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Article

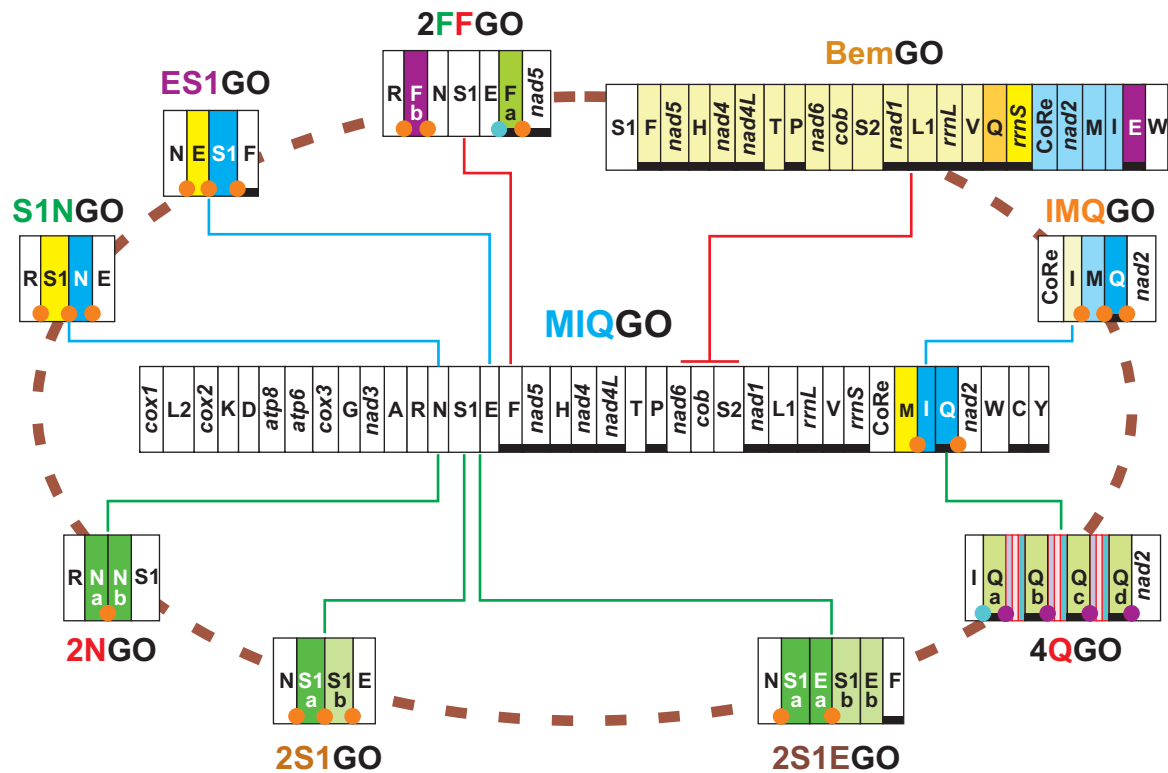
Macrostructural Evolution of the Mitogenome of Butterflies (Lepidoptera, Papilionoidea)

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Graphical abstract



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Simple Summary: Papilionoidea is a superfamily of Lepidoptera encompassing about 19,000 species. In the present work, we study the evolution of the structure of the mitogenome of these lepidopterans. The mechanisms generating the eight arrangements known for Papilionoidea were investigated analysing the movements of different mitochondrial genes. Five newly sequenced/assembled mitogenomes were included in our analysis involving more than 600 genomes. We provide new findings that help to understand the evolution of the gene orders MIQGO, IMQGO, 2S1GO, ES1GO and S1NGO in different butterflies. We demonstrate that the evolution of the 2S1GO in Lycaenidae followed a complicated pathway with multiple events of duplication and loss of *trnS1* and changes in anticodon. We describe two new gene orders 2FFGO and 4QGO for *Ampittia subvittatus* (Hesperiidae) and *Bhutanitis thaidina* (Papilionidae).

Abstract: The mitogenome of the species belonging to the Papilionoidea (Lepidoptera) is a double stranded circular molecule containing the 37 genes shared by Metazoa. Eight mitochondrial gene orders are known in the Papilionoidea. MIQGO is the plesiomorphic gene order for this superfamily, while other mitochondrial arrangements have a very limited distribution. 2S1GO gene order is an exception and is present in several Lycaenidae and one species of Hesperiidae. We studied the macrostructural changes generating the gene orders of butterflies by analysing a large data set (611 taxa) containing 5 new mitochondrial sequences/assemblies and 87 de novo annotated mitogenomes. Our analysis supports a possible origin of the intergenic spacer *trnQ-nad2*, characterising MIQGO, from *trnM*. We showed that the homoplasious gene order IMQGO, shared by butterflies, species of ants, beetles and aphids, evolved through different transformational pathways. We identify a complicated evolutionary scenario for 2S1GO in Lycaenidae, characterised by multiple events of duplication/loss and change in anticodon of *trnS1*. We show that the gene orders ES1GO and S1NGO originated through a tandem duplication random loss mechanism. We describe two novel gene orders. *Ampittia subvittatus* (Hesperiidae) exhibits the gene order 2FFGO, characterised by two copies of *trnF*, one located in the canonical position and a second placed in the opposite strand between *trnR* and *trnN*. *Bhutanitis thaidina* (Papilionidae) exhibits the gene order 4QGO, characterised by the quadruplication of *trnQ*.

Keywords: Papilionoidea; butterflies; mitogenomics; mitochondrial structural evolution; new gene orders



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1. Introduction

The mitochondrial genome (hereafter mitogenome) of all species of Lepidoptera (Insecta) sequenced so far is a double stranded circular molecule spanning 15–18 Kilo-base pairs [1]. The lepidopteran mitogenome encodes the standard 37 genes of the Insecta and more generally of bilaterian Metazoa [1]. This set includes 13 protein-encoding genes, 22 tRNAs, 2 ribosomal rRNA (small and large subunits) and a control Region (Figure 1) [1].

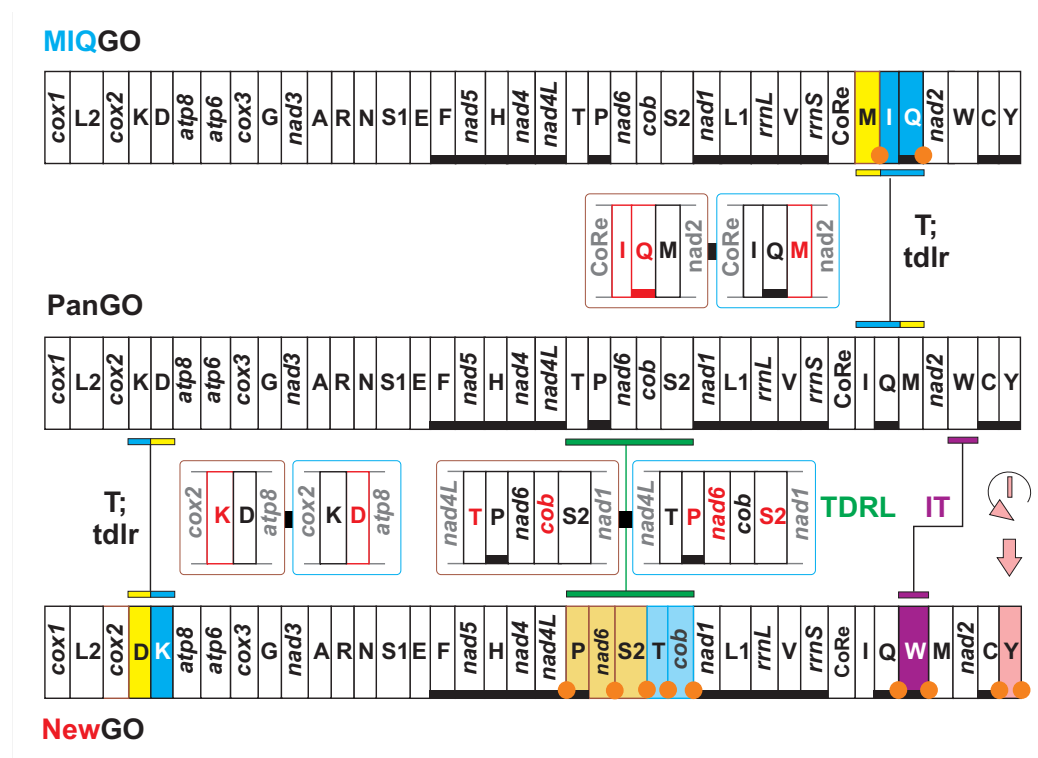


Figure 1. Mitochondrial macro-structural rearrangements. On top, transformational pathway from the gene order PanGO to the gene order MIQGO. On bottom, mechanisms generating a hypothetical new gene order. NewGO: hypothetical gene order generated by the rearrangements depicted in Figure 1. PanGO is linearized starting from *cox1*. The genes encoded on the plus strand (orientation from right to left in Figure 1) are black-boxed, while those encoded on the minus strand (orientation from left to right in Figure 1) are underlined and black-boxed. Nomenclature: *atp6* and *atp8*: ATP synthase subunits 6 and 8; *cob*: apocytochrome b; *cox1*–3: cytochrome c oxidase subunits 1–3; *nad1*–6 and *nad4 L*: NADH dehydrogenase subunits 1–6 and 4 L; *rrnS* and *rrnL*: small and large subunit ribosomal RNA (rRNA) genes; X: transfer RNA (tRNA) genes, where X is the one-letter abbreviation of the corresponding amino acid, in particular L1 (CTN codon family) L2 (TTR codon family), S1 (AGN codon family) S2 (TCN codon family); CoRe: Control Region. I: inversion; IT: inverse transposition, T: transposition event. tdlr: tandem duplication random loss mechanism producing the observed rearrangement. TDRL, Tandem Duplication Random Loss move. Gene/genes, transposed relative to PanGO, is/are shown with a yellow/blue background. The extra copy of every gene that is lost in the genomic rearrangement is red-boxed. Genes located immediately upstream/downstream the rearranged genes, and, possibly, partly involved by the genomic change are grey-boxed. An orange dot marks an intergenic spacer present in a position associated with a genomic rearrangement.

The disposition of genes, i.e., the gene order, along the mitogenomic sequence is not fixed (Figure 1). This dynamic behaviour of the mitogenomic structure can generate multiple gene orders within the same clade (e.g., [1,2]). With respect to a reference gene order, novel/alternative gene orders are generated through events of transposition, inversion and inverse-transposition of one or multiple genes (Figure 1). The transposition of single genes/cluster of adjacent genes can be explained through a tandem duplication random loss model [3,4]. The inversion of a gene, which implies its repositioning on the opposite DNA filament, can be modelled through an intramitochondrial recombination mechanism [5]. The inverse-transposition can be due to a combination of the mechanisms described above [2].

More complicated structural rearrangements can involve large segments of the mitogenome (e.g., [2,6,7]). Papetti et al. [7] reviewed the many models developed for explaining these rearrangements. However, most of them do not apply to known gene orders for butterflies (see below). We cite here only the Tandem Duplication Random Loss

move that allows explaining the simultaneous transposition of multiple non-adjacent genes (Figure 1) [8–10].

The 37 mitochondrial genes can be arranged in a huge number of gene orders (i.e., $37! = 1.367 \times 10^{43}$ or $38!$ if the control region is also included), assuming that the movement of every gene is equally probable [1,2]. However, this scenario is unrealistic because the movements occur preferentially along specific pathways and some genes, especially the tRNAs, are much more mobile than others [1,2,6]. The reduction in the possible rearrangements increases the probability of convergent evolution in gene orders. Convergence can be limited to the sharing of local homoplastic gene-dispositions or involve the full gene order (e.g., [2,6,11]).

When a gene order becomes a molecular landmark defining a clade in a unique manner, it assumes the role of mitogenomic apomorphy. However, not every gene order is a mitochondrial apomorphy [2]. The degree of rearrangement of a gene order plays a key role for reaching this status. If the level of rearrangement is very high and involves multiple genes and large genomic segments, the probability that homoplasious identical gene orders appear in unrelated taxa is minimal/null [2]. Conversely, low levels of mitogenomic rearrangements are prone to generate fully homoplasious gene orders (e.g., [2,6,11]).

The clade, Pancrustacea, containing Insecta plus Crustacea, is characterized by a gene order (hereafter listed as PanGO) that sets them apart from all other animals [12,13]. PanGO is widespread in Insecta, but different gene orders originating from its rearrangements occur in various taxa (e.g., [1]). The number of alternative gene orders and their level of rearrangement have a very uneven distribution among insect orders [1,11].

Within Lepidoptera, PanGO is found only in a few moth families that branch off at the base of the lepidopteran phylogenetic tree [14,15]. Most of the lepidopteran mitogenomes sequenced to date exhibit a gene order (hereafter listed as MIQGO) characterised by a transposition of *trnM*, *trnI* and *trnQ* with respect to PanGO (Figure 1) (e.g., [15,16]). The formation of MIQGO can be explained through a tandem duplication random loss mechanism (Figure 1) [3,4,17].

MIQGO is shared by a very large clade including Ditrysia (the biggest lineage of Lepidoptera), Tischeriidae and the Australian Palaephatoidea [15]. MIQGO has been hypothesised to be a potential synapomorphy for the phyletic group exhibiting it [15]. However, some ants (Hymenoptera), some bush-crickets (Orthoptera) and a praying mantis (Mantodea) exhibit MIQGO in their mitogenomes [6,11,18,19]. Even if homoplasious, MIQGO characterises a large phyletic lineage within the Lepidoptera. However, its value as a clade-delimiting character and its range of applicability is determined within a phylogenetic contest [2]. Here, we introduce the concept of mito-signature. A mito-signature is a mitochondrial feature, and its possible modifications, which characterises all the taxa located downstream to a node in a phylogenetic tree. According to this definition, a mito-signature is not necessarily an apomorphy, as it happens for the MIQGO.

The superfamily Papilionoidea is a large clade of the Lepidoptera order encompassing about 19,000 species [20]. This superfamily contains butterflies belonging to the families Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Riodinidae; the skippers (so named because they tend to skip from place to place with a quick flap of the wings) belonging to the family Hesperidae and the moth-like species to the family Hedyllidae [20–25].

MIQGO characterises the mitogenomes of most of the butterflies sequenced to date (Figure 1), but some butterflies have different gene orders. Distribution of the alternative gene orders is limited to terminal and sub-terminal branches of the Papilionoidea phylogenetic tree (Figures 1 and 2; Table 1).

2NGO, *Odontoptilum angulatum*



2S1GO, *Tagiades vajuna*



2S1GO, Lycaneidae (partim); *Ctenoptilum vasava*



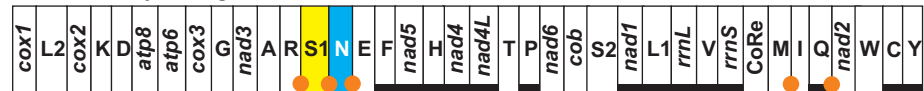
IMGO; *Euripus nyctelius*; species of ants, beetles and aphids



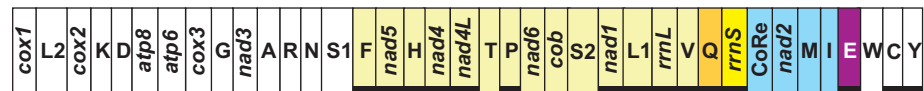
ES1GO, *Megathymus* genus



S1GO, *Erynnis* genus



BemGO, *Bematistes* subgenus



	P	M	I	S	E	B
PanGO	100					
MIQGO	90	100				
IMQGO	95	95	100			
S1NGO	85	95	90	100		
ES1GO	85	95	90	90	100	
BemGO	37	37	37	35	37	100

Figure 2. Alternative known gene orders and their distribution in butterflies. Genes characterizing each of genomic arrangements are presented with a coloured background. Green background, duplicated genes; yellow-orange/blue (different shades) background, transposed genes; purple background, gene that experienced and inverse-transposition. Nomenclature of genes as in Figure 1. On bottom, matrix-presenting scores (expressed in percentage) relative to shared common intervals among selected gene orders.

