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SHORT COMMUNICATION

Mitogenomes of two neotropical bird species and the multiple independent origin of mitochondrial gene orders in Passeriformes

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Abstract

At least four mitogenome arrangements occur in Passeriformes and differences among them are derived from an initial tandem duplication involving a segment containing the control region (CR), followed by loss or reduction of some parts of this segment. However, it is still unclear how often duplication events have occurred in this bird order. In this study, the mitogenomes from two species of Neotropical passerines (*Sicalis olivascens* and *Lepidocolaptes angustirostris*) with different gene arrangements were first determined. We also estimated how often duplication events occurred in Passeriformes and if the two CR copies demonstrate a pattern of concerted evolution in Sylvioidea. One tissue sample for each species was used to obtain the mitogenomes as a byproduct using next generation sequencing. The evolutionary history of mitogenome rearrangements was reconstructed mapping these characters onto a mitogenome Bayesian phylogenetic tree of Passeriformes. Finally, we performed a Bayesian analysis for both CRs from some Sylvioidea species in order to evaluate the evolutionary process involving these two copies. Both mitogenomes described comprise 2 rRNAs, 22 tRNAs, 13 protein-codon genes and the CR. However, *S. olivascens* has 16,768 bp showing the ancestral avian arrangement, while *L. angustirostris* has 16,973 bp and the remnant CR2 arrangement. Both species showed the expected gene order compared to their closest relatives. The ancestral state reconstruction suggesting at least six independent duplication events followed by partial deletions or loss of one copy in some lineages. Our results also provide evidence that both CRs in some Sylvioidea species seem to be maintained in an apparently functional state, perhaps by concerted evolution, and that this mechanism may be important for the evolution of the bird mitogenome.

Keywords *Lepidocolaptes angustirostris* · *Sicalis olivascens* · Mitochondrial genome · Anchored Phylogenomics

Introduction

When Desjardins and Moraes [1] described the first complete mitochondrial genome of a bird, the domestic chicken (*Gallus gallus*), they showed the existence of a new mitogenome arrangement among the chordates. Since

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then, hundreds of avian mitogenomes have been described and seven new arrangements have been discovered (see [2]). Differences among these arrangements are related to the number of copies and arrangements comprising the control region (CR), likely a result of CR and flanking region tandem duplications followed by partially random deletions or loss of one copy [3–5]. Although the mitochondrial DNA appears to be under selection for compactness, leading to loss of function and elimination of additional gene copies [6], several studies have showed some evidence for the maintenance of function in both CRs, demonstrating a pattern of concerted evolution (e.g. [7]). In these cases, stabilizing selection or gene conversions have been evoked to explain the high degree of similarity between the two CRs (e.g. [8]).

Based on evolutionary process, Gibb et al. [4] proposed the following nomenclature for avian mitochondrial gene orders: ancestral or standard avian [1], duplicate $tRNA^{Thr}$-CR (d_tThr-CR) [9], duplicate CR (d_CR) [7], and remnant CR2 (r_CR2) [3]. In the ancestral avian gene order, the gene arrangement comprises cytochrome b (Cytb)/$tRNA^{Thr}$/$tRNA^{Pro}$/ND6/$tRNA^{Glu}$/CR/$tRNA^{Phe}$. The segment from the end of Cytb to CR is tandem duplicated in d_tThr-CR. In d_CR and r_CR2, the first segment from $tRNA^{Pro}$ to $tRNA^{Glu}$ and the second segment from the end of Cytb to $tRNA^{Thr}$ are reduced or deleted. Additionally, in d_CR, both CR (CR1 and CR2) are complete and putatively functional, while in r_CR2, duplicated CR2 is degenerated. The last avian gene order described (hereafter tPro-CR) differs from the d_tThr-CR as $tRNA^{Thr}$ is not part of the duplicated segment or it is degenerated after duplication [10].

Although the standard avian gene arrangement has been found in many species from several orders, the other derived gene arrangements have been only found in some species/genera of few orders. For instance, the d_$tRNA^{Thr}$-CR have found in Procellariiformes [4, 9] and Bucerotiformes [11], d_CR in Psittaciformes [7] and Piciformes [4], r_CR2 in Tinamiformes and Cuculiformes [3, 12, 13], and dd_$tRNA^{Thr}$ in Charadriiformes [14]. Other two gene order were exclusively described for some Ardeidae species [2]. However, according to Schirtzinger et al. [8], the study of the evolutionary dynamics of avian mitogenome organization remains an ongoing challenge for the field of molecular evolution. Furthermore, only few orders were investigated in relation to evolutionary history of mitogenome rearrangements based on mapping these characters onto phylogenies (e.g. [7, 8]).

