to the thighs, while *M. madagascariensis* has red on the hind limbs through the thighs.

### 2.2. Samples preparation, laboratory and bioinformatic steps

Specimens were anaesthetized and subsequently euthanised in MS-222 solution after collection by hand. Thigh tissues were taken from the specimens using laboratory clamp and chisel, and then put and conserved in RNAlater. The specimens are fixed in 90% ethanol and preserved in 70% ethanol. The vouchers specimens with the field number DRV-9601 were deposited at the herpetological collection of the Mention "Zoologie et Biodiversité Animale" of the University of Antananarivo (UADBA-A).

Tissue extraction, sequencing and bioinformatics steps were done following Peso- Fernández et al. (2016). Dissected tissues from the specimen were conserved in RNAlater. Nucleic acids amount was quantified with Qubit HS and normalized to make sure that samples were sufficient

and we had enough high molecular weight DNA for sequencing. NEBNext Ultra RNA kit for Illumina (Illumina Inc., San Diego, CA) was used to create an RNAseq library which was quantified and size estimated with a Bioanalyzer 2100 High Sensitivity DNA chip. This RNAseq library was then reversely transcribed in DNA and a DNA library was created with the Kit NEBNext Ultra for Illumina. The DNA library was sequenced on 1/2 lane on Illumina HiSeq (2 x 100 bp pair-end reads). The bioinformatic steps including quality control and trimming with Trimmomatic v 0.32.2 (Bolger, Lohse, & Usadel, 2014) were executed. The sequences were assembled with Trinity v 2.0.6 (Haas et al., 2013) and MITObim (Hahn, Bachmann, & Chevreur, 2013) was used to complete the genome reconstruction. Genome annotation was done using part of the mitogenome of *Mantella baroni* and the complete sequence of mitogenome for *Mantella madagascariensis*, both available in genbank (accession numbers: AB239567 and AB212225).

### 2.3. Phylogenetic analyses

The mitochondrial gene Cytochrome b (Cytb) was used to identify and to explore the matrilineal genealogy of the *Mantella baroni* alongside the other 15 species of *Mantella*. Each species is represented by three different Cytb sequences from individuals collected at different places in Madagascar. Those sequences were obtained from genbank. The Cytochrome b genes were aligned in Mega version 6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) and Bayesian Interference (BI) phylogenetic analyses were performed. The model of evolution was suggested by the value of Akaike's information criterion (AIC) and hierarchical likelihood ratio test (hLRT) calculated with PAUP\*4.0b10 (Swofford, 2003) and MrModeltest version 2 (Nylander, 2004). The BI analysis was conducted on MrBayes with four chains running for 25 million Markov chain Monte Carlo (MCMC) generations and sampling frequency of 1,000. 5 million generations were discarded as burn in, after convergence checking.

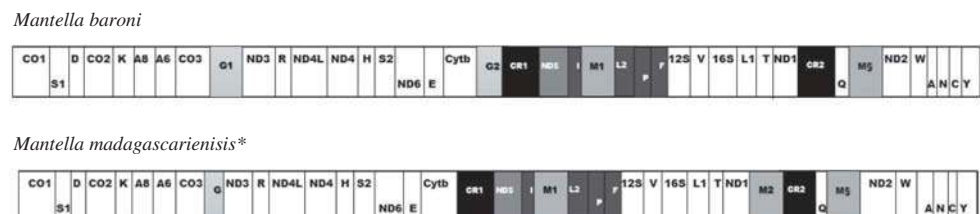
### 3. Results

*Mantella baroni* mitogenome information has been deposited in genbank under the accession number MH141597 (NC\_039758). The mitogenome has a size of 20,945 bp (21 kbp) with 13 protein-coding genes, 23 tRNA coding genes including tRNA methionine coding genes (M1 and M2), the RNAt-Met pseudogene (M5) and two Control Regions (CR1 and CR2) (Figure 1). The two control regions share the same sequences of TAS (Terminal Associated Sequence) and CSB (Conserved Sequence Bloc) (Figure 2), yet, CR2 is different from CR1 by having the sequence of tRNA-M2 gene at the beginning. Indeed, CR2 sequence starts at 6,225 bp and ends at 8,392 bp. And, with 70 bp of size the tRNA-M2 gene starts at 6,241 bp and stops at 6,310 bp. Hence, M2 is included within the CR2. The tRNA-M2 gene is not represented in Figure 1 but in Figure 2 as part of CR2.