Table 1. Gene orders, distribution in Papilionoidea and key features.

Name	Taxonomic Distribution	Key Feature
PanGO	Not present in Papilionoidea	apomorphic gene order for Pancrustacea
MIQGO	standard gene order for Papilionoidea	<i>trnM</i> , <i>trnI</i> and <i>trnQ</i> transposed with respect to PanGO
2NGO	<i>Odontoptilum angulatum</i> (Hesperiidae)	gene arrangement identical to MIQGO + 2 <i>trnN</i>
2S1EGO	<i>Tagiades vajuna</i> (Hesperiidae)	gene arrangement identical to MIQGO + 2 (<i>trnS1</i> + <i>trnE</i>)
2S1GO	<i>Caenoptillum vasava</i> (Hesperiidae), species of Lycaneinidae	gene arrangement identical to MIQGO + 2 <i>trnS1</i>
IMQGO	<i>Euripus nyctelius</i> (Nymphalidae)	<i>trnI</i> , <i>trnM</i> transposed (IM vs. MI) with respect to MIQGO
S1NGO	<i>Erynnis</i> genus (Hesperiidae)	<i>trnS1</i> , <i>trnN</i> transposed (S1N vs. NS1) with respect to MIQGO
ES1GO	<i>Megathymus</i> genus (Hesperiidae)	<i>trnE</i> , <i>trnS1</i> transposed (ES1 vs. S1E) with respect to MIQGO
BemGO	<i>Acraea</i> genus, subgenus <i>Bematistes</i> (Nymphalidae)	major structural rearrangement encompassing multiple genes with respect to MIQGO
2FFGO	<i>Ampittia subvittatus</i> (Hesperiidae)	two copies of <i>trnF</i> located on opposite strands
4QGO	<i>Bhutanitis thaidina</i> (Papilionidae)	gene arrangement identical to MIQGO + 4 <i>trnQ</i>

We can divide the alternative gene orders into three types (Figure 2). Three gene orders with the same arrangement of MIQGO but presenting an extra copy of one or two adjacent tRNAs belong to the first type. The mitogenome of the skipper *Odontoptilum angulatum* exhibits a duplication of *trnN* (2NGO) [26]. Two contiguous copies of the *trnS1* and *trnE* occur in the mitogenome of the skipper *Tagiades vajuna* (2S1EGO) [27]. Finally, two copies of *trnS1* (2S1GO) appear independently in the mitogenomes of the skipper *Caenoptillum vasava* [28] and of several lycaenid butterflies (e.g., [29,30], present paper).

Three gene orders, characterised by a single event of transposition involving 2–3 contiguous tRNAs, form the second type. The arrangement IMQGO, where the *trnI* and *trnM* are transposed (IM vs. MI) with respect to MIQGO, is found in the mitogenome of the nymphalid *Euripus nyctelius* [31]. IMQGO is a homoplastic rearrangement as it also occurs in ants of the genus *Camponotus*, in curculionid beetles of the genus *Trypodendron*, and in the aphids of the family Pemphigidae [11,32,33]. A transposition of *trnS1* and *trnN* (S1N vs. NS1) characterises the arrangement S1NGO of the mitogenome of the skipper *Erynnis montanus* [34]. S1NGO is present in the mitogenome of other *Erynnis* species as described below. Finally, the giant-skipper of the *Megathymus* genus show an ES1GO arrangement, where *trnE* and *trnS1* are transposed (ES1 vs. S1E) with respect to MIQGO. The evolution of ES1GO is analysed in the present paper.

Only one gene order is known for being very different from MIQGO in Papilionoidea. Within the genus *Acraea* (Nymphalidae, Heliconinae), the four species of the subgenus *Bematistes* sequenced to date have a mitogenome exhibiting a major structural rearrangement (BemGO) encompassing multiple genes [35] (Figures 2 and S1). The complexity of the rearrangement occurring in the mitogenomes of *Bematistes* is evident provided that the reconstructed evolutionary pathway leading to this gene order implies the occurrence of the inverse-transposition of a gene (*trnE*) and two tandem duplication random loss moves (Figure S1). The peculiarity of BemGO is corroborated by the low percentage (37%) of shared common intervals with MIQGO (Figure 2). We will not analyse BemGO further in the present work, but we predict that it will prove to be a true synapomorphy for *Bematistes*.

Irrespective of the gene order, neighbour genes located on the same strand or opposite strands can be adjacent, overlapped or spaced by a variable number of nucleotides forming intergenic spacers (ISPs). DNA slippage during genome replication or mitogenomic rearrangements produce the ISPs [2,7,16]. The occurrence of a set of ISPs, congruent with the predicted evolutionary pathway leading to the formation of a specific gene order, is the first level of support for the proposed mitogenomic evolutionary scenario (e.g., [2,7]). The presence in the sequences of these ISPs of remnants of the genes involved in the rearrangement is more compelling evidence to confirm the transformational pathway generating a studied gene order (e.g., [2,7]).

Currently near complete/complete mitogenomes are available in the GenBank for more than 400 species of Papilionoidea and for some of them multiple sequences exist. This mitogenomic coverage is unmatched by any other lepidopteran Superfamily. Taking advantage of the large amount of data, we performed an in-depth analysis of the structural evolution of Papilionoidea mitogenome, both at a micro- and macro-structural level. We analysed more than 500 mitochondrial genomic sequences of Papilionoidea and about 140 outgroups were selected among the most closely related taxa [20]. We also sequenced the mitogenomes of two endangered butterflies, *Parnassius apollo* and *Lopinga achine*. Finally, we assembled the mitogenomes of the skippers *Erynnis brizo brizo*, *Gesta gesta* and *Ephyriades brunnea brunnea*, starting from available Illumina reads [36], as these taxa are key species for better understanding the evolution of SINGO within the HesperIIDae [37] (see below).

In this paper, we present the results of our research focusing on the macro-structural evolution of Papilionoidea mitogenome. In particular, we present new findings on: (a) the evolution of the key ISP *trnQ-nad2* characterizing MIQGO, (b) the transformational pathways generating IMQGO, ES1GO and S1NGO, (c) the complicated evolutionary scenario behind the occurrence of 2S1GO in Lycaenidae, and (d) the discovery of two new gene orders 2FFGO and 4QGO, and the genomic mechanisms producing them.

2. Materials and Methods

2.1. Sequencing of Two New Mitogenomes

One specimen of *Parnassius apollo* (Linnaeus, 1758) (Voucher: FPa02. Collection data: Italy, Trento Province, Caoria, 46°11'41.39" N 11°41'4.11" E, 28–30 June 2008, collected by E. Negrisolò) and one specimen of *Lopinga achine* (Scopoli, 1763) (Voucher: FLA03. Collection data: Italy, Trento Province, Tonadico, Villa Welsperg, 29 June 2008, 46°11'54.81" N 11°52'13.22" E, collected by E. Negrisolò) were collected in the Natural Park Parco di Paneveggio—Pale di San Martino (Trentino Province, Italy). Collection of the specimens was carried out according to Italian national rules on protected species and with the permission of the Paneveggio—Pale San Martino Park. Total DNA was isolated using the ZR Genomic DNA-Tissue Midiprep (Zymo Research corp., Irvine, CA, USA) Kit. DNA quality was assessed through electrophoresis. The amplification and sequencing of both genomes were carried out through a combination of universal and species-specific designed primers [38,39]. The sequencing of purified PCR products was performed at the BMR Genomics service (Padova, Italy) on automated DNA sequencers mostly employing the primers used for PCR amplification. Chromatograms were visualised and corrected with the Chromas 2.5.1 software (Technelysium Ltd., City of Brisbane, Australia). The assembly of the whole genome sequences was performed with the SeqMan II program from the Lasergene software package (DNASTar, Madison, WI, USA). The two new mitogenomes are available in GenBank through the accession numbers ON087695 (*L. achine*) and ON087696 (*P. Apollo*).

2.2. Assembly of New Mitogenomes for Three Skippers (HesperIIDae)

The raw reads for *Erynnis brizo brizo* (GenBank accession number: SRR7174469), *Gesta gesta* (GenBank accession number: SRR7174466) and *Ephyriades brunnea brunnea* (GenBank accession number: SRR7174465) were downloaded from the European Nucleotide Archive (ENA) public database in FASTQ format. The software FastQC [40] was used to assess the quality of the reads and the presence of adapter sequences. We used SPAdes v-3.15.3 [41] to assemble, de novo, the complete genome (nuclear and mitochondrial scaffolds) of the three samples. SPAdes v-3.15.3 was run under the careful mode. This option runs Mismatch-Corrector, a post-processing tool that uses the BWA tool to correct the assemblies using the Illumina reads. The unique contig corresponding to the mitogenome (mtDNA) was identified by BLASTn [42] search against the gene sequences of the previously assembled and annotated mitogenome of *Erynnis montanus*.

2.3. Data Set Creation and Standardized Annotation of Mitogenomes

Initially, a starting data set including 511 near complete/complete mitochondrial genomes of Papilionoidea plus 138 complete mitogenomes of lepidopteran outgroups was created (Tables S1–S3). Outgroups were selected among the most closely related lineages to the superfamily Papilionoidea [20] in order to maximize taxonomic diversity at the genus level. In particular, all the superfamilies within the Apoditrysia clade, for which mitogenomes were available, were included in the starting data set. Outgroup species exhibiting the MIQGO were preferred over other gene orders because the bulk of mitogenomes of Papilionoidea exhibit this gene order and we wanted to make the comparisons of mitogenomic features as simple as possible.

The availability of new mitogenomes in the GenBank is a continuous process, occurring at an unpredictable pace. The partial/complete sequences of Papilionoidea available in the GenBank before 1 April 2021 were included in all analyses carried out for the present work. If available, multiple mitogenomes of the same butterfly species were included for investigating the intraspecific behaviour (Table S1). Some relevant sequences, available only later, were included only in selected analyses.

To ensure a standardised annotation of 649 mitogenomes forming the starting data set, all original annotations were checked and sometimes modified. We followed the well-established guidelines for a homogeneous annotation of mitogenomes (e.g., [6,16,43]). Several mitogenomes of butterflies are available in the GenBank as sequences without annotation. We produced new complete annotations for these sequences (Table S1).

To annotate/re-annotate the mitogenomes, we followed the strategy described in detail in previous open access works, carried out by our group [2,6,16,44]. Boundaries of every gene were further checked according to the guidelines provided by Cameron [43]. Transfer RNA genes were identified using the tRNAscan-SE program [45] or recognised manually as sequences with the appropriate anticodon and capable of folding into the typical cloverleaf secondary structure of tRNAs [46]. The validity of these predictions was further enhanced by comparison, based on multiple alignment and structural information, with published orthologous counterparts. The boundaries of the ribosomal *rrnL* and *rrnS* genes were determined by comparison to the orthologous counterparts present in the mtDNAs of the species already sequenced, as well as structural information implied by direct modelling (data not presented here). In this paper, the strand encoding the majority of genes is listed as plus-strand. Conversely, the strand encoding the minority of genes is listed here as minus strand.

After the process of checking and reannotation, 38 mitogenomes of Papilionoidea were excluded as it was impossible to correct evident errors in their sequence (Table S2). Thus, the final data set included 611 mitogenomes. Within this set, 138 sequences were from species belonging to outgroups (Table S3), while 473 mitogenomes belonged to 393 species of Papilionoidea (Table S1).

2.4. Sequence Heterogeneity Assessment

The GC-skew = $(G - C)/(G + C)$ and AT-skew = $(A - T)/(A + T)$ [47] were used to measure the base compositional differences among mitogenomes. The skews were calculated with Excel (Microsoft TM).

2.5. Phylogenetic Analyses

A phylogenetic analysis was performed on a data set limited to species belonging to Lycaneinae + Riodinidae (Table S1), because a reference tree was necessary to plot the evolution of 2S1GO (see below). Initially, each set of the 13 orthologous proteins, encoded in the mitogenome (Figure 1) was aligned with the MAFFT software [48,49]. Then, these alignments were concatenated in a single multiple alignment. The latter was analysed according to the maximum likelihood (ML) [50]. ML trees were computed with the program IQ-TREE 2.1.3 [51]. A phylogenomic partition model was used [52]. The optimal partitioning scheme in addition to the best fitting evolutionary model were selected with

the ModelFinder method implemented in the program IQ-TREE 2.1.3 [53]. In the tree searching analyses, fifty independent runs were performed in order to avoid/minimize the possibility of becoming entrapped in sub-optimal trees. The ultrafast bootstrap test (UFBT) was performed to assess the statistical support of ML tree topologies (10,000 replicates) [54]. Finally, a neighbour-joining tree (NJ) was created through the analysis of the alignment of *trnS1* genes of Lycaenidae + Riodinidae. The NJ-tree was computed by applying the evolutionary model Kimura 2-parameter model [50] with the MEGA software [55]. The interior branch test (1000 replicates) was computed for the NJ-tree [56].

2.6. Macrostructural Evolution in the Mitogenome of Butterflies

Pairwise comparisons between different gene orders were performed with the CREx program [8]. This software analyses genomic rearrangement pathways using common intervals [8–10]. A common interval is a subset of genes that appear consecutively in two (or more) gene orders being investigated [8]. Initially, the number of shared common intervals (SCI) for each pair of gene orders was computed with CREx. The control region was included in the computation of values of shared common intervals. The computed values were transformed in percentages with Excel (Microsoft™). Percentages were used to evaluate the level of conservation among different gene orders [6]. Highly divergent gene orders share low percentages of shared common intervals (e.g., [2,6]).

The CREx program models rearrangements involving transpositions, inversions and inverse transpositions, in addition to the more complicated tandem duplications random loss moves [3–5,8–10]. In a tandem duplications random loss move, a tandem duplication of a continuous segment of genes occurs. Thus, the original segment and its copy are arranged consecutively. This duplication is followed by the loss of one copy of each redundant gene [10]. Multiple genes simultaneously change their position in a tandem duplications random loss move.

The online version of the ClustalW program available at the PRABI Rhone-Alpes Bioinformatics Centre NPS@ (<http://www.prabi.fr/>, accessed on 5 April 2022) was used to perform the alignments of ISPs and the genes involved in the mitogenomic rearrangements. The alignments were improved manually by visual inspection.

3. Results and Discussion

3.1. Mitogenomes: New Sequences, New Assemblies and New Annotations

We sequenced/assembled the complete mitogenomes of *Parnassius apollo* (Papilionidae), *Lopinga achine* (Nymphalidae), *Ephyriades brunnea brunnea* (Hesperiidae), and *Erynnis brizo brizo* (Hesperiidae), and the near complete genome of *Gesta gesta* (Hesperiidae). Size, AT-, GC- contents and skews are provided in Table S1. The five mitogenomes contained the canonical 37 genes of insect mitogenomes [1]. MIQGO characterises the mitogenome of *P. apollo*, *L. achine*, *E. brunnea brunnea* and *G. gesta*, while S1NGO occurs in the sequence of *E. brizo brizo* (see below). The five new genomes were included in the data set used in this paper to study the structural macroevolution of the mitogenome of butterflies. We annotated, de novo, 87 mitogenomes. Fifty-four sequences represented new species while the remaining sequences belonged to taxa with at least one mitogenome available (Table S1).