Particularly in Passeriformes, > 200 mitogenomes have been described, representing 47 of the 100 families of this order. The increase in the number of mitogenomes described results from the advent of next generation sequencing and due to the application of this methodology in phylogenetic studies on birds. As part of searching for a greater number of loci to increase resolution of the evolutionary relationships in birds, many authors have described bird mitogenomes as a byproduct of these studies (e.g. [15]).

At least four different mitogenome arrangements occur in Passeriformes. The ancestral avian arrangement has been found in the basal lineage of Passeriformes (*Acanthisitta chloris*) [16] and in several supraorders, such as Meliphagoidea, Corvoidea and Passeroidea (e.g. [17]). The other three arrangements showed a more restricted taxonomic distribution and have been only found in all suboscine (d_CR and r_CR2), in some Sylvioidea species (d_CR and r_CR2), and in *Notiomystis cincta* and *Turdus philomelos* (tPro-CR) (see [10]). While the presence of similar rearrangements in suboscines and more derived lineages of oscines (e.g. Sylvioidea) indicates independent origin of these rearrangements, it is still unclear how often mitochondrial duplication events have occurred more generally in Passeriformes and in particular within Sylvioidea.

In this paper, we describe the complete mitochondrial genome of two species of Neotropical passerines with different gene arrangements: the greenish yellow finch (*Sicalis olivascens*, Thraupidae), which showed the ancestral avian arrangement, and the narrow-billed woodcreeper (*Lepidocolaptes angustirostris*, Dendrocolaptidae), which showed the r_CR2 arrangement. In addition, we investigate the distribution of these arrangements on a mitogenome Bayesian phylogenetic tree of Passeriformes to estimate how often duplication events have occurred in this order and if the two CR copies show a pattern of concerted evolution in some Sylvioidea species.

Materials and methods

DNA extraction, library preparation, enrichment, and sequencing

Total DNA was extracted from vouchered tissue samples using DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol. One tissue sample for each species was used in this work: *S. olivascens* sample (MACN1307, Catamarca, Argentina) deposited in the Museo Argentino de Ciencias Naturales and *L. angustirostris* sample (MPEG 68210; São João dos Patos, Maranhão, Brazil) deposited in the Museu Paraense Emílio Goeldi. Mitogenome sequences were obtained as a by-product of the anchored hybrid enrichment approach described by Lemmon et al. [18] and Prum et al. [19]. Illumina indexed genomic libraries were prepared using a protocol modified (see details in [18]) from Meyer and Kircher [20] in a Beckman–Coulter Biomek® FXP Liquid-handling robot. A single pool containing 16 libraries (more specimens of *L. angustirostris* were included for phylogeographic analysis) was then enriched for the target using an Agilent Custom SureSelect kit (Agilent Technologies)

containing a single pool of all probes described in Lemmon et al. [18]. All procedures were performed by the Center for Anchored Phylogenomics (http://www.anchoredphylogeny.com). The enriched library pool was then 250 bp paired-end Illumina Hiseq 2000 sequenced on one PE150 Illumina HiSeq2000 lane by the Translational Science Laboratory in the College of Medicine at Florida State University.

Mitogenome assembly and annotation

Paired reads were merged following Rokyta et al. [21]. Reads failing to meet the probability criterion were kept separate but still used in the assembly. Merged and unmerged reads were aligned to the reference mitogenomes using GENEIOUS 4.7 [22]: for *S. olivascens* and *L. angustirostris* was used the *Thraupis episcopus* (GenBank: KM078765) and *Mionectes oleagineus* (GenBank: NC024682) mitogenome as reference, respectively. Complete mitochondrial genomes were annotated based on the automatic methods of DOGMA [23] using default parameters and manually adjusted based on comparisons to the respectively reference mitogenomes using GENEIOUS.