There are the presence of other duplicated genes other than tRNA methionine coding genes in *M. baroni* mitogenome, which are:

- The tRNA-Ser (S1 and S2) coding genes located between CO1-tRNA-Asp and tRNA-His-ND6,
- The tRNA-Leu (L1 and L2) coding genes with L1 placed between 16S-tRNA-Trp and L2 between M1-tRNA-Pro and
- The tRNA-Gly (G1 and G2) coding genes found between CO3-ND3 and Cytb-CR1.

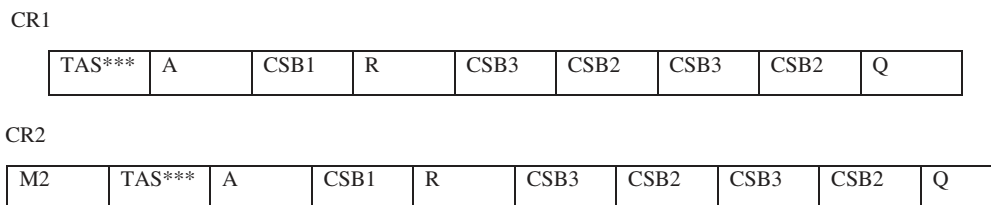
**Figure 1. Mitochondrial genome feature and organization of *Mantella baroni* compared to *M. madagascariensis*.**



\* Reference: Kurabayashi et al., 2006

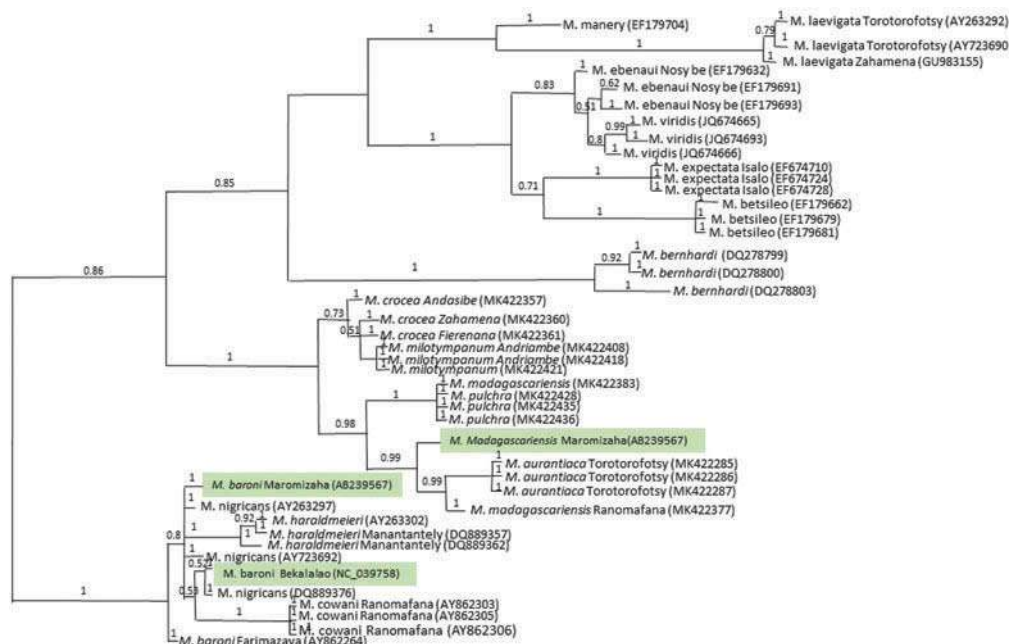
**Figure 2. Mitochondrial genome elements of the Control Regions of *Mantella baroni*.**

TAS: Terminal Associated Sequence and CSB: Conserved Sequence BlocTAS  
 \*\*\* Three-time repetition of TAS sequence.