3.2. Some Statistics on Mitogenomes of Butterflies

The average size of the mitogenomes of butterflies was $15,303.35 \pm 224.69$ bases. The range of variation spanned from 14,964 bases (*Protesilaus protesilaus*, Papilionidae) to 17,733 (*Hesperia comma*, Hesperiidae). Mitogenomes were A + T-rich (%-average = $80.49\% \pm 0.95$) with a range of variation spanning from 76.01% (*Acraea (Telchinia) polis*, Nymphalidae) to 83.13% (*Hesperia comma*, Hesperiidae). On the other hand, the G + C content (%-average = $19.48\% \pm 0.95$) varied from 16.87% (*H. comma*) to 23.98% (*A. polis*). The mitogenomes exhibit AT-skews (AT-skew-average = -0.077 ± 0.018) ranging from -0.021 (*Cethosia cyane*, Nymphalidae) to 0.017 (*Hypolimnas bolina*, Nymphalidae). The GC-skews were always negative (GC-skew-average = -0.214 ± 0.024) and ranged from -0.326 (*Acraea*

(*Bematistes alcinoe*, Nymphalidae) to -0.149 (*Dodona durga*, Riodinidae). All the species listed above exhibited the MIQGO arrangement with only exception represented by *A. alcinoe* (BemGO) (Figures 1 and 2). Three of the four *Bematistes* butterflies exhibited the lowest GC-skews (Table S1). This result would suggest an impact of the BemGO genomic arrangement on the GC-skew. However, *Isoteinon lamprospilus*, a skipper with MIQGO, shows a GC-skew lower than the fourth *Bematistes* butterfly, i.e., *Acraea (Bematistes) poggei*. Very similar GC-skews characterised the mitogenomes of other butterflies presenting MIQGO (Table S1). These results suggest that the analysed different gene orders have a minimal/null effect on GC-skews. The homogenous behaviour of the statistics presented above also supports the view that no mitogenome experienced inversion of the control region, an event that is not immediately evident but can be detected through the computations of AT- GC-skews, which exhibit a change in the sign of their values if the control region is located on the opposite strand [57]. The statistics computed for the mitogenomes of outgroups mirrored those of butterflies, suggesting a homogenous behaviour within the analysed data set (Table S3).

3.3. The Origin of the Intergenic Spacer *trnQ-nad2* in the Mitogenome of Butterflies

Mitogenomes of butterflies sequenced to date, exhibiting MIQGO, have an intergenic spacer located between *trnQ* and *nad2* (ISP *trnQ-nad2*) (Figure 1). This ISP was produced during the formation of MIQGO. Some authors think that the ISP *trnQ-nad2* is a remnant of a partly duplicated *nad2* that was generated during the rearrangement process [58–60]. Supporters of this hypothesis provided pairwise alignments of the ISPs *trnQ-nad2* and *nad2s* [58–60]. In these comparisons, the ISPs *trnQ-nad2* aligned with different portions (hereafter segments) of the *nad2* with percentage of identity for each pair varying from 62% to 78%. The search of these segments for the new mitogenomes of *P. apollo* and *L. achine* provided ambiguous results (Alignments S1–S2). In both species, at least two distinct *nad2* segments were identified with very similar percentages of identity (67.44% vs. 68.89%, *P. apollo*; 68.33% vs. 70.97%; *L. achine*), and further segments with percentages of identity >62% occurred in both butterflies. Because this behaviour could be peculiar for the new mitogenomes, the same search was repeated for species of Hesperiiidae including all taxa analysed by Kim et al. [60]. Results were very similar to those obtained for *P. apollo* and *L. achine* and multiple distinct segments of *nad2* were identified with very similar percentages of identity for each species (Alignments S3–S8). In some cases, the newly identified *nad2* segments exhibited higher percentages of identity than those identified by Kim et al. [60]. In *Choaspes benjaminii*, the segments B (percentage of identity = 78.08%) and E (percentage of identity = 78.08%) outperformed the D segment (percentage of identity = 69.32%) identified as the best match by Kim et al. [60] (Alignment S3). The same behaviour was observed for *Lobocla bifasciatus* and *Carterocephalus silvicola*. Thus, multiple distinct segments with very similar percentages of identity can be identified in each comparison (Alignments S4 and S7). The positioning of these segments along *nad2* is very variable, spanning most of the gene length.

A detailed analysis of the *nad2* segments vs. ISP *trnQ-nad2* alignments identified some common patterns (Alignments S1–S8). (a) The number of identical contiguous positions is low/very low and ranges from two to 13 (e.g., *C. benjaminii*, segment B), with 3–8 representing the more common condition. (b) Nucleotide composition of conserved positions is A/T-rich, in agreement with the compositional bias of butterflies' mitogenomes. (c) The motifs of conserved nucleotides are, in many cases, not unique and have multiple counterparts scattered along the whole mitogenomic sequence.

If the *nad2* segment with the best percentage of identity is regarded as the true orthologous counterpart of the ISP *trnQ-nad2*, the following evolutionary scenario must be hypothesised.

First, the formation of MIQGO generated a near complete/complete second upstream copy of *nad2* that remained largely unchanged in the last common ancestor of Papilionoidea, and in the successive ancestors of families, subfamilies and so on. This behaviour is

necessary because the ISPs *trnQ-nad2* align, according to the best percentage of identity scores, with very different segments, covering the whole length of *nad2*. Second, the shrinking of the pseudo-*nad2* leading to ISP *trnQ-nad2* must have occurred suddenly in the most recent ancestors of current species, because the length of these spacers is usually smaller than 100 nucleotides.

This evolutionary scenario is not plausible as it implies a very different behaviour of the lepidopteran mitogenome, i.e., a long period of stasis with the duplicated *nad2* transmitted through most of the branches of the tree, followed by very rapid shrinking in the sub-terminal/terminal nodes. Until now, there has been no evidence of this double behaviour in any studied animal mitogenome. The hypothesis of a long-lasting presence for a second near complete *nad2* is further contradicted by the fact that there is a strong selective pressure towards the maintenance of a compact mitogenome and constant gene content [7]. Finally, by assuming the origin of ISP *trnQ-nad2* from distinct segments of *nad2*, aligning them should not be possible as they represent the remnants of different non-orthologous segments of *nad2*, as identified through pairwise comparisons.

The analysis of the mitogenome of the praying mantis *Humbertiella nada*, which shares with butterflies the homoplastic MIQGO, allowed us to hypothesise a different evolutionary scenario for the ISP *trnQ-nad2* origin. The ISP *trnQ-nad2* of *H. nada* contains in its sequence a pseudogene of *trnM* (Figure 3).

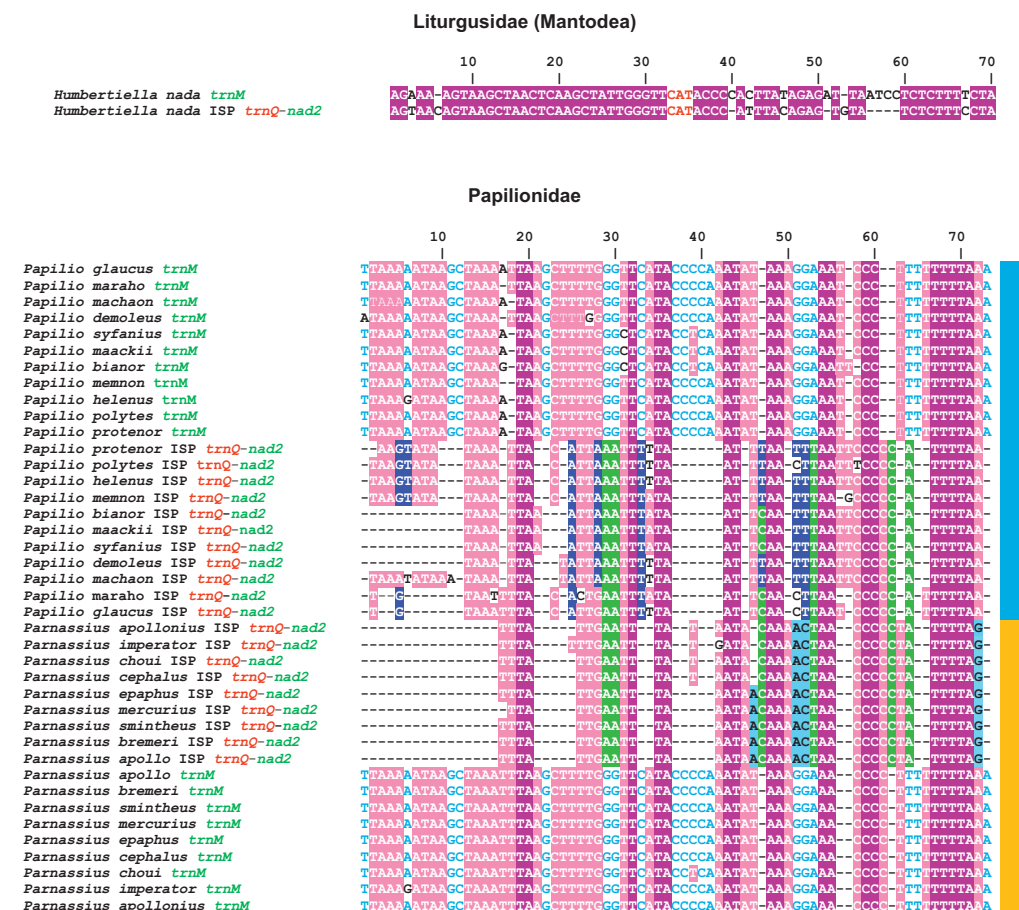


Figure 3. Alignments of *trnMs* vs. ISPs *trnQ-nad2*. On top, pairwise alignment of *trnM* vs. ISPs *trnQ-nad2* in *Humbertiella nada*. On bottom, multiple alignments of *trnMs* and ISPs *trnQ-nad2* of butterflies of the genera *Papilio* (Paapilioninae) and *Parnassius* (Parnassiinae). Deep-purple background, fully conserved nucleotide; pink background, majority rule (>50%) conserved nucleotide; cyan-coloured position, conserved nucleotide in *trnMs*; dark-blue background, conserved position in ISPs *trnQ-nad2* of *Papilio* species; light-blue background, conserved position in ISPs *trnQ-nad2* of *Parnassius* species; green-background conserved position in ISPs *trnQ-nad2* of *Papilio* + *Parnassius*.

The ISP *trnQ-nad2* and *trnM* of *H. nada* share the identical segment AGTAAGCTAACT-CAAGCTATTGGGTCATACCC, spanning 33 nucleotides, including the anticodon CAT. The probability that an identical segment of this size appeared independently in ISP *trnQ-nad2* and *trnM* is null, as shown in other animal mitogenomes [7].

This finding prompted us to test the hypothesis that in lepidopteran mitogenomes, during the homoplastic genomic rearrangement that generated MIQGO, *trnM* also contributed to the formation of the ISP *trnQ-nad2* (Figure 1). To test this alternative evolutionary scenario, multiple alignments were created at different taxonomic levels containing the ISP *trnQ-nad2* and *trnM* sequences (Figure 3; Alignments S9–S12).

Before discussing the outputs of these alignments in detail, an explanation is necessary. The nucleotide substitution process is not limited by constraints in the sequences of ISP *trnQ-nad2*; conversely, the *trnMs* are under strong purifying selection. This opposite behaviour suggests that there should be a rapid/very rapid drop in the percentage of fully conserved positions when alignments are carried out at a taxonomic rank higher than genus. To mitigate this problem, we evaluated the pattern of conservation through a majority rule approach. Thus, we considered not only invariable positions but also positions conserved in more than 50% of the aligned sequences.

The analysis of the *trnMs* vs. ISPs *trnQ-nad2* alignments revealed some general features (Figure 3, Alignments S9–S12). (a) ISPs *trnQ-nad2* and *trnMs* can be aligned up to the level of families that are sister taxa (e.g., Lycaenidae and Riodinidae) (Alignment S10). We did not attempt a global alignment at a superfamily level. Within Papilionidae the level of conserved positions is 68.49% (50/73) (Figure 3). This alignment included butterflies of the genera *Parnassius* and *Papilio*, two taxa belonging to two distinct subfamilies that diverged around 59 MYA [61]. Within the Hesperidae, the conserved positions account for the 55.67% (54/97) and the aligned sequences are derived from butterflies of different subfamilies that share a last common ancestor around 65 MYA old [61] (Alignment S9). Within the subfamily Pierinae, the analysed species share a last common ancestor dating back to 55.25 MYA [61] and the conserved positions represent the 64.47% (49/76) (Alignment S11). Within Lycaenidae and Riodinidae (Alignment S10), sharing a last common ancestor 97.36 MYA old [60], the conserved positions represent the 60.52% (46/76). Thus, the percentage of conserved positions is always above 50%. (b) ISPs *trnQ-nad2* and *trnMs* share conserved motifs in their alignments.

(c) The ISPs *trnQ-nad2* exhibit levels of conservation that make an origin from different mitochondrial segments implausible, as outlined in the examples listed below (Figure 3; Alignments S9–S12). Within Lycaenidae, the ISPs *trnQ-nad2* alignment exhibits 76.92% of conserved positions (50/65). Within Hesperidae, the conserved positions account for 62.36% (58/93). Within Lycaenidae + Riodinidae, the conserved positions are 63.23% (43/68). Finally, hypothesising an origin from *trnM* is also a good fit to the actual size of most of ISPs *trnQ-nad2* and does not require very asymmetrical behaviour, i.e., a long stasis followed by a very rapid gene shrinking, necessary if *nad2* is considered the true ancestor of ISP *trnQ-nad2*.

Our findings exhibit some limitations. In contrast to what was found in the praying mantis *H. nada*, we did not identify within the alignments a unique DNA segment shared only by *trnMs* and ISPs *trnQ-nad2*. Furthermore, the sequence motifs shared by *trnMs* and ISPs *trnQ-nad2* are short, ranging from two to seven nucleotides, and not unique. The formation of the ISP *trnQ-nad2* is a very old event in the mitogenomes of Lepidoptera [15] and as mentioned above, this spacer does not have any structural constraint preventing it from changing quickly and not homogeneously in the different lineages of butterflies.

Our results provide support to the origin of ISP *trnQ-nad2* from the *trnM*. However, this support is not undisputable, and alternative evolutionary scenarios can be put forward to explain the percentages of identical nucleotides shared by ISPs *trnQ-nad2* and *trnMs*. We believe that the *trnM* is a better candidate than *nad2* for explaining the origin of ISP *trnQ-nad2* in Lepidoptera. However, future analyses and more compelling evidence will be necessary to definitely prove or disprove this hypothesis.

3.4. Different Transformational Pathways Generate the Homoplasic IMQGO

As mentioned above, IMQGO is the homoplasic rearrangement shared by the nymphalid *E. nyctelius*, the ants of genus *Camponotus*, the beetles of the genus *Trypodendron* (Curculionidae), and the aphids of the family Pemphigidae. However, IMQGO appeared in these taxa following different transformational pathways (Figure 4).

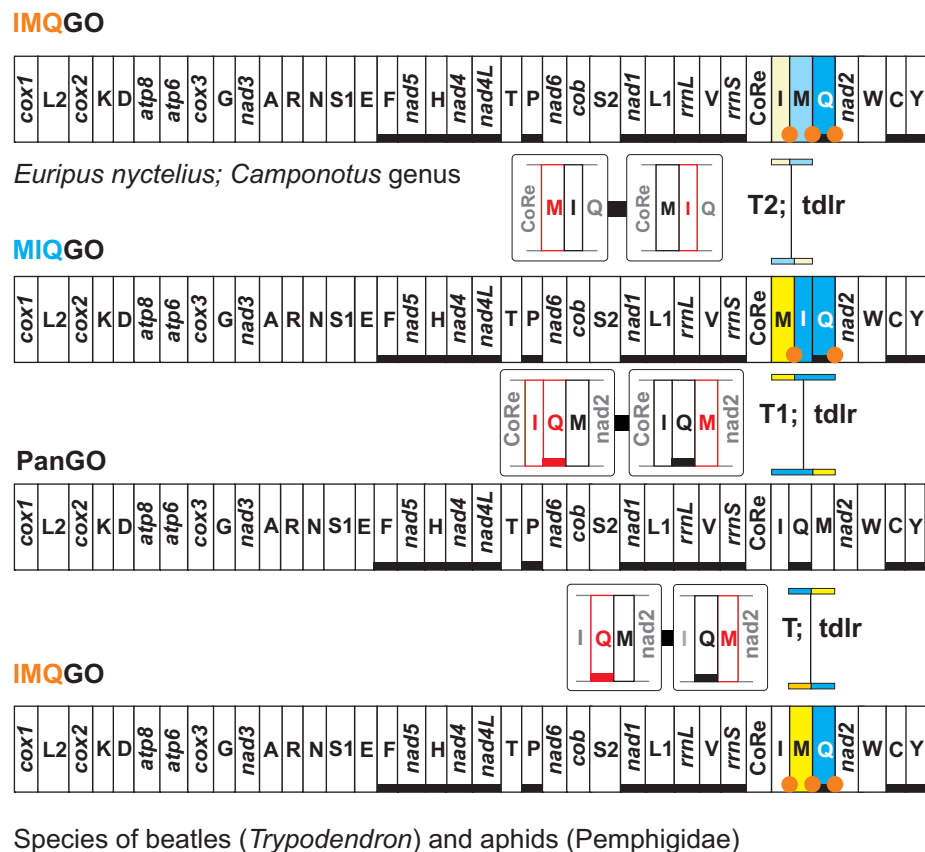


Figure 4. Mitochondrial transformational pathways generating IMQGO. Gene colours, nomenclature, as well mechanisms responsible for rearrangements depicted and labelled as in Figure 1.