Alignment and bayesian phylogenetic inference

For the phylogenetic study we used an ingroup of 68 taxa, which included at least one representative of most families of Passeriformes for which mitogenomes have previously been described. We followed the taxonomic arrangement described in the Handbook Birds of the World [24]. With the exception of the two mitogenomes described here, the mitogenome sequences were obtained from GenBank. One Psittaciformes mitogenome (*Nestor notabilis*, GenBank: AY325307) was chosen as outgroup because it is the sister group of passerines based on previous molecular phylogenies (e.g. [19]). Sequences were aligned using MUSCLE v3.6 [25] with default parameters on GENEIOUS.

For Bayesian phylogenetic inference of mitogenomes, we used only the protein-coding genes. The one exception is the ND6 gene which was excluded because it was included in the region involved in the mitochondrial genome rearrangement analysis. The total alignment consisted of 10,826 bp obtained from 12 protein-codon genes. Three partitioning schemes were considered: all genes, partitioned by gene or partitioned by codon position. The optimal partitioning scheme (partitioned by codon position) was selected by PARTITIONFINDER v1.1.1 [26] under the Bayesian information criterion. The general time-reversible model was unlinked across partitions. Among-site rate variation was modeled using a discrete gamma distribution with four categories and a proportion of invariant sites. The approximation of posterior tree distributions was obtained by Markov Chain Monte Carlo (MCMC) using MRBAYES v.3.2 [27]. Multiple four chain Metropolis-coupled analyses (with default heating) were run 20,000,000 generations, sampling every 2000 generations. Run convergence and parameter posteriors were assessed using TRACER v1.5. The first 20% of the sampled trees were discarded as burn-in and the remaining trees were used to construct a 50% majority-rule consensus tree. The gene order arrangement was mapped on Bayesian phylogenetic tree using MESQUITE v.3.04 [28], considering four discrete categories: ancestral (0), duplicate CR (1), remnant CR2 (2) and tPro-CR (3). We used the parsimony criterion (Fitch) to reconstruct the ancestral state on the Bayesian phylogenetic tree. Finally, we also performed a Bayesian analysis for both CRs (CR1 and CR2) from some Sylvioidea species in order to evaluate the evolutionary

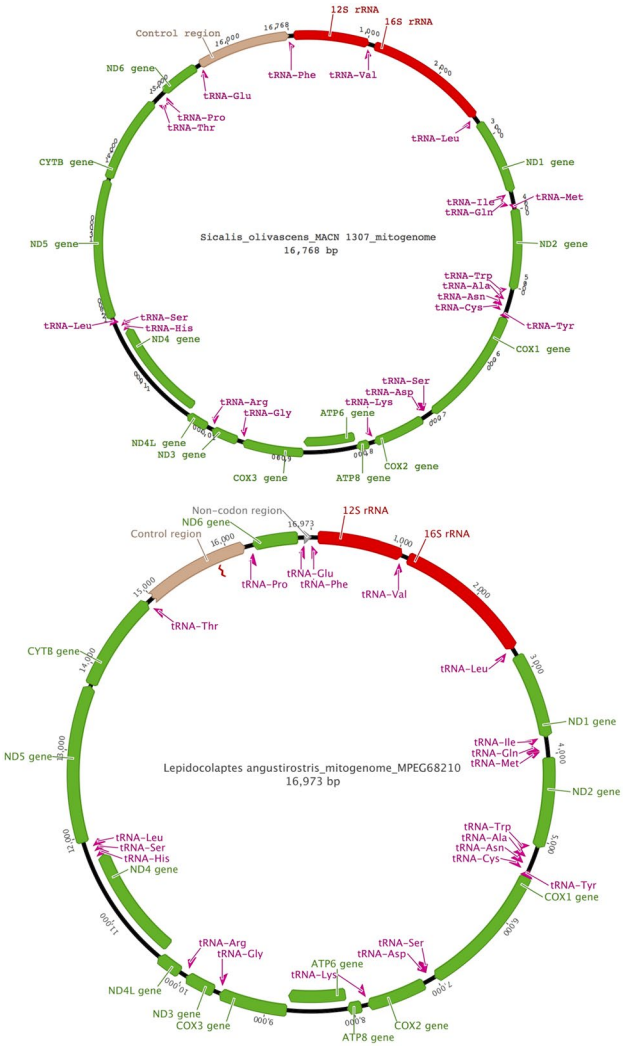

Fig. 1 Gene maps of the *S. olivascens* (top) and *L. angustirostris* (bottom) mitochondrial genomes. COX1-3 indicates cytochrome c oxidase subunits 1–3; CYTB, cytochrome b; ATP6–8, ATPase subunits 6 and 8; ND1–6/4L, NADH dehydrogenase subunits 1–6/4L. Transfer RNA genes are designated by single-letter amino acid codes (Tables S1 and S2)

process involving the two CRs copies. The latter analysis was performed using multiple four chain Metropolis-coupled analyses (with default heating) with 20,000,000 generations, sampling every 2000 generations using MRBAYES.