**Figure 3. Phylogenetic relationships between *Mantella* species.**

Node numbers show Bayesian posterior probabilities. *M. baroni* collected in Bekalalao-Andasibe discussed in this paper present the accession number NC\_039758.



The abbreviations were taken from <https://www.bioinformatics.org/sms/iupac.html>. Serine (S), Aspartique Acid (D), Lysine (K), Glycine (G), Arginine (R), Histidine (H), Glutamique Acid (E), Isoleucine (I), Methionine (M), Proline (P), Phenylalanine (F), Valine (V), Threonine (T), Glutamine (Q) Tryptophane (W), Alanine (A), Asparagine (N), Cysteine (C), Tyrosine (Y). CO for cytochrome oxidase with sub-units 1, 2 and 3, A8 and A6 for ATP synthetase sub-units 8 and 6, ND for Nicotinamide dehydrogenase (NADH) sub-units 1, 2, 3, 4, 4L, 5 and 6, Cytb for cytochrome b, CR1 and CR2 correspond to Control Region 1 and 2, MS for Methionine pseudogene. S1 for Serine with the start codon UCN, S2 AGY with Y corresponding to A or T, L1 for Leucine with the start codon UUN, N corresponds to A, T, G or C, L2 CUN. 12S and 16S represent rRNAs sub-units 12S and 16S. The genome feature represented at the bottom of the columns are found on the light strand and those at the top on the heavy strand of the mitochondrial DNA.

Phylogenetic analysis (Figure 3) recover *M. baroni* grouped within the *Mantella cowani* group, together with *M. nigricans*, *M. haraldmeieri* and *M. cowani*. The result support the presence of *M. baroni* within *M. cowani* group.

#### 4. Discussion

##### 4.1. *Mantella baroni* mitochondrial genome size

The mitogenomes of *Mantella* are among the largest found in vertebrates. *M. baroni* mitogenome has 21kbp, close to the 23kbp genome of *Mantella madagascariensis* (Kurabayashi et al.,

2006). The large size of *M. baroni* and *M. madagascariensis* genomes are mainly due to the presence of two long control regions which are considered to be the largest segments within mitogenomes (Boore, 1999). Why *Mantella* have such large genomes is not yet known. The striking similarity in *M. baroni* and *M. madagascariensis* coloration and pattern as well as their toxicity are thought to be a case of Müllerian mimicry since the two species occur sympatrically and belong to two different clades (Chiari et al., 2004; Rojas, 2017; Schaefer et al., 2002; Vences, Chiari, Raharivololoniaina, & Meyer, 2004). Our finding raises the question on whether the long genome size found in these two species is related to their aposematic coloration pattern and the use of Müllerian mimicry as a defensive strategy or their capacity to eat and secrete alkaloids. Available mitogenomes in genbank for representatives of Dendrobatidae, the south American poison dart frogs, have sizes ranging from 14kbp to 18kbp, within the normal size of other anurans. Both Dendrobatidae and Mantellidae have aposematic bright colorations and alkaloid skin secretion, which evolved independently in different continents, but *Mantella* genomes are much larger. Hence, whether large size genomes in *Mantella* may be related to aposematism and alkaloid defense is unclear, as is not paralleled by dendrobatid frogs.

The large mitogenome size in the *Mantella* is uncommon, because small mitogenomes have been proposed to be evolutionarily advantageous than larger ones due to a faster replication rate (Kurabayashi et al., 2006). This is because small mitogenomes replicate faster than larger ones hence allow for more rapid adaptations. In the case of *Mantella* it may be that a compensation exists to allow a fast replication by the presence of the multiple origins of replication for the light strand ( $O_L$ ) and the presence of two CRs which function as origin of replication for the heavy strand ( $O_H$ ) (Kumazawa, Ota, Nishida, & Ozawa, 1998; Kurabayashi et al., 2006; Yokobori, Fukuda, Aoyama, Nakamura, & Oshima, 2004). To understand the implications of this long genome size in the evolution of *Mantella* and the Mantellidae, the genome size and structure of representatives across the tree life of Mantellids should be investigated.