Two events of transposition generated the final arrangement in *E. nyctelius* and the ants of the genus *Camponotus*. MIQGO is the plesiomorphic condition for both butterflies and ants [6,15]. Thus, there was not a direct transformational pathway going from PanGO to IMQGO for ants and lepidopterans. Conversely, a single event of transposition generated IMQGO in beetles and aphids (Figure 4). The analysis of the ISPs, associated with IMQGO, supports this single-step evolutionary scenario due to the presence of undisputed remnants of the copies of genes lost during the rearrangement (Figure 4; Alignments S13 and S14) [2,7]. Within the ISP, *trnQ-nad2* and ISP *trnM-trnQ* are present remnants of the copies of *trnM* and *trnI* that were lost in transposition. A remnant of the control region was identified in the ISP *trnM-trnQ* of *Trypodendron signatum* (Alignment S14). The presence of the control region remnant does is not surprising if we consider that any rearrangement process for the integrity of the moving genes must also involve portions of the genes located upstream/downstream to the genomic segment interested by the macro-structural change. Interestingly, IMQGO is structurally more similar to PanGO than MIQGO, as revealed by the percentages of shared common intervals (Figure 2).

3.5. The Evolution of 2S1GO within the Lycaenidae

Several lycaenid butterflies share with the skipper *C. vasava* the homoplasic 2S1GO (Figure 2), which implies the occurrence of two contiguous copies of *trnS1* (this pa-

per) [28–30]. To study the evolution of 2S1GO in Lycaenidae, the multiple alignment of *trnS1s* belonging to the 611 taxa was generated. The alignment was carried out using the secondary structure determined for *trnS1* (Alignment S15) as a template. A phylogenetic tree was computed for the *trnS1s* of Riodinidae + Lycaenidae using the software MEGA (Figure 5).

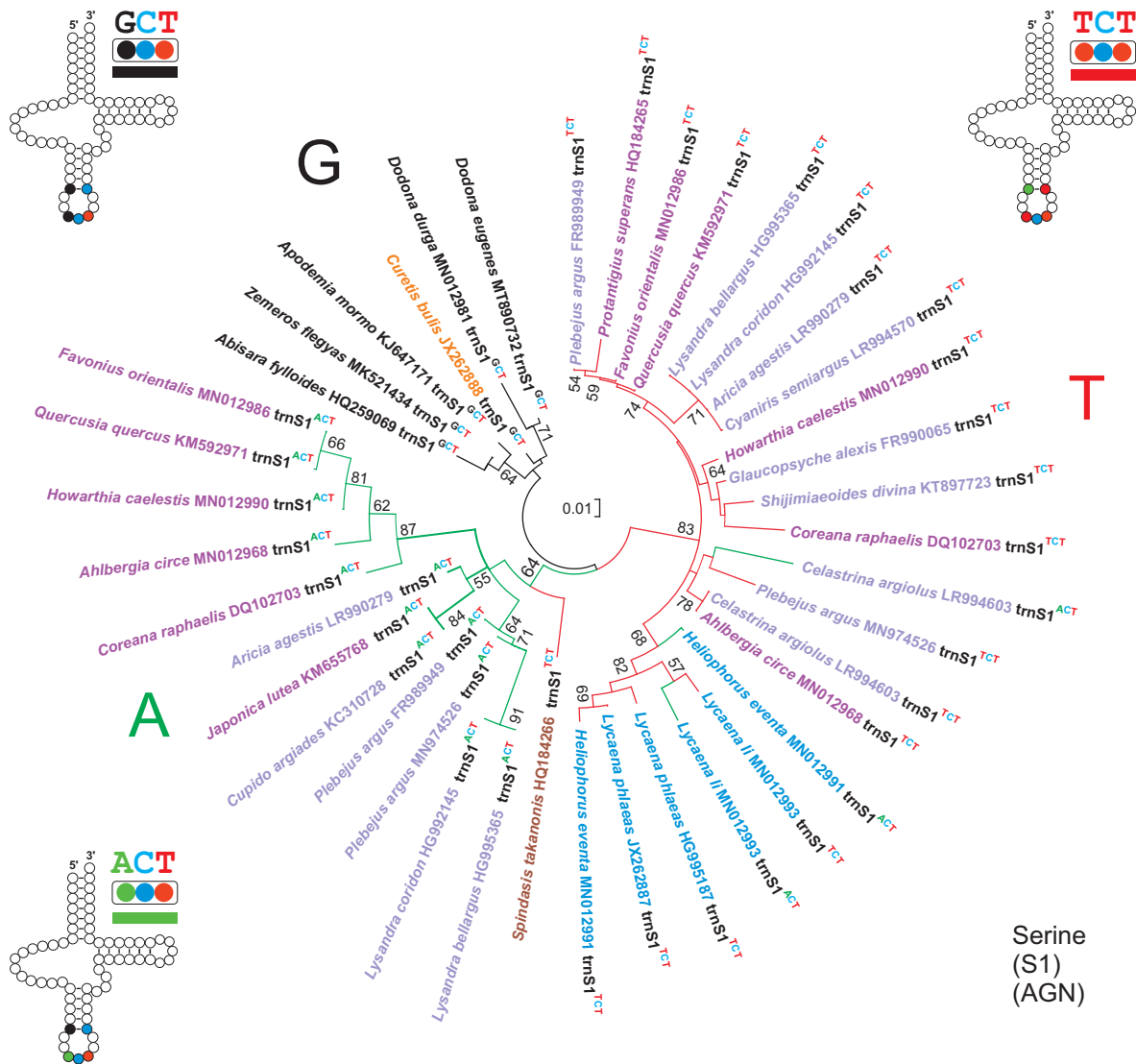


Figure 5. Phylogenetic relationships among *trnS1s* of Riodinidae + Lycaenidae. Neighbour-joining tree computed with MEGA. Number on branches are values, expressed in percentage, of the interior branch test. Secondary structures are provided for different types of *trnS1*. Names of species of Lycaenidae belonging to different subfamilies are coloured as follows: orange, Curetinae; cyan, Lycaeninae; light brown, Aphnaeinae; light-purple, Theclinae; light lilac, Polyommatinae.

Finally, the distribution and the type of *trnS1* present in the different lycaenid butterflies was mapped in the ML reference phylogeny (Figure 6).

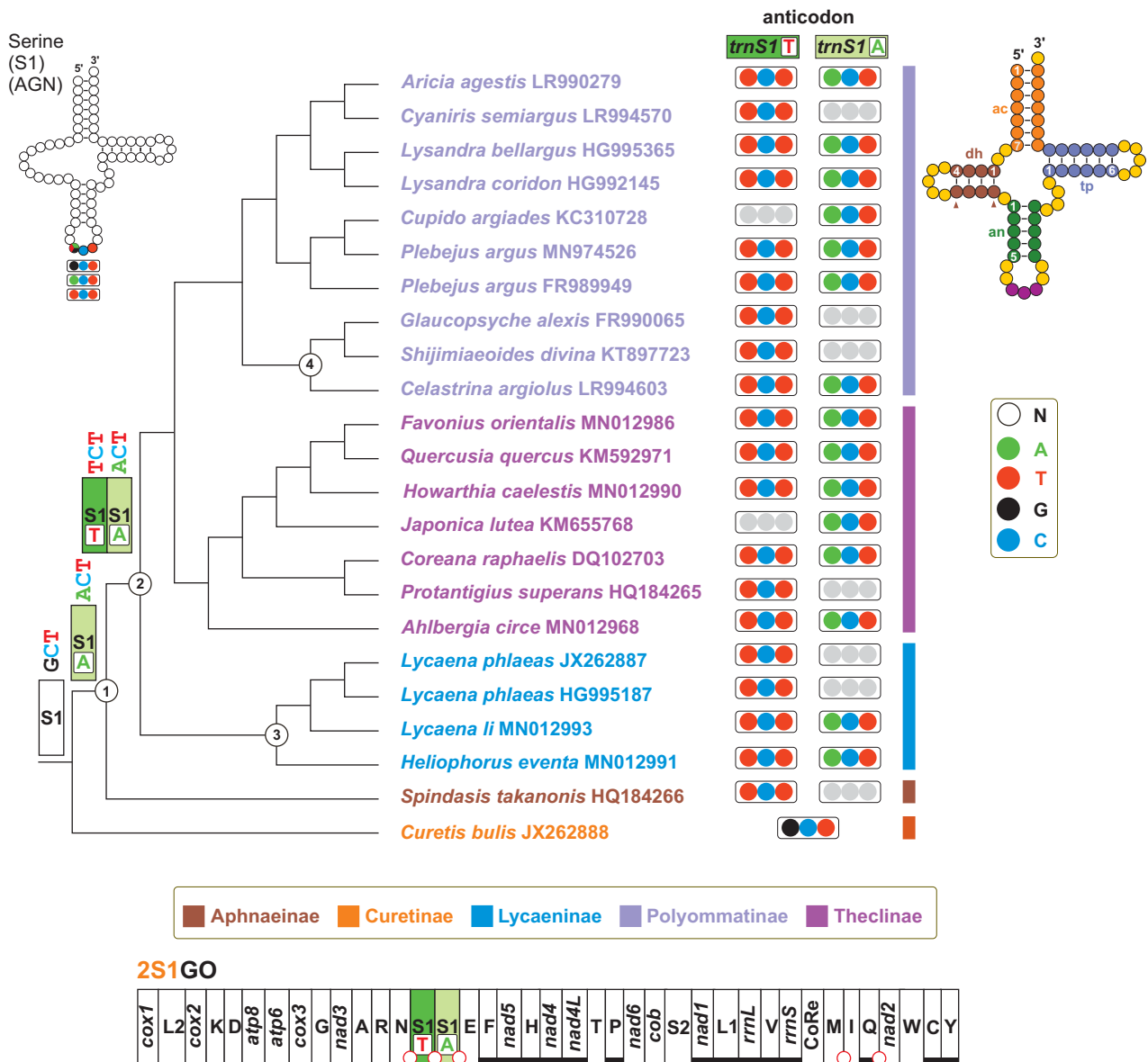


Figure 6. Evolution of *trnS1* in Lycaenidae. Tree, obtained with a ML analysis, depicting the phylogenetic relationships among analysed Lycaenidae. For reason of clarity, only the tree topology of the ingroup is provided. Outgroups (five species of Riodinidae) as well as UFBT node support (always 100%) are not presented. On top right, the secondary structure of *trnS1*: ac, acceptor stem; dh, DHU stem; an anticodon stem; tp, T Ψ C stem (nomenclature as in Montelli et al. [62]).

Outputs of these combined analyses revealed that the evolution of 2S1GO in Lycaenidae followed an unexpected, complicated pathway.

In Lepidoptera, the *trnS1* sequences exhibit three different anticodons, i.e., *trnS1*^{GCT}, *trnS1*^{ACT} and *trnS1*^{TCT} (Alignment S15). The *trnS1*^{GCT} is the most widespread and represents the plesiomorphic condition, while the alternative *trnS1*^{ACT} and *trnS1*^{TCT} appears independently through a process of parallel evolution in butterflies/moths belonging to different families (some examples are provided in Alignment S15).

Among the Lycaenidae sequenced so far, only *Curetis bulis* exhibits the *trnS1*^{GCT} (Figures 5 and 6). All other species have one *trnS1*^{ACT}/*trnS1*^{TCT} or both. When both *trnS1* types occur, they are always arranged in the 5'–3' order *trnS1*^{TCT}–*trnS1*^{ACT} (Figure 6). The phylogenetic analysis performed on the *trnS1* of Lycaenidae + Riodinidae identified three major clusters: A, G and T (Figure 5). Only *trnS1*^{GCT} sequences are included in cluster G.

Only $trnS1^{ACT}$ sequences belong to cluster A. The $trnS1^{TCT}$ of *Spindasis takanonis* is a sister taxon of this cluster. Finally, cluster T includes all other lycaenid $trnS1^{TCT}$ sequences, and the $trnS1^{ACT}$ of *Lycaena li*, *Heliophorus eventa* and *Celastrina argiulus*. These three species have a second $trnS1^{TCT}$ located within the cluster T (Figure 5). Irrespective of the anticodon type, all $trnS1$ sequences belonging to cluster T exhibit a fully compensatory base change in the an5 fifth base-pair of anticodon stem (i.e., G–C vs. A–T) (Figure 6; Alignment S15) [62]. This change is considered fully compensatory because it does not alter the secondary structure of $trnS1$ [62]. Among the 611 sequences analysed here, which belong to 586 lepidopteran species, the same fully compensatory base change is present also in the skipper *Ochlodes venatus* (Hesperiidae) and in the moth *Eogystia hippophaecolus* (Cossidae), while all other taxa exhibit the plesiomorphic condition A–T. Interestingly, both *O. venatus* and *E. hippophaecolus* have a $trnS1^{TCT}$ sequence (Alignment S15).

Combining the phylogenetic output with the structural features exhibited by the $trnS1$ sequences and the distribution of different anticodon types in lycaenid butterflies, an evolutionary scenario was reconstructed and is described in the steps (A–G) detailed below. Some parts of this transformational pathway are well corroborated, while others are tentatively sketched (Figure 6).

- (A) The common ancestor of Lycaenidae had a single $trnS1^{GCT}$ in its mitogenome, a condition that is still present in *C. bulis*, which belong to the Curetinae, the sister group of all other Lycaenidae [23]. The $trnS1^{GCT}$ of *C. bulis* also shows the plesiomorphic condition for all the nucleotide pairs in the stems of the secondary structure (Alignment S15).
- (B) First, a shift from the $trnS1^{GCT}$ to $trnS1^{ACT}$, favoured by the fact that it is a transition from G to A, occurred in the ancestor of the not-Curetinae Lycaenids (node 1, of Figure 6). Representative of this early change are the $trnS1s^{ACT}$ contained in cluster A, which exhibit mostly a plesiomorphic condition in their nucleotide sequences (Alignment S15).
- (C) Successively, in the common ancestor of the Lycaeninae, Polyommatainae and Theclinae, a duplication of $trnS1^{ACT}$ occurred and was followed by a shift in the TCT anticodon in the upstream copy (node 2, Figure 6). In this ancestral $trnS1s^{TCT}$ the G–C fully compensatory change also appeared, characterizing the 5an-pair of the anticodon stem (Figure 6; Alignment S15). The G–C change, present in the 5an-pair, has an extremely limited distribution among the 586 lepidopteran species studied here (Tables S1 and S3), which are representative of quite a large taxonomic diversity within Lepidoptera [20]. Thus, we regard it as highly improbable that this type of change occurred independently multiple times in Lycaenidae.
- (D) Within Polyommatainae and Theclinae, multiple independent losses interested both $trnS1^{ACT}$ and $trnS1s^{TCT}$.
- (E) Within the Lycaeninae (node 3, Figure 6), the ancestral $trnS1^{ACT}$ was lost, but successively $trnS1s^{TCT}$ was duplicated and one copy reverted to $trnS1^{ACT}$. The latter is present in *Lycaena li* and *Heliophorus eventa*, while it was successively lost in *Lycaena phlaeas*. This transformational pathway is supported by the fact that (1), irrespective to the anticodon type, the $trnS1s$ of Lycaeninae share and unique nucleotide pattern in their TΨC loop. (2) The Lycaeninae $trnS1s^{ACT}$ are nested within the T cluster and share the G–C pair in the an5 position of the anticodon stem with all the $trnS1s^{TCT}$ (Figure 5; Alignment S15).
- (F) The ancestral $trnS1^{ACT}$ was lost in a subclade of Polyommatainae (node 4; Figure 6), but in *Celastrina argiulus* there occurred a duplication of $trnS1^{TCT}$ and successive transformation of the downstream copy in $trnS1^{ACT}$.
- (G) A shift from $trnS1^{ACT}$ to $trnS1s^{TCT}$ occurred in the branch leading to *Spindasis takanonis*. In favour of this reconstruction is that the $trnS1^{TCT}$ of *S. takanonis* is sister taxon to cluster A and retains the plesiomorphic A–T pair in its anticodon stem (Figure 5; Alignment S15).

