The complete mitochondrial genome of red-fronted parrot (\textit{Poicephalus gulielmi}) revealed a new gene rearrangement within the order \textit{Psittaciformes}

Adam Dawid Urantówka\textsuperscript{a}, Aleksandra Kroczak\textsuperscript{b} and Paweł Mackiewicz\textsuperscript{b}

\textsuperscript{a}Department of Genetics, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland; \textsuperscript{b}Department of Genomics, Faculty of Biotechnology, Wroclaw University, Wroclaw, Poland

\textbf{ABSTRACT}

Vertebrate mitogenomes are thought to be selected for compactness. Therefore, the increasing number of avian mitogenomes comprising duplicated regions is surprising. Such regions were proposed for at least 26 parrot genera based on the length of PCR products. However, complete mitogenomes with the duplications were shown only for six genera. These duplications evolved probably from the ancestral \text{tRNA\textsuperscript{THR}}/\text{tRNA\textsuperscript{PRO}}/\text{ND6}/\text{tRNA\textsuperscript{GLU}}/\text{CR} and were subjected to subsequent degeneration. Here, we report the mitogenome of \textit{Poicephalus gulielmi} (the subfamily Psittacinae) with a unique duplication \text{tRNA\textsuperscript{THR}}/\text{pseudoND6}/\text{CR1}/\text{tRNA\textsuperscript{PRO}}/\text{ND6}/\text{tRNA\textsuperscript{GLU}}/\text{CR2}. This region is different from all other identified regions and resembles mostly the arrangements in \textit{Amazona} and \textit{Pionus} from the subfamily Arinae.

A typical mitogenome of vertebrates includes only one control region and the same set of 37 genes coding for two ribosomal RNAs, 13 proteins, and 22 tRNAs (Lavrov 2007). The coherent gene content makes that mitogenomes are still regarded to be under selection for compactness. However, the growing number of fully sequenced avian mitochondrial genomes has revealed that rearrangements and partial duplications occur in different lineages.

Recent studies also provided evidences for the persistence of duplicated CRs in parrot mitochondria. So far, 26 parrot genera were found to have a duplicated CR based on differences in the length of mitogenome fragments obtained in PCR (Schirtzinger et al. 2012). However, complete mitogenomes with duplicated elements were sequenced only for six genera: \textit{Amazona}, \textit{Forpus}, \textit{Melopsittacus}, \textit{Pionus}, \textit{Prioniturus}, and \textit{Psittacus} (Urantowka et al. 2013, 2016, 2017a; Eberhard and Wright 2016; Urantówka and Mackiewicz 2016). All identified genome rearrangements preserve two control regions and seem to evolve from the ancestral tandem duplication of \text{tRNA\textsuperscript{THR}}/\text{tRNA\textsuperscript{PRO}}/\text{ND6}/\text{tRNA\textsuperscript{GLU}}/\text{CR} fragment followed by degeneration and/or loss of some genes (Eberhard and Wright 2016).

So far, \textit{Psittacus erithacus} is the only representative of the Psittacinae subfamily with the known complete mitogenome sequence. This subfamily comprises also another genus \textit{Poicephalus}, which in contrast to monotypic \textit{Psittacus}, is the most species-rich and widely distributed in Africa. \textit{Poicephalus} is very morphologically diverse (Forshaw 2010) and several of its species (\textit{fuscicolis}, \textit{gulielmi}, \textit{senegalus}, \textit{flavifrons}, and \textit{meyeri}) are further divided into subspecies (Gill and Donsker 2017). Therefore, this parrot offers an interesting possibility to study mechanisms of speciation and emergence of new lineages. The previously recognized \textit{Poicephalus robustus} taxon was recently divided into \textit{r. robustus} (Cape parrot) and two other subspecies for which a new species \textit{Poicephalus fuscicolis} (brown-necked parrot) was established: \textit{f. fuscicolis} and \textit{f. suahelicus} (Gill and Donsker 2017, see also for Hockey et al. 2005; Coetzer et al. 2015 for such proposition). This separation is also indicated by our results (Figure 1). However, this classification was questioned by some taxonomists because of small number of markers used. Therefore, further analyses of complete mitochondrial genomes are necessary to resolve this taxonomic proposition because results for single individual markers can be biased and produce inconsistent phylogenies (Urantówka et al. 2017a).