#### 4.2. Gene organization in the *Mantella baroni* mitochondrial genome

The mitogenome of *M. baroni* presents two tRNA-Glycine (G1 and G2) coding genes. The tRNA-Gly gene (G1) has a length of 69bp and is located between CO3 and ND3. tRNA-Gly gene G1 has been reported in all Archaeobatrachians and Neobatrachians (Mantellidae, Rhacoporidae, Ranidae and Bufonidae) (Kurabayashi et al., 2008). G1 gene sequence and the tRNA-glycine gene found in *M. madagascariensis* have 95.65% of nucleotide similarity. The partial mitogenome of *M. baroni* reported before is from the Cytb gene to the ND2 gene which does not include the tRNA glycine gene. This situation has not permitted the comparison between G1 in this mitogenome and the tRNA-Gly in the partial genome of *M. baroni*. The second tRNA-Gly G2 gene in this mitogenome sizing 70bp and located between Cytb and CR1 has not been observed in *M. madagascariensis*, in any Mantellids partial genomes available in genbank nor in any anurans mitogenomes sequenced to date. Furthermore, the portion of mitogenome of *M. baroni* captured in Maromizaha and sequenced by Kurabayashi et al. (2006) from Cytb to ND2 does not have this G2 element that should be found between Cytb and CR1. Thus, this feature constitutes a new gene arrangement within the Mantellids. Maromizaha is located around the Mantadia National Park in Andasibe, Madagascar (Rakotondravony, 2004) near Bekalalao where we captured the specimens of *M. baroni*. Our result might suggest that there is a signature, certain degree of genetic differentiation between populations of *M. baroni*. G1 and G2 in this mitogenome have 55% of nucleotide similarity.

The mitogenome of *M. baroni* presents a translocation of the tRNA-Methionine (M2) coding gene in CR2 (Figure 2). For *M. madagascariensis* mitogenome, the sequence of M2 gene begins at 10,955 bp and stops at 11,024 bp (Kurabayashi et al., 2006). CR2 sequence for *M. madagascariensis* comes after M2 and starts at 11,025 bp and ends at 13,298 bp. *M. baroni* portion of mitogenome sequenced by Kurabayashi et al. (2006) has its sequence of M2 located from 7,302 bp to 7,371 bp and CR2 from 7,372 bp to 9,466 bp. So similar to *M. madagascariensis* mitogenome, M2 sequence for *M. baroni* portion of mitogenome is located before CR2. However, for *M. baroni* mitogenome reported here, M2

is located within CR2. CR2 starts and stops from 6,225 bp to 8,392 bp and M2 from 6,241 to 6,310 bp within *M. baroni* mitogenome. We hypothesize that the presence of the second tRNA-Met gene sequence M2 in CR2 is caused by the duplication of the tRNA-glycine gene in the genome. This situation has led to the translocation of the M2 gene in the CR2 in order to maintain the 22 tRNA genes characterizing all metazoans. Indeed, genome is usually under selection pressure for size minimization (Yokobori et al., 2004) and for maintaining the number of tRNAs characteristic of metazoans. The M2 gene sequence reported here and in the partial mitogenome of *M. baroni* are identical with 100% of nucleotide similarity and 92.86% of similarity with *M. madagascariensis* M2. The similarity reported for M2 in *M. baroni* partial genome and *M. madagascariensis* mitogenome is 92.9% (Kurabayashi et al., 2006) which is almost the same as reported here (92.86%) for M2 in the mitogenomes of *M. baroni* and *M. madagascariensis*. The tRNA methionine (M1) in *M. baroni* genome has 100% nucleotide similarity with M1 in *M. baroni* partial genome and 92.86 similarity with M1 in *M. madagascariensis* mitogenome. M1 in this genome has 91.66% nucleotide similarity with M2. The similarity between M1 and M2 in the mitogenome of *M. baroni* is higher than reported in *M. madagascariensis* mitogenome which was 77.1% (Kurabayashi et al., 2006).