The *trnS1^{ACT}* of *Japonica lutea* (KM655768) is identical to the *trnS1^{ACT}* of *Cupido argiades* (KC310728), which belongs to the different subfamily Polyommatainae. Furthermore, the *J. lutea trnS1^{ACT}* (KM655768) lacks a very peculiar fully compensatory base change (A–T vs. T–A) in the tp1-pair of the TYC stem, which is present in all other *trnS1s^{ACT}* of Theclinae (Figure 6; Alignment S15). All this is very unusual and needs further corroboration.

The evolutionary scenario depicted above shows a very complex transformational pathway with repeated events of duplications and loss of the different types of *trnS1*, as well as changes between the ACT and TCT anticodons. Not all steps described above are fully settled and a more complete taxon sampling is necessary to obtain a well-defined complete picture. Irrespective of some level of uncertainty, what clearly emerges is that the Lycaenidae are unique among butterflies and, more in general, Lepidoptera, for the high plasticity of their *trnS1*. This plasticity was present in the common ancestor of the non-Curetinae lycaenids and remained in the descendants, as proved by the multiple changes described above. Finally, the presence of this high plasticity suggests that *trnS1s* must be excluded from phylogenetic analyses because there is no guarantee that the relationship of orthology is fulfilled even among sequences sharing the same anticodon.

3.6. Two Mito-Signatures for the Genera *Erynnis* and *Megathymus*

To our best knowledge, the evolution of ES1GO in the skippers of the *Megathymus* genus has never been studied in detail. S1NGO was unrecognised by the group that sequenced the *Megathymus* mitogenomes [63]. Indeed, these authors wrote that the sequences exhibit “... a gene order typical for mitogenomes of Lepidoptera”. The transformational pathway leading from MIQGO to ES1GO is shown in Figure 7.

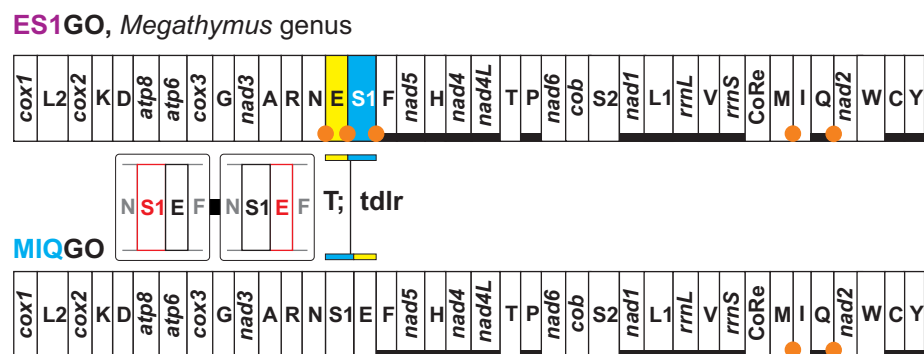


Figure 7. Mitochondrial transformational pathway generating ES1GO. Gene colours, nomenclature as well mechanisms responsible of rearrangements depicted and labelled as in Figure 1.

A single tandem duplication random loss event can be assumed to be responsible for the appearance of this macro-structural change and a set of ISPs congruent with this transformational pathway is present in all the *Megathymus* mitogenomes. Reliable remnants of the genes involved in the tandem duplication random loss event, which would have better supported this transformational pathway [2,7], could not be found due to the rapid shortening and change in sequences associated with these intergenic spacers. Currently, ES1GO is known only for butterflies belonging to *Megathymus*, and its phylogenetic distribution represents a mito-signature for the genus. ES1GO does not show a highly rearranged gene disposition. Indeed, it shares 95% of shared common intervals with MIQGO (Figure 2). With current limited sampling, it is not possible to define unambiguously ES1GO as a true synapomorphy. Better genomic coverage is necessary to fix this point.

Initially, S1NGO was described for *Erynnis montanus* [34]. Our annotation/re-annotation and new assembly of the mitogenomes of *Erynnis tages*, *Erynnis popoviana* and *Erynnis brizo brizo* revealed that they share S1NGO with *E. montanus* (Figure 8).

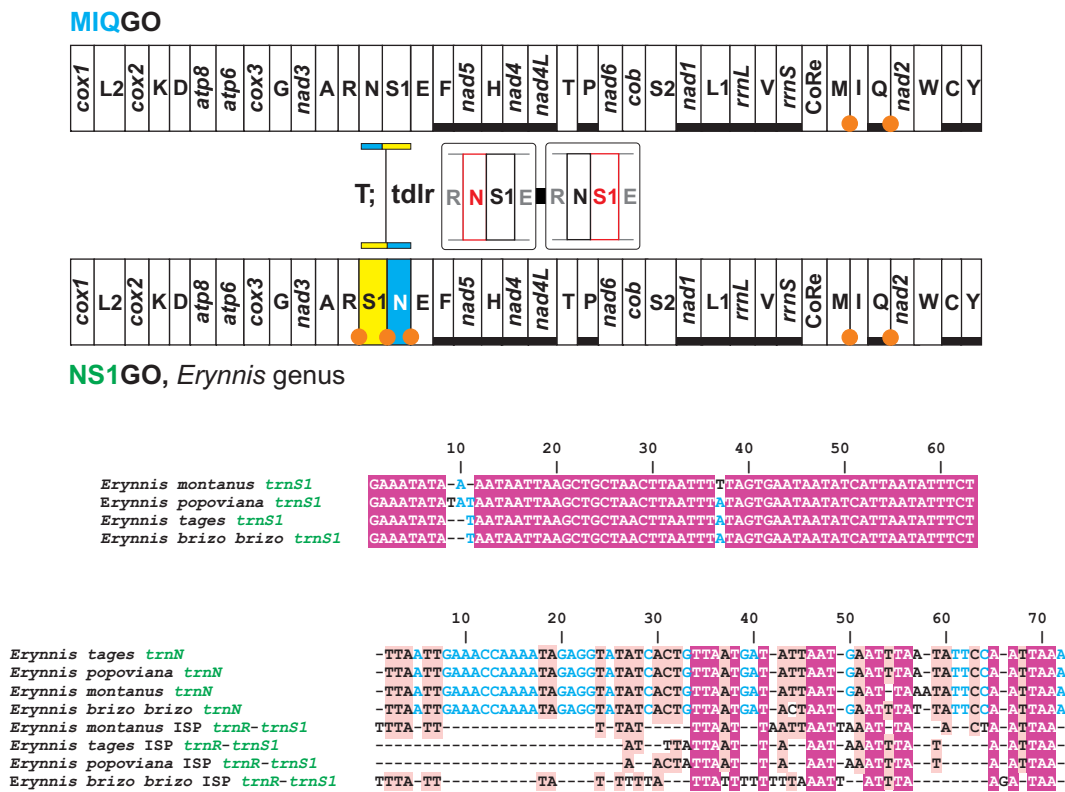


Figure 8. Mitochondrial transformational pathway generating NS1GO. Gene colours, nomenclature as well mechanisms responsible of rearrangements depicted and labelled as in Figure 1. On bottom, multiple alignments of *trnS1s* and *trnNs* vs. ISPs *trnR-trnS1* in species belonging to the genus *Erynnis*. Deep-purple background, fully conserved nucleotide; pink background, majority rule (>50%) conserved nucleotide; cyan-coloured position, conserved nucleotide in *trnMs*.

The presence of S1NGO in the mitogenome of *E. popoviana* was unrecognized in the paper describing it [37]. However, the comparison of the *trnN* and *trnS1* sequences, identified during reannotation, with the orthologous counterparts of other *Erynnis* species provides compelling evidence in favour of this genomic arrangement (Figure 8). The occurrence of a single tandem duplication random loss event was hypothesised to explain the appearance of S1NGO [34]. All mitogenomes exhibiting S1NGO present ISPs in the segment interested by the tandem duplication random loss event (Figure 8). Furthermore, we identified possible remnants of the *trnNs* within the ISPs *trnR-trnS1*, even if in this case the evidence is not conclusive. All these findings corroborate the hypothesised tandem duplication random loss transformational pathway [2,7].

Currently, S1NGO is known only for the genus *Erynnis*. Indeed, *Gesta gesta* and *Ephyriades brunnea brunnea*, which resulted in the taxa with an available mitogenome, most closely related to the genus *Erynnis* in the very recent phylogeny of Xiao et al. [37], who presents the standard MIQGO. Available taxonomic coverage, combined with the low level of rearrangement of S1NGO that shares 95% of shared common intervals with MIQGO (Figure 2), suggests considering for the moment this gene order as a structural mitosignature, awaiting further corroboration to be possibly elevated to true synapomorphy.

3.7. FFGO and 4QGO: Two Novel Gene Orders for the Papilionoidea

Two novel gene orders were identified through the annotation of multiple mitogenomes, available as unannotated sequences in the GenBank (Table S1).

The first new arrangement (2FFGO) characterises the mitogenome of the skipper *Ampittia subvittatus* (Hesperiidae, Hesperinae) (Figure 9).

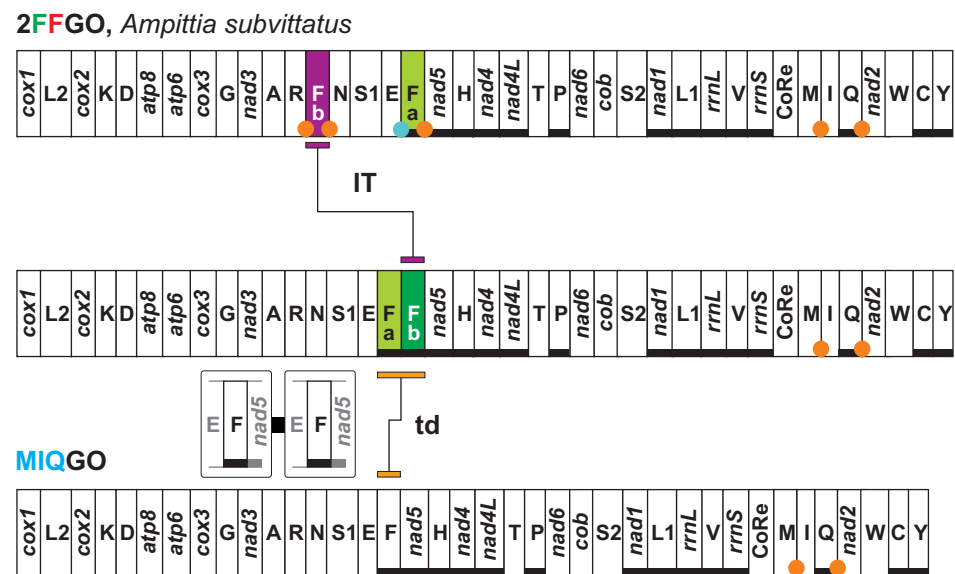


Figure 9. Mitochondrial transformational pathway generating 2FFGO. Gene colours, nomenclature as well mechanisms responsible of rearrangements depicted and labelled as in Figures 1 and 2.

Currently, 2FFGO is known only for this species as the congeneric *Ampittia dioscorides* exhibits the standard MIQGO. 2FFGO is characterised by the presence of two copies of *trnF*, located on opposite strands (Figure 9). Both *trnFa* (69 nucleotides) and *trnFb* (66 nucleotides) can perfectly fold and produce their secondary structure (Alignment S16). Their sequence identity is very high (92.28%), with most of the different nucleotides located in the TΨC loop (Alignment S16). The transformational pathway leading from MIQGO to 2FFGO implies the first duplication of *trnF*, followed by the reverse transposition of the downstream *trnFb* copy to a final placement between *trnR* and *trnS1*. It could be argued that after duplication took place, it was *trnFa* and not *trnFb* that moved between *trnR* and *trnS1*. The *trnE* and *trnFa* of *A. subvittatus* overlap for two nucleotides, mirroring the behaviour observed for *trnE* and *trnF* of *A. dioscorides*. This shared disposition favours the assumption that the partial superposition of this pair of tRNAs is the plesiomorphic condition for the genus *Ampittia*. The overlap of the two genes makes it more complicated to hypothesise the inverse transposition of one of the two without affecting the structure of the other, something that is not observed in the mitogenome of *A. subvittatus*. Thus, we favour in our reconstruction the reverse transposition of *trnFb*, which was free to move without affecting any other adjacent gene.

The mitogenome of the butterfly *Bhutanitis thaidina* (Papilionidae, Parnassiinae) presents a quadruplication of *trnQ* that generates the 4QGO arrangement (Figure 10).

This novel gene order does not occur in the congeneric *Bhutanitis mansfieldi* that shows the standard MIQGO (Table S1). To identify the transformational pathway leading to 4QGO, it is first necessary to analyse the intergenic spacers ISP *trnQa-trnQb*, ISP *trnQb-trnQc*, and ISP *trnQc-trnQd* (Figure 10; Alignment S17). These spacers share an identical sequence that can be divided into four segments: A–D (Alignment S17). The four segments unambiguously align with the ISP *trnQd-nad2* (A), a 3′ portion of control region (B); the 3′ end of *trnM* (C), and *trnI* (D). A second point to consider is that *trnI* and *trnQa* overlap for three nucleotides at their respective 3′ end, a condition they share with the *trnI* and *trnQ* pair of *B. mansfieldi*.

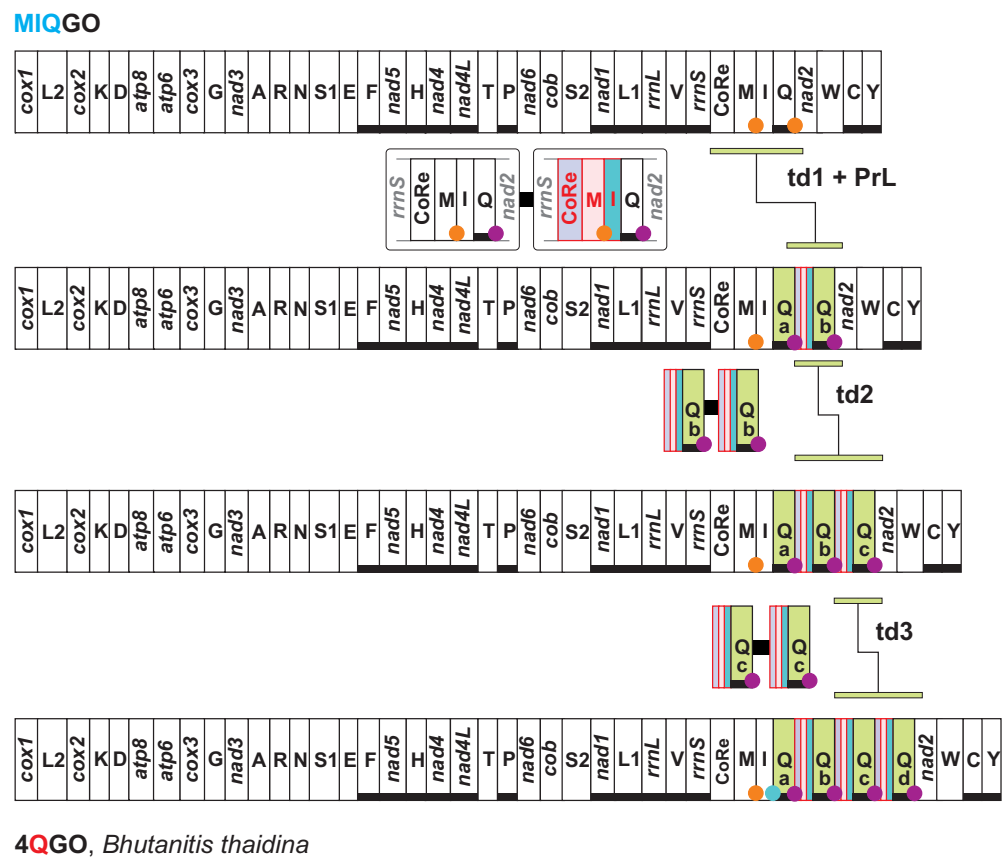


Figure 10. Mitochondrial transformational pathway generating 4QGO. Gene colours, nomenclature as well mechanisms responsible of rearrangements depicted and labelled as in Figures 1 and 2. PrL, partial random loss; td, tandem duplication.