Therefore, to enrich the set of molecular markers for future study of \textit{Poicephalus} diversification, we obtained the sequence of mitogenome from \textit{Poicephalus gulielmi} (accession number MF977813). Interestingly, the gene order found in the duplicated region \text{tRNA\textsuperscript{THR}}/\text{pseudoND6}/\text{CR1}/\text{tRNA\textsuperscript{PRO}}/\text{ND6}/\text{tRNA\textsuperscript{GLU}}/\text{CR2} is different from that identified by Eberhard and Wright (2016) for closely related \textit{Psittacus erithacus}: \text{tRNA\textsuperscript{THR}}/\text{CR1}/\text{tRNA\textsuperscript{PRO}}/\text{ND6}/\text{tRNA\textsuperscript{GLU}}/\text{CR2}. The gene order in \textit{Poicephalus gulielmi} differs from any other previously identified and resembles mostly the arrangement characteristic of \textit{Amazona} (Eberhard et al. 2001) and \textit{Pionus} from the subfamily Arinae: \text{tRNA\textsuperscript{THR}}/\text{pseudoND6}/\text{psuedotRNA\textsuperscript{GLU}}/\text{CR1}/\text{tRNA\textsuperscript{PRO}}/\text{ND6}/\text{tRNA\textsuperscript{GLU}}/\text{CR2}.

The morphology of the analysed captive individual is typical of \textit{gulielmi} species, which is undoubtedly confirmed in
In PhyloBayes (Lartillot and Philippe 2004), we applied CAT an alternative position of clades in the PhyloBayes tree. The length of branches (the dashed lines) leading to outgroup sequences was shortened five times. The values at nodes, in the order shown, indicate posterior probabilities found in MrBayes (MB) and PhyloBayes (PB) as well as SH-aLRT (SH) and non-parametric bootstrap (BP) percentages calculated in IQ-TREE. The phylogenetic tree obtained in MrBayes for the concatenated alignment of 16S rRNA and COI genes (1133 bp) indicating that the studied individual (bolded) belongs to Poicephalus gulielmi. Its sequences show the smallest genetic distance (0.49%) to Poicephalus gulielmi gulielmi. The individual is a male kept in culture in Poland (Kosiciak town) and naturally this species inhabits Central Africa. The blood sample, from which DNA was isolated, is available for further researches in the laboratory at the Department of Genetics in Wroclaw University of Environmental and Life Sciences under the number AUPMAK11. The blue arrow indicates culture in Poland (Kosičak town) and including all available sequences sampled for each generation. The last 30,000 trees from each chain were collected to compute posterior consensus trees after reaching convergence. In the case of IQ-TREE (Nguyen et al. 2015), we used separate nucleotide substitution models for two partitions as suggested by ModelFinder (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017). In SH-aLRT bootstrap analysis, 10,000 replicates were assumed, and in non-parametric bootstrap, 1000 replicates were applied. The tree demonstrates the significant separation of Poicephalus robustus and fuscicollis, which were formerly classified to one species. In comparison to result by Coetzter et al. (2015), the presented tree includes larger support values as well as groups together P. rueppelli and P. meyeri, and next adds to this clade P. cryptoxanthus. All these species distinguish from other studied Poicephalus species by smaller body length, 21 and 22 cm in comparison to 28 and 33 cm (Forshaw 2010). The grouping P. rueppelli and P. meyeri agrees also with their common morphological feature, i.e. yellow feathers on the leading edge of the wings.