The presence of 23 tRNA coding genes, the duplication of the tRNA-Met gene (M1 and M2) with the pseudogene tRNA-Met (MS) and the presence of two CRs in the *M. baroni* mitogenome have only been found in *Mantella*. The tRNA-Ser (S1 and S2) and tRNA-Leu (L1 and L2) gene duplication are shared by *M. baroni* and *M. madagascariensis*. The tandem duplication of tRNA-Thr, tRNA-Pro, tRNA-Leu, tRNA-Glu and tRNA-Met genes are found in *M. baroni* and *M. madagascariensis* mitogenomes. It has been hypothesized that gene duplication and pseudogene apparition arose from duplication-deletion events which are considered to be the principal factors of evolution leading to the apparition of new gene organization within a genome (Kurabayashi et al., 2008, 2006). The gene cluster tRNA Isoleucine (I), Methionine (M1), Leucine (CUN or L2), Proline (P) and Phenylalanine (F) or IMLPF, between ND5-12S and the presence of ARNt-Threonine (Thr or T) between ARNt-Leu (UUR or L1)-ND1 are observed in *M. baroni* and *M. madagascariensis* mitogenomes. The cluster IMLPF is a feature characteristic of *Mantella* species while other Mantellids and Archeobatrachians have the gene cluster IMLTPF located between ND5 and 12S rRNA (Kurabayashi et al., 2008).

The position of O<sub>L</sub> in the WANCY region (WAN-O<sub>L</sub>-CY) is a feature shared between *M. baroni* and all other Mantellids. This feature is found in Archeobatrachians which present the primitive gene organization within the anurans (Kurabayashi et al., 2006; Pabijan, Spolsky, Uzzel, & Szymura, 2008; Roe et al., 1985; San Mauro et al., 2004). This suggests that all Mantellids including *Mantella baroni* have conserved the ancestral organization for O<sub>L</sub> position in the WANCY region. The position of ND5 after CR is also a feature shared within all the Mantellids including *Mantella baroni*.

In conclusion, *M. baroni* mitogenome with its 21Kbp is one of the largest genome invertebrates. It is unclear if this size increment in respect to other mantellids, is related to aposematism or alkaloid secretion, as dendrobatids have regular size genomes, yet still are valid hypothesis to explore. The mitogenome of *M. baroni* shows the same pattern as the mitogenome of *M. madagascariensis* and is characterized by the duplication of the tRNA glycine coding gene and the translocation of tRNA methionine M2 coding gene in the Control Region CR2. This is the first time this gene duplication is reported in anurans. It is interesting as a perspective for future study to sequence the mitogenomes of *M. baroni* populations from different areas of Madagascar to look at genomic feature and pattern between metapopulations. The phylogenetic tree obtained in this study support a close relationship between *M. baroni* and *M. nigricans* as reported by Crottini, Orozco-terWengel, Rabemananjara, Hauswaldt, and Vences (2019). Sequencing other *Mantella* such as *M. nigricans* mitogenome is recommended for future finding to solve the evolutionary pattern of the *Mantella* species.

#### Acknowledgements

We are grateful to the following institutions for the administrative and logistic supports: Mention Zoologie et Biodiversité Animale, Faculté des Sciences Université d'Antananarivo,

Museo Nacional de Ciencias Naturales and the Ministère de l'environnement et des forêts. We thank the local guides as well as the lab technicians for their great assistance in the field and during laboratory work. We thank Dr. David Vieites

and the Spanish Ministerio de Economía y Competitividad (FEDER, EU), grant reference: CGL2013-40924-P, and the Agencia Española de Investigación (AEI/FEDER, EU), grant reference: CGL2017-89898-R. for the financial support during the study.

#### Funding

This research has received grants of the Spanish Ministerio de Economía y Competitividad (FEDER, EU), grant reference: CGL2013-40924-P, and the Agencia Española de Investigación (AEI/FEDER, EU), grant reference: CGL2017-89898-R.

#### Competing Interests

The authors declare no competing interests.

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#### Cover Image

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#### Citation information

Cite this article as: Complete mitochondrial genome of the Malagasy poison frog *Mantella baroni* through RNAseq, Yasmireilda Richard, David R. Vieites & Fanomezana Mihaja Ratsoavina, *Cogent Biology* (2019), 5: 1679327.

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