By combining all these data, we sketched the transformational pathway presented in Figure 10. The formation of 4QGO started with the duplication of a genomic segment spanning from the control region to *trnQ*. Successively, a partial random loss event coupled with the shortening of the DNA sequence occurred within the duplicated segment. This process produced the ISP *trnQa-trnQb* containing the four segments A-D. The next step was a duplication of the segment ISP *trnQa-trnQb* + *trnQb*, which generated the ISP *trnQb-trnQc* + *trnQc*. The last step was, again, a duplication of the segment the ISP *trnQb-trnQc* + *trnQc* that produced the final 4QGO. Alternatively, a second duplication of ISP *trnQa-trnQb* + *trnQb* would have created the same 4QGO arrangement. Multiple identical copies of a tRNA and the upstream spacer are not a common phenomenon in insect mitogenomes. However, *B. thaidina* is not the only example. Four identical copies of *trnL2* + upstream spacer occur in the mitogenome of the Eumenid wasp *Abispa ephippium* [64]. 2FFGO and 4QGO were identified in two non-annotated mitogenomic sequences available in the GenBank (Table S1). The sequencing of more mitogenomes of *A. dioscorides* and *B. thaidina* is desirable in the near future. This would provide independent corroboration to our findings.

3.8. Two Clusters of tRNAs Are the Hot Spots of Mitogenomic Diversity in Butterflies

With respect to PanGO, most of the gene orders present in butterflies (Figures 1, 2, 9 and 10) involve duplication, transposition and inverse-transposition of one to three genes located within the two main clusters (ARNS1EF and MIQ) of tRNAs in the mitogenome of butterflies. BemGO represents the only exception (Figures 2 and S1). Our results corroborate and extend previous findings that have identified the two hot spots of mitogenomic rearrangements in butterflies in these two clusters, and, more generally, in insects (e.g., [1]).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/insects13040358/s1>, Figure S1: Mitochondrial transformational pathway generating BemGO; Table S1: Mitogenomes of Papilionoidea included in the final data set; Table S2: Mitogenomes of Papilionoidea excluded from the final data set; Table S3: Mitogenomes of Outgroups included in the final data set; Alignment S1: *nad2* vs. ISP *trnQ-nad2* pairwise alignments in *Lopinga achine* (Nymphalidae, Satyrinae); Alignment S2: *nad2* vs. *trnQ-nad2* ISP pairwise alignments in *Parnassius apollo* (Papilionidae, Parnassiinae); Alignment S3: *nad2* vs. ISP *trnQ-nad2* pairwise alignments in *Choaspes benjaminii* (KJ629164) (Hesperiidae, Coliadinae); Alignment S4: *nad2* vs. ISP *trnQ-nad2* pairwise alignments in *Lobocla bifasciatus* (KJ629166) (Hesperiidae, Eudaminae); Alignment S5: *nad2* vs. ISP *trnQ-nad2* pairwise alignments in *Euschemon rafflesia* (KY513288) (Hesperiidae, Euschemoninae); Alignment S6: *nad2* vs. ISP *trnQ-nad2* pairwise alignments in *Potanthus flavus* (KJ629167) (Hesperiidae, Hesperinae); Alignment S7: *nad2* vs. ISP *trnQ-nad2* pairwise alignments in *Carterocephalus silvicola* (KJ629163) (Hesperiidae, Heteroptera); Alignment S8: *nad2* vs. ISP *trnQ-nad2* pairwise alignments in *Daimio tethys* (KJ629165) (Hesperiidae, Tagiadinae); Alignment S9: Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the family Hesperiidae; Alignment S10: Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the families Lycaenidae and Riodinidae; Alignment S11: Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the subfamily Pierinae; Alignment S12: Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the subfamily Satyrinae (Nymphalidae); Alignment S13: *trnM* vs. ISP *trnQ-nad2* alignment and *trnI* vs. ISP *trnM-trnQ* alignment in species of the family Pemphigidae (Hemiptera); Alignment S14: *trnM* vs. ISP *trnQ-nad2* alignment and CoRe 3'p + *trnI* vs. ISP *trnM-trnQ* 5'p alignment in species of the family Curculionidae (Coleoptera); Alignment S15: Multiple alignment of *trnS1* sequences of selected species of Lepidoptera; Alignment S16: Multiple alignment of *trnF* sequences in Hesperiidae and pairwise alignment of *trnFa* and *trnFb* in *Ampittia subvittatus*; Alignment S17: Pairwise alignments of portions of ISP *trnQa-trnQb* with ISP *trnQd-nad2*, CoRe 3'-end, *trnM* and *trnI*; Pairwise alignment of ISPs *trnQ-nad2* in species of *Bhutanitis*.

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References

1. Cameron, S.L. Insect mitochondrial genomics: Implications for evolution and phylogeny. *Annu. Rev. Entomol.* **2014**, *59*, 95–117. [[CrossRef](#)] [[PubMed](#)]
2. Basso, A.; Babbucci, M.; Pauletto, M.; Riginella, E.; Patarnello, T.; Negrisolo, E. The highly rearranged mitochondrial genomes of the crabs *Maja crispata* and *Maja squinado* (Majidae) and gene order evolution in Brachyura. *Sci. Rep.* **2017**, *7*, 4096. [[CrossRef](#)] [[PubMed](#)]
3. Moritz, C.; Dowling, T.E.; Brown, W.M. Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. *Annu. Rev. Ecol. Syst.* **1987**, *18*, 269–292. [[CrossRef](#)]
4. Boore, J.L. The duplication/random loss model for gene rearrangement exemplified by mitochondrial genomes of Deuterostome animals. In *Comparative Genomics: Empirical and Analytical Approaches to Gene Order Dynamics, Map Alignment and the Evolution of Gene Families*; Sankoff, D., Nadeau, J.H., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; pp. 133–147. [[CrossRef](#)]
5. Downton, M.; Campbell, N.J.H. Intramitochondrial recombination—is it why some mitochondrial genes sleep around? *Trends Ecol. Evol.* **2001**, *16*, 269–271. [[CrossRef](#)]
6. Babbucci, M.; Basso, A.; Scupola, A.; Patarnello, T.; Negrisolo, E. Is it an ant or a butterfly? Convergent evolution in the mitochondrial gene order of Hymenoptera and Lepidoptera. *Genome Biol. Evol.* **2014**, *6*, 3326–3343. [[CrossRef](#)] [[PubMed](#)]
7. Papetti, C.; Babbucci, M.; Dettai, A.; Basso, A.; Lucassen, M.; Harms, L.; Bonillo, C.; Heindler, F.M.; Patarnello, T.; Negrisolo, E. Not frozen in the ice: Large and dynamic rearrangements in the mitochondrial genomes of the Antarctic Fish. *Genome Biol. Evol.* **2021**, *13*, evab017. [[CrossRef](#)]
8. Bernt, M.; Merkle, D.; Ramsch, K.; Fritzsche, G.; Perseke, M.; Bernhard, D.; Schlegel, M.; Stadler, P.F.; Middendorf, M. CREx: Inferring genomic rearrangements based on common intervals. *Bioinformatics* **2007**, *23*, 2957–2958. [[CrossRef](#)] [[PubMed](#)]
9. Bernt, M.; Merkle, D.; Middendorf, M. An algorithm for inferring mitogenome rearrangements in a phylogenetic tree. In *Comparative Genomics; RECOMB-CG 2008 LNB 5267*; Nelson, C.E., Vialette, S., Eds.; Springer: Berlin/Heidelberg, Germany, 2008; pp. 143–157. [[CrossRef](#)]
10. Bernt, M.; Middendorf, M. A method for computing an inventory of metazoan mitochondrial gene order rearrangements. *BMC Bioinform.* **2011**, *12* (Suppl. S9), S6. [[CrossRef](#)]
11. Moreno-Carmona, M.; Cameron, S.L.; Prada Quiroga, C.F. How are the mitochondrial genomes reorganized in Hexapoda? Differential evolution and the first report of convergences within Hexapoda. *Gene* **2021**, *791*, 145719. [[CrossRef](#)]
12. Boore, J.L.; Collins, T.M.; Stanton, D.; Daehler, L.L.; Brown, W.M. Deducing arthropod phylogeny from mitochondrial DNA rearrangements. *Nature* **1995**, *376*, 163–165. [[CrossRef](#)]
13. Boore, J.L.; Lavrov, D.V.; Brown, W.M. Gene translocation links insects and crustaceans. *Nature* **1998**, *392*, 667–668. [[CrossRef](#)]
14. Cao, Y.Q.; Ma, C.; Chen, J.Y.; Yang, D.R. The complete mitochondrial genomes of two ghost moths, *Thitarodes renzhiensis* and *Thitarodes yunnanensis*: The ancestral gene arrangement in Lepidoptera. *BMC Genom.* **2012**, *13*, 276. [[CrossRef](#)]
15. Timmermans, M.J.T.M.; Lees, D.C.; Simonsen, T.J. Towards a mitogenomic phylogeny of Lepidoptera. *Mol. Phylogenet. Evol.* **2014**, *79*, 169–178. [[CrossRef](#)] [[PubMed](#)]
16. Salvato, P.; Simonato, M.; Battisti, A.; Negrisolo, E. The complete mitochondrial genome of the bag-shelter moth *Ochrogaster lunifer* (Lepidoptera, Notodontidae). *BMC Genom.* **2008**, *9*, 331. [[CrossRef](#)] [[PubMed](#)]
17. Bernt, M.; Braband, A.; Schierwater, B.; Stadler, P.F. Genetic aspects of mitochondrial genome evolution. *Mol. Phylogenet. Evol.* **2013**, *69*, 328–338. [[CrossRef](#)]
18. Yang, J.; Ye, F.; Huang, Y. Mitochondrial genomes of four katydid (Orthoptera: Phaneropteridae): New gene rearrangements and their phylogenetic implications. *Gene* **2016**, *575*, 702–711. [[CrossRef](#)]
19. Vieira, G.A.; Prosdocimi, F. Accessible molecular phylogenomics at no cost: Obtaining 14 new mitogenomes for the ant subfamily Pseudomyrmecinae from public data. *PeerJ* **2019**, *7*, e6271. [[CrossRef](#)]
20. Mitter, C.; Davis, D.R.; Cummings, M.P. Phylogeny and evolution of Lepidoptera. *Annu. Rev. Entomol.* **2017**, *62*, 265–283. [[CrossRef](#)]
21. Breinholt, J.W.; Earl, C.; Lemmon, A.R.; Lemmon, E.M.; Xiao, L.; Kawahara, A.Y. Resolving relationships among the megadiverse butterflies and moths with a novel pipeline for anchored phylogenomics. *Syst. Biol.* **2018**, *67*, 78–93. [[CrossRef](#)] [[PubMed](#)]
22. Condamine, F.L.; Nabholz, B.; Clamens, A.-L.; Dupuis, J.R.; Sperling, F.H. Mitochondrial phylogenomics, the origin of swallowtail butterflies, and the impact of the number of clocks in Bayesian molecular dating. *Syst. Entomol.* **2018**, *43*, 460–480. [[CrossRef](#)]
23. Espeland, M.; Breinholt, J.; Willmott, K.R.; Warren, A.D.; Vila, R.; Toussaint, E.F.A.; Maunsell, S.C.; Aduse-Poku, K.; Talavera, G.; Eastwood, R.; et al. A Comprehensive and dated phylogenomic analysis of butterflies. *Curr. Biol.* **2018**, *28*, 770–778. [[CrossRef](#)] [[PubMed](#)]
24. Kawahara, A.Y.; Plotkin, D.; Espeland, M.; Meusemann, K.; Toussaint, E.F.A.; Donath, A.; Gimmich, F.; Frandsen, P.B.; Zwick, A.; Dos Reis, M.; et al. Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 22657–22663. [[CrossRef](#)] [[PubMed](#)]
25. McCullagh, B.S.; Alexiuk, M.R.; Payment, J.E.; Hamilton, R.V.; Lalonde, M.M.L.; Marcus, J.M. It’s a moth! It’s a butterfly! It’s the complete mitochondrial genome of the American moth-butterfly *Macrosoma conifera* (Warren, 1897) (Insecta:Lepidoptera: Hedyliidae)! *Mitochondrial DNA B* **2020**, *5*, 3633–3635. [[CrossRef](#)] [[PubMed](#)]