Results and discussion

A total of 47,255 reads were mapped to *S. olivascens* (average coverage = 732 reads) while 63,814 reads were mapped to *L. angustirostris* (average coverage = 644 reads). Genbank accessions are KY628988 and KY628989, respectively.

Both mitogenomes described comprise 2 rRNAs, 22 tRNAs, 13 protein-codon genes and the CR (Fig. 1). However, *S. olivascens* showed the ancestral avian mitogenome order (T/P/ND6/E/CR), while *L. angustirostris* showed the r_CR2 arrangement (Fig. 1), as expected for dendrocolaptids. In the latter, CR1 is found between $tRNA^{Thr}$ and $tRNA^{Pro}$ and there is also an extra 76 bp non-coding region (CR2) flanked by $tRNA^{Glu}$ and $tRNA^{Phe}$.

Mitogenome phylogeny of Passeriformes

The bayesian reconstruction yielded a well-supported topology with high support (posterior probabilities > 0.98) in most nodes (Fig. 2). Evolutionary relationships across the majority of passerine taxa are consistent with those previously described (e.g. [17]), sustaining a basal split between Tyranni (suboscine) and Passeri (oscine). The Tyranni clade is composed of two main lineages corresponding to Old (*Smithornis* and *Pitta*) and New World (*Lepidocolaptes, Cnemotriccus* and *Mionectes*) suboscines. The Lyrebird *Menura* is the sister lineage of the other oscines, while Passeroidea (finch-like birds) is the most recently derived lineage. However, in contrast, Acanthisittidae was not recovery as the basal lineage of Passeriformes, but the clade support was low.