Figure 1. The phylogenetic tree obtained in MrBayes for the concatenated alignment of 16S rRNA and COI genes (1133 bp) indicating that the studied individual (bolded) belongs to Poicephalus gulielmi. Its sequences show the smallest genetic distance (0.49%) to Poicephalus gulielmi gulielmi. The individual is a male kept in culture in Poland (Kosiciak town) and naturally this species inhabits Central Africa. The blood sample, from which DNA was isolated, is available for further researches in the laboratory at the Department of Genetics in Wroclaw University of Environmental and Life Sciences under the number AUPMAK11. The blue arrow indicates culture in Poland (Kosičak town) and including all available sequences sampled for each generation. The last 30,000 trees from each chain were collected to compute posterior consensus trees after reaching convergence. In the case of IQ-TREE (Nguyen et al. 2015), we used separate nucleotide substitution models for two partitions as suggested by ModelFinder (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017). In SH-aLRT bootstrap analysis, 10,000 replicates were assumed, and in non-parametric bootstrap, 1000 replicates were applied. The tree demonstrates the significant separation of Poicephalus robustus and fuscicollis, which were formerly classified to one species. In comparison to result by Coetzter et al. (2015), the presented tree includes larger support values as well as groups together P. rueppelli and P. meyeri, and next adds to this clade P. cryptoxanthus. All these species distinguish from other studied Poicephalus species by smaller body length, 21 and 22 cm in comparison to 28 and 33 cm (Forshaw 2010). The grouping P. rueppelli and P. meyeri agrees also with their common morphological feature, i.e. yellow feathers on the leading edge of the wings.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

This work was supported by the National Science Centre Poland (Narodowe Centrum Nauki, Polska) under Grant No. 2015/17/B/NZ8/02402.

References

Chernomor O, von Haeseler A, Minh BQ. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol. 65:997–1008.

Coetzter WG, Downs CT, Perrin MR, Willows-Munro S. 2015. Molecular systematics of the Cape Parrot (Poicephalus robustus): implications for taxonomy and conservation. PLoS One. 10:e013376.

Eberhard JR, Wright TF, Bermingham E. 2001. Duplication and concerted evolution of the mitochondrial control region in the parrot genus Amazona. Mol Biol Evol. 18:1330–1342.

Eberhard JR, Wright TF. 2016. Rearrangement and evolution of mitochondrial genomes in parrots. Mol Phylogenet Evol. 94:34–46.

Forshaw JM. 2010. Parrots of the world. London: A&C Black Publishers Ltd; p. 328.

Gill F, Donsker D, editors. 2017. IOC World Bird List (v 7.3). DOI:10.14344/IOCML7.3.
Hockey PAR, Dean WRJ, Ryan PG, editors. 2005. Robert’s birds of Southern Africa. 7th ed. Cape Town: John Voelcker Bird Book Fund.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 14:587–589.

Lanfear R, Calcott B, Ho SY, Guindon S. 2012. Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol. 29:1695–1701.

Lartillot N, Philippe H. 2004. A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. Mol Biol Evol. 21:1095–1109.

Lavrov DV. 2007. Key transitions in animal evolution: a mitochondrial DNA perspective. Integr Comp Biol. 47:734–743.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32:268–274.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 61:539–542.

Schritzinger EE, Tavares ES, Gonzales LA, Eberhard JR, Miyaki CY, Sanchez JJ, Hernandez A, Müeller H, Graves GR, Fleischer RC, et al. 2012. Multiple independent origins of mitochondrial control region duplications in the order Psittaciformes. Mol Phylogenet Evol. 64:342–356.

Urantowka AD, Hajduk K, Kosowska B. 2013. Complete mitochondrial genome of endangered Yellow-shouldered Amazon (Amazona barbodens): two control region copies in parrot species of the Amazona genus. Mitochondrial DNA. 24:411–413.

Urantowka AD, Krocza A, Mackiewicz P. 2016. Complete mitochondrial genome of the greater Antillean parrot Amazona ventralis (Hispaniolan amazon). Mitochondrial DNA B. 1:1:864–866.

Urantowka AD, Krocza A, Mackiewicz P. 2017a. Complete mitochondrial genome of bronze-winged parrot (Pionus chalcopterus chalcopterus, Psittaciformes). Mitochondrial DNA B. 2:744–746.

Urantowka AD, Krocza A, Mackiewicz P. 2017b. The influence of molecular markers and methods on inferring the phylogenetic relationships between the representatives of the Arini (parrots, Psittaciformes), determined on the basis of their complete mitochondrial genomes. BMC Evol Biol. 17:166.

Urantowka AD, Mackiewicz P. 2016. The first complete mitochondrial genome sequence from the blue-headed parrot (Pionus menstruus menstruus): a representative for the genus. Mitochondrial DNA B. 1:891–892.