26. Liu, J.; Xiao, J.; Hao, X.; Yuan, X. Unique duplication of *trnN* in *Odontoptilum angulatum* (Lepidoptera: Pyrginae) and phylogeny within HesperIIDae. *Insects* **2021**, *12*, 348. [CrossRef]
27. Liu, F.F.; Li, Y.P.; Jakovlić, I.; Yuan, X.F. Tandem duplication of two tRNA genes in the mitochondrial genome of *Tagiades vajuna* (Lepidoptera: HesperIIDae). *Eur. J. Entomol.* **2017**, *114*, 407–415. [CrossRef]
28. Hao, J.; Sun, Q.; Zhao, H.; Sun, X.; Gai, Y.; Yang, Q. The complete mitochondrial genome of *Ctenoptilum vasava* (Lepidoptera: HesperIIDae: Pyrginae) and its phylogenetic implication. *Int. J. Genom.* **2012**, *2012*, 328049. [CrossRef]
29. Kim, I.; Lee, E.M.; Seol, K.Y.; Yun, E.Y.; Lee, Y.B.; Hwang, J.S.; Jin, B.R. The mitochondrial genome of the Korean hairstreak, *Coreana raphaelis* (Lepidoptera: Lycaenidae). *Insect Mol. Biol.* **2006**, *15*, 217–225. [CrossRef]
30. Zhou, Y.; Wang, S.; Wang, N.; Liang, Z.; Zhong, H.; Liu, Y.; Liang, B. Phylogenetic inference of *Plebejus argus* (Lepidoptera: Lycaenidae) using its complete mitochondrial genome with an extra copy of tRNA^{Ser}. *Mitochondrial DNA B Resour.* **2020**, *5*, 1584–1585. [CrossRef]
31. Xuan, S.; Song, F.; Cao, L.; Wang, J.; Li, H.; Cao, T. The complete mitochondrial genome of the butterfly *Euripus nyctelius* (Lepidoptera: Nymphalidae). *Mitochondrial DNA A* **2016**, *27*, 2563–2565. [CrossRef]
32. Kim, M.J.; Hong, E.J.; Kim, I. Complete mitochondrial genome of *Camponotus atrox* (Hymenoptera: Formicidae): A new tRNA arrangement in Hymenoptera. *Genome* **2016**, *59*, 59–74. [CrossRef]
33. Park, J.; Kwon, W.; Park, J. The complete mitochondrial genome of *Camponotus concavus* Kim & Kim, 1994 (Hymenoptera: Formicidae). *Mitochondrial DNA B* **2019**, *4*, 1243–1244. [CrossRef]
34. Wang, A.R.; Jeong, H.C.; Han, Y.S.; Kim, I. The complete mitochondrial genome of the mountainous duskywing, *Erynnis montanus* (Lepidoptera: HesperIIDae): A new gene arrangement in Lepidoptera. *Mitochondrial DNA* **2014**, *25*, 93–94. [CrossRef] [PubMed]
35. Timmermans, M.J.T.M.; Lees, D.C.; Thompson, M.J.; Sáfián, S.; Brattström, O. Mitogenomics of ‘Old World *Acraea*’ butterflies reveals a highly divergent ‘*Bematistes*’. *Mol. Phylogenet. Evol.* **2016**, *97*, 233–241. [CrossRef] [PubMed]
36. Li, W.; Cong, Q.; Shen, J.; Zhang, J.; Hallwachs, W.; Janzen, D.H.; Grishin, N.V. Genomes of skipper butterflies reveal extensive convergence of wing patterns. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 6232–6237. [CrossRef]
37. Xiao, J.; Liu, J.; Ma, L.; Hao, X.; Yu, R.; Yuan, X. Mitogenomes of nine Asian skipper genera and their phylogenetic position (Lepidoptera: HesperIIDae: Pyrginae). *Insects* **2022**, *13*, 68. [CrossRef]
38. Simon, C.; Frati, F.; Beckenbach, A.; Crespi, B.; Liu, H.; Flook, P. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **1994**, *87*, 651–704. [CrossRef]
39. Simon, C.; Buckley, T.R.; Frati, F.; Stewart, J.B.; Beckenbach, A.T. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. *Ann. Rev. Eco. Evol. Syst.* **2006**, *37*, 545–579. [CrossRef]
40. Andrews, S. FastQC: A Quality Control Tool for High Throughput Sequence Data. Available online: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc> (accessed on 1 March 2022).
41. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.; Nikolenko, S.; Pham, S.; Pribelski, A.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef]
42. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]
43. Cameron, S.L. How to sequence and annotate insect mitochondrial genomes for systematic and comparative genomics research. *Syst. Entomol.* **2014**, *39*, 400–411. [CrossRef]
44. Negrisolo, E.; Babbucci, M.; Patarnello, T. The mitochondrial genome of the ascalaphid owlfly *Libelloides macaronius* and comparative evolutionary mitochondriomics of neuropterid insects. *BMC Genom.* **2011**, *12*, 221. [CrossRef] [PubMed]
45. Lowe, T.M.; Eddy, S.R. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **1997**, *25*, 955–964. [CrossRef] [PubMed]
46. Wolstenholme, D.R. Animal mitochondrial DNA: Structure and evolution. *Int. Rev. Cytol.* **1992**, *141*, 173–216. [CrossRef]
47. Perna, N.T.; Kocher, T.D. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *J. Mol. Evol.* **1995**, *41*, 353–358. [CrossRef] [PubMed]
48. Katoh, K.; Misawa, K.; Kuma, K.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef] [PubMed]
49. Katoh, K.; Kuma, K.; Toh, H.; Miyata, T. MAFFT version 5: Improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* **2005**, *33*, 511–518. [CrossRef] [PubMed]
50. Felsenstein, J. *Inferring Phylogenies*; Sinauer Associates: Sunderland, MA, USA, 2004; pp. 1–664.
51. Minh, B.Q.; Schmidt, H.A.; Chernomor, O.; Schrempf, D.; Woodhams, M.D.; von Haeseler, A.; Lanfear, R. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **2020**, *37*, 1530–1534. [CrossRef] [PubMed]
52. Chernomor, O.; von Haeseler, A.; Minh, B.Q. Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.* **2016**, *65*, 997–1008. [CrossRef] [PubMed]
53. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; von Haeseler, A.; Jermini, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 587–589. [CrossRef] [PubMed]

54. Hoang, D.T.; Chernomor, O.; von Haeseler, A.; Minh, B.Q.; Vinh, L.S. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **2018**, *35*, 518–522. [[CrossRef](#)]
55. Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **2011**, *28*, 2731–2739. [[CrossRef](#)] [[PubMed](#)]
56. Nei, M.; Kumar, S. *Molecular Evolution and Phylogenetics*; Oxford University Press: New York, NY, USA, 2000; p. 352.
57. Wei, S.-J.; Shi, M.; Chen, X.-X.; Sharkey, M.J.; van Achterberg, C.; Ye, G.Y.; He, J.-H. New views on strand asymmetry in insect mitochondrial genomes. *PLoS ONE* **2010**, *5*, e12708. [[CrossRef](#)] [[PubMed](#)]
58. Kim, M.I.; Baek, J.Y.; Kim, M.J.; Jeong, H.C.; Kim, K.G.; Bae, C.H.; Han, Y.S.; Jin, B.R.; Kim, I. Complete nucleotide sequence and organization of the mitogenome of the redspotted apollo butterfly, *Parnassius bremeri* (Lepidoptera: Papilionidae) and comparison with other lepidopteran insects. *Mol. Cells* **2009**, *28*, 347–363. [[CrossRef](#)] [[PubMed](#)]
59. Kim, M.J.; Wan, X.; Kim, K.G.; Hwang, J.S.; Kim, I. Complete nucleotide sequence and organization of the mitogenome of endangered *Eumenis autonoe* (Lepidoptera: Nymphalidae). *Afr. J. Biotechnol.* **2010**, *9*, 735–754. [[CrossRef](#)]
60. Kim, J.M.; Whang, A.R.; Park, J.S.; Kim, I. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperidae) and phylogenetic reconstruction of Lepidoptera. *Gene* **2014**, *549*, 97–112. [[CrossRef](#)] [[PubMed](#)]
61. Chazot, N.; Wahlberg, N.; Lucci Freitas, A.V.; Mitter, C.; Labandeira, C.; Sohn, J.C.; Kumar Sahoo, R.; Seraphim, N.; de Jong, R.; Heikkilä, M. Priors and posteriors in Bayesian timing of divergence analyses: The age of butterflies revisited. *Syst. Biol.* **2019**, *68*, 797–813. [[CrossRef](#)]
62. Montelli, S.; Peruffo, A.; Patarnello, T.; Cozzi, B.; Negrisolo, E. Back to water: Signature of adaptive evolution in cetacean mitochondrial tRNAs. *PLoS ONE* **2016**, *11*, e0158129. [[CrossRef](#)]
63. Zhang, J.; Cong, Q.; Fan, X.; Wang, R.; Wang, M.; Grishin, N.V. Mitogenomes of giant-skipper butterflies reveal an ancient split between deep and shallow root feeders. *F1000 Res.* **2017**, *6*, 222. [[CrossRef](#)]
64. Cameron, S.L.; Downton, M.; Castro, L.R.; Ruberu, K.; Whiting, M.F.; Austin, A.D.; Diement, K.; Stevens, J. Mitochondrial genome organization and phylogeny of two vespidae wasps. *Genome* **2008**, *51*, 800–808. [[CrossRef](#)]

Article

Macrostructural Evolution of the Mitogenome of Butterflies (Lepidoptera, Papilionoidea)

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Supplementary Materials

Figure S1: Mitochondrial transformational pathway generating BemGO. **Table S1:** Mitogenomes of Papilionoidea included in the final data set. **Table S2:** Mitogenomes of Papilionoidea excluded from the final data set; **Table S3:** Mitogenomes of Outgroups included in the final data set. **Alignment S1:** *nad2* vs ISP *trnQ-nad2* pairwise alignments in *Lopinga achine* (Nymphalidae, Satyrinae). **Alignment S2:** *nad2* vs *trnQ-nad2* ISP pairwise alignments in *Parnassius apollo* (Papilionidae, Parnassiinae). **Alignment S3:** *nad2* vs ISP *trnQ-nad2* pairwise alignments in *Choaspes benjaminii* (KJ629164) (Hesperiidae, Coliadinae). **Alignment S4:** *nad2* vs ISP *trnQ-nad2* pairwise alignments in *Lobocla bifasciatus* (KJ629166) (Hesperiidae, Eudaminae). **Alignment S5:** *nad2* vs ISP *trnQ-nad2* pairwise alignments in *Euschemon rafflesia* (KY513288) (Hesperiidae, Euschemoninae). **Alignment S6:** *nad2* vs ISP *trnQ-nad2* pairwise alignments in *Potanthus flavus* (KJ629167) (Hesperiidae, Hesperinae). **Alignment S7:** *nad2* vs ISP *trnQ-nad2* pairwise alignments in *Carterocephalus silvicola* (KJ629163) (Hesperiidae, Heteropterinae). **Alignment S8:** *nad2* vs ISP *trnQ-nad2* pairwise alignments in *Daimio tethys* (KJ629165) (Hesperiidae, Tagiadinae). **Alignment S9:** Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the family Hesperiidae. **Alignment S10:** Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the families Lycaenidae and Riodinidae. **Alignment S11:** Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the subfamily Pierinae. **Alignment S12:** Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the subfamily Satyrinae (Nymphalidae). **Alignment S13:** *trnM* vs ISP *trnQ-nad2* alignment and *trnI* vs ISP *trnM-trnQ* alignment in species of the family Pemphigidae (Hemiptera). **Alignment S14:** *trnM* vs ISP *trnQ-nad2* alignment and CoRe 3'p + *trnI* vs ISP *trnM-trnQ* 5'p alignment in species of the family Curculionidae (Coleoptera). **Alignment S15:** Multiple alignment of *trnS1* sequences of selected species of Lepidoptera. **Alignment S16:** Multiple alignment of *trnF* sequences in Hesperiidae and pairwise alignment of *trnFa* and *trnFb* in *Ampittia subvittatus*. **Alignment S17:** Pairwise alignments of portions of ISP *trnQa-trnQb* with ISP *trnQd-nad2*, CoRe 3'-end, *trnM* and *trnI*; Pairwise alignment of ISPs *trnQ-nad2* in species of *Bhutanitis*.



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BemGO, *Bematistes* subgenus

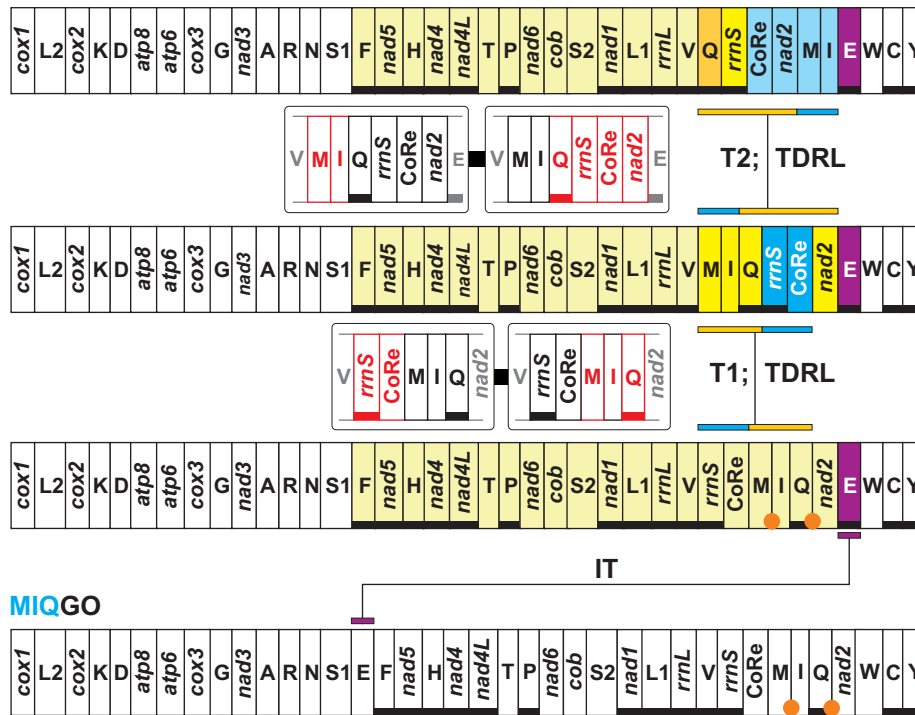


Figure S1. Mitochondrial transformational pathway generating **BemGO**. **MIQGO** and **BemGO** are linearized starting from *cox1*. The genes encoded on the plus strand (orientation from right to left in Figure 1) are black-boxed, while those encoded on the minus strand (orientation from left to right in Figure 1) are underlined and black-boxed. Nomenclature: *atp6* and *atp8*: ATP synthase subunits 6 and 8; *cob*: apocytochrome b; *cox1-3*: cytochrome c oxidase subunits 1–3; *nad1-6* and *nad4 L*: NADH dehydrogenase subunits 1–6 and 4 L; *rrnS* and *rrnL*: small and large subunit ribosomal RNA (rRNA) genes; X: transfer RNA (tRNA) genes, where X is the one-letter abbreviation of the corresponding amino acid, in particular L1 (CTN codon family) L2 (TTR codon family), S1 (AGN codon family) S2 (TCN codon family); CoRe: Control Region. **IT**: inverse transposition, **T**: transposition event. **tdrl**: tandem duplication random loss mechanism producing the observed rearrangement. **TDRL**, Tandem Duplication Random Loss move. Genes transposed relative to **MIQGO** are shown with a yellow-orange/blue (different shades) background. Genes that experienced an inverse-transposition are presented with purple background. Genes duplicated are presented with a green background. The extra copy of every gene that is lost in the genomic rearrangement is red-boxed. Genes located immediately upstream/downstream the rearranged genes, and, possibly, partly involved by the genomic change are grey-boxed. An orange dot marks an intergenic spacer present in a position associated to a genomic rearrangement.