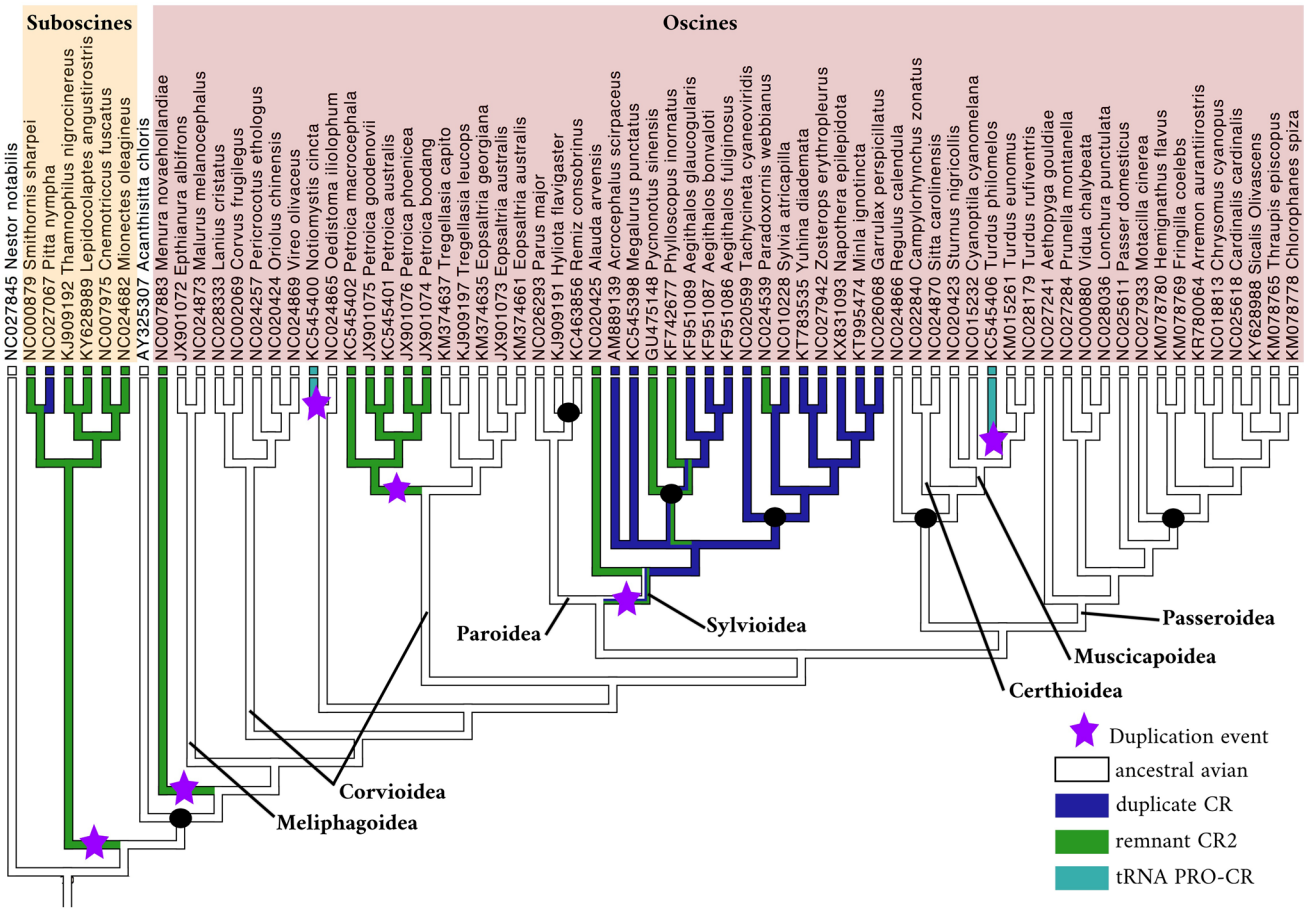

Fig. 2 Ancestral state reconstruction of the avian mitochondrial gene arrangements onto the Bayesian phylogenetic tree of Passeriformes. Most nodes showed posterior probability distribution higher than 0.98 (values < 0.98 are indicate by black circles). Numbers before the species names are the GenBank accessions. Bicolor or tricolor branches show that both gene arrangements are equally parsimonious as ancestral state

Evolution of gene rearrangements in Passeriformes

Reconstruction of the ancestral state of gene rearrangements revealed that the standard avian order is the ancestral state in Passeriformes and the most frequent arrangement in this order (Fig. 2). Furthermore, considering the most commonly suggested model for gene rearrangement where one duplication event is followed by the reduction or loss of one copy (e.g. [4]), this analysis also showed at least six independent duplication events: (I) in the basal diversification of Suboscines; (II) in Menuridae lineage; (III) in Notiomystidae lineage; (IV) in Petroica lineage; (V) in *T. philomelos* lineage; and (VI) in the basal diversification of Sylvioidea (Fig. 2). After duplication event, reduction or loss of parts of one copy (yielding r_CR2 arrangement) was observed to occur at least in four of these lineages, but likely initiating independently in different families of Sylvioidea. Although unlikely, there may have been a single duplication event in the basal diversification of Passeriformes followed by multiples independently reversals to standard avian order. According to Gibb et al. [4], gene duplications can be stable for long evolutionary periods due to continued gene conversion which eventually may revert to the original order.

In most Suboscines species studied and *M. novaehollandiae*, the CR2 was smaller than 301 bp in size (range from 76 to 301, Fig. S1) and this small segment aligned fragmented-way with different region of the suboscines' CRs (data not shown). This pattern is as expected for the derived arrangement r_CR2, in which most parts of the segments involved in the duplication are deleted or gradually degenerated [3]; only a small portion of the ancestral CR remains. The only exception was observed in the CR2 of *Pitta nympha*, which is the longest segment (1136 bp) and did not align with any suboscines CR analyzed. In addition, the 3′ segment is composed by around 25 bp tandemly repeated around 25 times.

In contrast, the CR2 observed in the derived arrangements of the Sylvioidea clade showed signs of drastic reduction only in four (Alaudidae, Pycnonotidae, Phylloscopidae and Timaliidae) of the eight families studied (Fig. 2 and Fig. S1). In the other species of this clade, CR2 were either a little longer, or similar in size, than the respective CR1. In all these latter cases, the duplicated CR (CR2) showed high similarity with their respective CRs (data not shown) and contained many of the conserved primary sequence motifs (e.g. F, D and C boxes, and CSB-1) that are typical of functional CRs in birds [29] and other vertebrates [30]. Furthermore, the CR2 of these Sylvioidea species are more closely related to paralogous segments (respective CRs) than the orthologous ones (Fig. 3). Thus, our data supports the hypothesis first raised by Singh et al. [5] that the duplication of the segment containing the CR occurred early on in

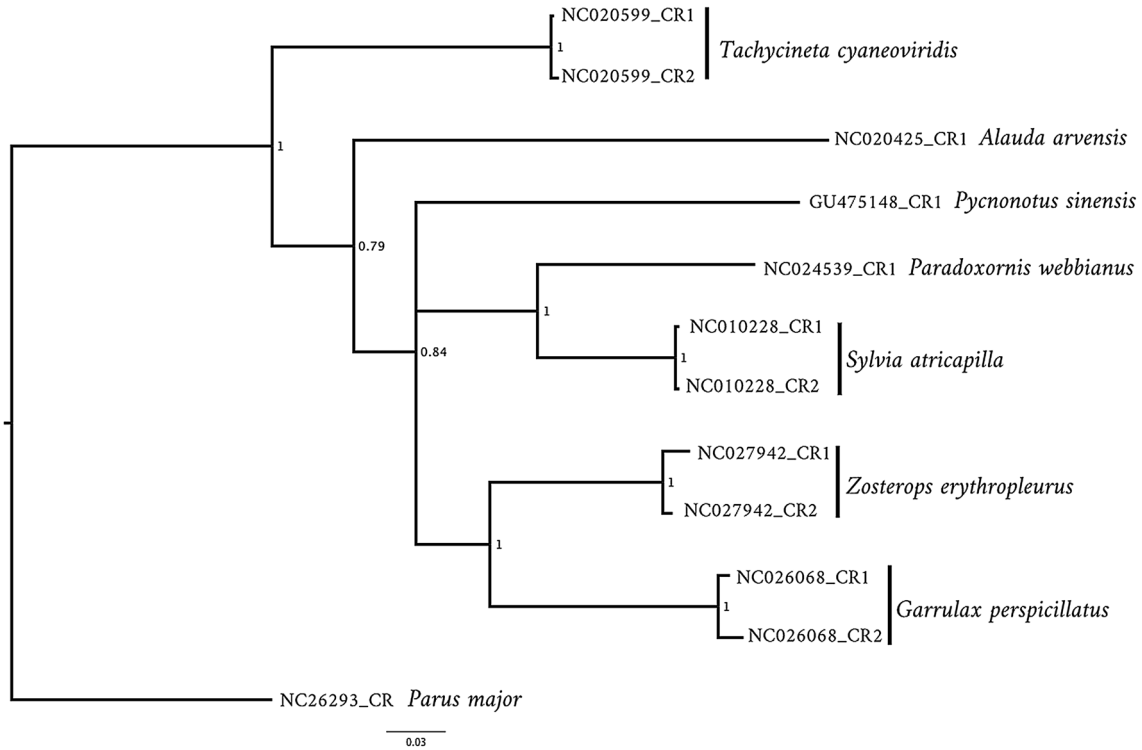

Fig. 3 Bayesian analysis of mitochondrial CRs (CR1) and duplicated CRs (CR2) of some Sylvioidea species. Posterior probabilities are given in each node. Numbers before the names are the Genbank accessions

Sylvioidea diversification and that both CRs of some Sylvioidea species may be maintained functional over evolutionary time, either through stabilizing selection or through occasional gene conversion as previously described in other bird orders (e.g. [7]).

In conclusion, our results reinforce the idea of multiple origins of some mitochondrial gene arrangements in Passeriformes, with ancestral state reconstruction suggesting at least six independent duplication events. Our results also reinforce evidence that both CRs in some Sylvioidea species seem to be maintained in an apparently functional state, perhaps by concerted evolution, but the molecular mechanisms involved should be further investigated.

Finally, our conclusions were based on almost exclusively in published mitogenome, and in few cases in incomplete genomes, such as *A. chloris*, and this may directly affect our findings if different genetic orders are found in some of these lineages.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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