Table S1. Mitogenomes of Papilionoidea included in the final data set

GO	SUPERFAMILY	FAMILY	SUBFAMILY	SPECIES	GenBank	C/P	Size	AT-skew	AT%	GC-skew	GC%	NOTE
MIQGO	Papilionoidea	Hedyliidae		<i>Macrosoma confifera</i> (Warren, 1897)	MT852025	C	15344	-0.008	81,680	-0.185	18,320	
MIQGO	Papilionoidea	Hesperiidae	Barcinae	<i>Apostictopterus fuliginosus</i> Leech, [1893]	MH985707	C	15417	-0.006	80,736	-0.227	19,264	
MIQGO	Papilionoidea	Hesperiidae	Barcinae	<i>Barca bicolor</i> (Oberthür, 1896)	MN012971	C	15577	0.007	79,444	-0.247	20,556	new annotation
MIQGO	Papilionoidea	Hesperiidae	Barcinae	<i>Barca bicolor</i> (Oberthür, 1896)	MH985708	C	15574	0.007	79,382	-0.249	20,611	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Burara striata</i> (Hewitson, 1867)	KY524446	C	15327	-0.020	80,127	-0.233	19,873	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Choaspes benjaminii</i> (Guérin-Méneville, 1843)	JX101620	C	15272	-0.008	80,782	-0.218	19,218	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Choaspes benjaminii</i> (Guérin-Méneville, 1843)	KJ629164	C	15300	0.002	80,111	-0.240	19,889	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Hasora anura</i> (de Niceville, 1889)	KF881049	C	15280	0.005	79,503	-0.223	20,491	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Hasora anura</i> (de Niceville, 1889)	KR189008	C	15290	0.001	79,529	-0.221	20,471	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Hasora badra</i> (Moore, 1857)	MK238674	C	15324	-0.007	79,718	-0.229	20,282	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Hasora chromus</i> (Cramer, 1782)	MN182753	C	15413	-0.007	79,641	-0.222	20,359	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Hasora vitta</i> (Butler, 1870)	KR076553	C	15282	-0.010	79,918	-0.222	20,082	
MIQGO	Papilionoidea	Hesperiidae	Coeliadinae	<i>Hasora vitta</i> (Butler, 1870)	MK238675	C	15290	-0.009	79,993	-0.220	20,007	
MIQGO	Papilionoidea	Hesperiidae	Eudaminae	<i>Achalarus lyciades</i> Geyer, 1832	CM009487	C	15612	-0.012	81,899	-0.190	18,101	
MIQGO	Papilionoidea	Hesperiidae	Eudaminae	<i>Achalarus lyciades</i> Geyer, 1832	KX249739	C	15612	-0.012	81,899	-0.190	18,101	
MIQGO	Papilionoidea	Hesperiidae	Eudaminae	<i>Lobocla bifasciata</i> (Bremer & Grey, 1853)	MN012996	C	15613	-0.025	81,349	-0.192	18,651	new annotation
MIQGO	Papilionoidea	Hesperiidae	Eudaminae	<i>Lobocla bifasciatus</i> (Bremer & Grey, 1853)	KJ629166	C	15366	-0.020	80,932	-0.212	19,068	
MIQGO	Papilionoidea	Hesperiidae	Euschemoninae	<i>Euschemon rafflesia</i> (Macleay, [1826])	KY513288	C	15447	-0.040	81,440	-0.205	18,560	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Agathymus mariae</i> (W. Barnes & Benjamin, 1924)	KY630504	C	15342	-0.004	79,983	-0.247	19,874	
2F1GO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Ampittia subvittatus</i> (Moore, 1878)	MN013003	P	15403	-0.015	80,283	-0.245	19,717	new annotation
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Astictopterus jama</i> Felder & Felder, 1860	MH763663	C	15430	-0.012	80,687	-0.238	19,313	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Erionota torus</i> Evans, 1941	MW586888	P	15987	-0.023	81,228	-0.222	18,772	new annotation
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Hesperia comma</i> (Linnaeus, 1758)	FR990041	C	17733	-0.001	83,128	-0.180	16,872	new annotation
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Isoteinon lamprospilus</i> Felder & Felder, 1862	MH763664	C	15430	-0.001	78,568	-0.283	21,432	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Lerema accius</i> (Smith, 1797)	KT598278	C	15338	-0.022	80,858	-0.209	19,142	
ES1GO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Megathymus beulahae</i> Scudder, 1872	KY630505	P	15412	0.004	79,101	-0.256	20,406	
ES1GO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Megathymus cofaqui cofaqui</i> (Strecker, 1876)	KY630503	P	15421	-0.012	80,274	-0.242	19,052	
ES1GO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Megathymus streckeri</i> (Skinner, 1895)	KY630501	C	15507	-0.011	80,764	-0.230	18,804	
ES1GO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Megathymus ursulae</i> D. Stallings and Turner, 1956	KY630502	C	15396	-0.008	80,508	-0.246	19,492	
ES1GO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Megathymus yuccae</i> (Boisduval & Leconte, 1837)	KY630500	C	15477	-0.003	80,474	-0.244	19,229	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Notocrypta curvifascia</i> (Felder & Felder, 1862)	MH763665	C	15546	-0.024	80,747	-0.228	19,253	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Ochlodes sylvanus</i> (Esper, 1777)	FR990152	C	17059	-0.009	82,889	-0.207	17,111	new annotation
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Ochlodes thibetana</i> (Oberthür, 1886)	MN013004	C	15773	-0.022	82,064	-0.203	17,936	new annotation
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Ochlodes venata</i> (Bremer & Grey, 1853)	HM243593	C	15622	-0.010	81,993	-0.183	18,007	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Parnara guttata</i> (Bremer & Grey, 1852)	JX101619	C	15441	-0.010	80,636	-0.218	19,364	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Pelopidas mathias</i> (Fabricius, 1798)	MW264491	C	15524	-0.009	80,901	-0.213	19,099	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Polytremis jigongi</i> J.Q. Zhu, Z.B. Chen & L.Z. Li, 2012	KP765762	C	15353	-0.016	80,942	-0.204	19,058	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Polytremis nascentis</i> (Leech, 1893)	KM981865	C	15392	-0.012	80,353	-0.213	19,647	
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Potanthus flavus</i> (Murray, 1875)	KJ629167	C	15267	-0.017	80,651	-0.217	19,349	
MIQGO	Papilionoidea	Hesperiidae	Heteropterinae	<i>Carterocephalus alcina</i> Evans, 1939	MN012974	P	15747	0.004	81,330	-0.192	18,664	new annotation
MIQGO	Papilionoidea	Hesperiidae	Heteropterinae	<i>Carterocephalus silvicola</i> Meigen, 1829	KJ629163	C	15765	0.006	80,298	-0.205	19,702	
MIQGO	Papilionoidea	Hesperiidae	Heteropterinae	<i>Heteropterus morpheus</i> (Pallas, 1771)	KF881050	C	15750	-0.001	81,308	-0.190	18,679	
MIQGO	Papilionoidea	Hesperiidae	Heteropterinae	<i>Leptalina unicolor</i> Bremer & Grey, 1853	MK265705	C	15854	0.016	81,241	-0.213	18,759	
MIQGO	Papilionoidea	Hesperiidae	Malazinae	<i>Malaza camides</i> (Hewitson, 1868)	MN919191	C	15380	0.005	79,870	-0.250	19,863	
MIQGO	Papilionoidea	Hesperiidae	Malazinae	<i>Malaza empyreus</i> (Mabille, 1878)	MN919190	C	16103	0.004	80,997	-0.229	19,003	
MIQGO	Papilionoidea	Hesperiidae	Malazinae	<i>Malaza fastuosus</i> (Mabille, 1884)	MK301537	C	15579	0.005	80,358	-0.235	19,391	
MIQGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Ephyriades brunnea brunnea</i> (Herrich-Schäffer, 1865)	SRR7174465	C	15645	-0.008	80,262	-0.196	18,498	de novo assembly
S1NGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Erynnis brizo brizo</i> (Boisduval & Leconte, [1837])	SRR7174469	C	15644	0.001	82,012	-0.178	17,988	de novo assembly
S1NGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Erynnis montanus</i> (Bremer, 1861)	MN012984	C	15477	-0.002	81,747	-0.183	18,253	new annotation
S1NGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Erynnis montanus</i> (Bremer, 1861)	KC659955	C	15530	-0.001	81,758	-0.180	18,242	
S1NGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Erynnis popoviana</i> Nordmann, 1851	MZ221165	C	15559	-0.003	81,798	-0.168	18,202	new annotation
S1NGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Erynnis tages</i> (Linnaeus, 1758)	LR990102	C	15507	-0.003	82,195	-0.178	17,805	new annotation
MIQGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Gesta gesta</i> (Herrich-Schäffer, 1863)	SRR7174466	P	15393	-0.007	81,043	-0.190	18,957	de novo assembly
MIQGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Pyrgus maculatus</i> (Bremer & Grey, 1853)	MN013011	C	15366	-0.005	80,620	-0.216	19,380	new annotation
MIQGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Pyrgus maculatus</i> (Bremer & Grey, 1853)	KP689265	C	15346	-0.003	80,679	-0.216	19,321	
MIQGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Pyrgus malvae</i> (Linnaeus, 1758)	BK013352	C	15380	-0.009	81,671	-0.190	18,329	
MIQGO	Papilionoidea	Hesperiidae	Tagiadininae	<i>Abraximorpha davidii</i> Mabille, 1876	MT371044	C	15469	-0.015	81,544	-0.211	18,456	
MIQGO	Papilionoidea	Hesperiidae	Tagiadininae	<i>Celaenorhinus maculosa</i> (C. & R. Felder, [1867])	KF543077	C	15282	0.001	79,872	-0.230	20,128	
MIQGO	Papilionoidea	Hesperiidae	Tagiadininae	<i>Daimio tethys</i> (Ménétries, 1857)	KJ629165	C	15350	-0.003	79,277	-0.239	20,723	
MIQGO	Papilionoidea	Hesperiidae	Tagiadininae	<i>Daimio tethys</i> (Ménétries, 1857)	KJ813807	C	15341	-0.002	79,154	-0.244	20,846	
2S1EGO	Papilionoidea	Hesperiidae	Tagiadininae	<i>Tagiades vajuna</i> Fruhstorfer, 1910	KX865091	C	15359	-0.021	79,706	-0.220	20,294	
MIQGO	Papilionoidea	Hesperiidae	Trapezitinae	<i>Rachelia extrusus</i> C. & R. Felder, 1867	MN919192	C	16114	-0.016	80,681	-0.247	18,977	
MIQGO	Papilionoidea	Lycanidae	Aphnaeinae	<i>Spindasis takanonis</i> (Matsumura, 1906)	HQ184266	C	15349	0.004	82,351	-0.214	17,649	
MIQGO	Papilionoidea	Lycanidae	Curetinae	<i>Curetis bulis</i> Westwood, [1851]	JX262888	C	15162	-0.040	81,381	-0.175	18,619	
2S1GO	Papilionoidea	Lycanidae	Lycaninae	<i>Heliophorus eventa</i> Fruhstorfer 1918	MN012991	P	15531	0.005	80,175	-0.228	19,825	new annotation
2S1GO	Papilionoidea	Lycanidae	Lycaninae	<i>Lycæna li</i> (Oberthür, 1886)	MN012993	C	15420	-0.015	81,291	-0.197	18,696	new annotation
MIQGO*	Papilionoidea	Lycanidae	Lycaninae	<i>Lycæna phlaeas</i> (Linnaeus, 1761)	HG995187	C	16221	-0.028	82,819	-0.168	17,181	new annotation
MIQGO*	Papilionoidea	Lycanidae	Lycaninae	<i>Lycæna phlaeas</i> (Linnaeus, 1761)	JX262887	C	15280	-0.033	82,356	-0.165	17,644	
2S1GO	Papilionoidea	Lycanidae	Polyommatae	<i>Aricia agestis</i> (Denis & Schiffermüller, 1775)	LR990279	C	15467	-0.004	82,117	-0.174	17,883	new annotation
2S1GO	Papilionoidea	Lycanidae	Polyommatae	<i>Celastrina argiolus</i> (Linné, 1758)	LR994603	C	15337	-0.002	82,011	-0.166	17,989	new annotation
MIQGO*	Papilionoidea	Lycanidae	Polyommatae	<i>Cupido argiades</i> (Pallas, 1771)	KC310728	C	15330	-0.011	81,807	-0.167	18,193	
MIQGO*	Papilionoidea	Lycanidae	Polyommatae	<i>Cyaniris semiargus</i> (Rottemburg, 1775)	LR994570	C	15275	-0.007	81,663	-0.175	18,337	new annotation
MIQGO*	Papilionoidea	Lycanidae	Polyommatae	<i>Glaucopsyche alexis</i> Poda, 1761	FR990065	C	15228	-0.010	82,690	-0.161	17,310	new annotation
2S1GO	Papilionoidea	Lycanidae	Polyommatae	<i>Lysandra bellargus</i> (Rottemburg, 1775)	HG995365	C	15577	0.009	80,908	-0.190	19,092	new annotation
2S1GO	Papilionoidea	Lycanidae	Polyommatae	<i>Lysandra coridon</i> (Poda, 1761)	HG992145	C	15437	0.007	80,793	-0.197	19,207	new annotation
2S1GO	Papilionoidea	Lycanidae	Polyommatae	<i>Plebejus argus</i> (Linnaeus, 1758)	FR999949	C	15467	-0.008	81,748	-0.173	18,252	new annotation
2S1GO	Papilionoidea	Lycanidae	Polyommatae	<i>Plebejus argus</i> (Linnaeus, 1758)	MN974526	C	15426	-0.006	82,128	-0.168	17,872	
MIQGO*	Papilionoidea	Lycanidae	Polyommatae	<i>Shijimiaooides divina</i> (Fixsen, 1887)	KT897723	C	15259	-0.005	82,450	-0.174	17,544	
2S1GO	Papilionoidea	Lycanidae	Theclinae	<i>Ahlbergia circe</i> (Leech, 1893)	MN012968	C	15452	-0.040	82,701	-0.155	17,292	

GO	SUPERFAMILY	FAMILY	SUBFAMILY	SPECIES	GenBank	C/P	Size	AT-skew	AT%	GC-skew	GC%	NOTE
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Euploea core</i> (Cramer, 1780)	KF590546	C	15192	-0.045	81,734	-0,187	18,253	
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Euploea midamus</i> (Linnaeus, 1758)	KJ866207	C	15187	-0.036	80,878	-0,213	19,122	
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Euploea mulciber</i> (Cramer, 1777)	HQ378507	C	15166	-0.038	81,426	-0,209	18,574	
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Idea leuconoe</i> Erichson, 1834	MN012994	C	15182	-0.050	80,740	-0,229	19,260	new annotation
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Idea leuconoe</i> Erichson, 1834	KR815449	C	15278	-0.027	80,456	-0,232	19,538	
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Ideopsis similis</i> (Linnaeus, 1758)	KJ476729	C	15200	-0.041	81,559	-0,210	18,434	
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Parantica aglea</i> (Stoll, 1782)	MH388505	C	15219	-0.035	79,578	-0,241	20,422	
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Parantica aglea aglea</i> (Stoll, 1782)	MN938921	C	15214	-0.035	79,578	-0,244	20,422	
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Parantica sita</i> (Kollar, 1844)	KF590544	C	15211	-0.032	80,488	-0,234	19,512	
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Parantica sita</i> (Kollar, 1844)	MG571524	C	15156	-0.035	80,496	-0,233	19,504	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Acraea) zetes</i> (Linnaeus, 1758)	KT371361	C	15228	-0.053	81,330	-0,190	18,670	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Alacria) parrhasia</i> (Fabricius, 1793)	KT371374	C	15261	-0.013	77,400	-0,255	22,600	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Alacria) penelope</i> Staudinger, 1896	KT371367	C	15271	-0.008	76,976	-0,269	23,024	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Alacria) perenna</i> Doubleday, 1847	KT371369	C	15315	-0.017	77,049	-0,220	22,951	
BemGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Bematistes) alcinoe</i> C. Felder & R. Felder, 1865	KT371373	C	15729	0.011	77,125	-0,326	22,875	
BemGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Bematistes) epaea</i> (Cramer, 1779)	KT371368	C	15712	0.004	79,213	-0,286	20,787	
BemGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Bematistes) poggei</i> Dewitz, 1879	KT371366	C	15361	-0.011	80,001	-0,280	19,999	
BemGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Bematistes) vestalis</i> C. Felder & R. Felder, 1865	KT371375	C	15350	-0.006	79,661	-0,303	20,339	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Rubraea) egina</i> (Cramer, 1775)	KT371359	C	15405	-0.042	80,221	-0,214	19,779	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Stephania) rogersi</i> Hewitson, 1873	KT371365	C	15274	-0.035	80,778	-0,215	19,222	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) acerata</i> Hewitson, 1874	KT371360	C	15281	-0.019	79,707	-0,223	20,293	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) bonasia</i> (Fabricius, 1775)	KT371364	C	15290	-0.012	78,764	-0,215	21,236	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) circeis</i> (Drury, 1782)	KT371362	C	15235	-0.013	77,217	-0,258	22,783	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) jodutta</i> (Fabricius, 1793)	KT371358	C	15272	-0.022	78,195	-0,211	21,805	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) kalinzu</i> Carpenter, 1936	KT371376	C	15260	-0.006	76,802	-0,261	23,198	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) lycoa</i> Godart, 1819	KT371370	C	15190	-0.021	77,762	-0,216	22,238	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) pharsalus</i> Ward, 1871	KT371363	C	15224	-0.023	79,020	-0,224	20,980	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) polis</i> Pierre, 1999	KT371371	C	15254	-0.001	76,013	-0,233	23,981	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Telchinia) serena</i> (Fabricius, 1775)	KT371372	C	15258	-0.016	79,467	-0,205	20,533	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Argynnis childreii</i> Gray, 1831	KF590547	C	15131	-0.021	80,041	-0,231	19,939	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Argynnis hyperbius</i> (Linnaeus, 1763)	MN012969	C	15158	-0.025	80,782	-0,219	19,211	new annotation
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Argynnis hyperbius</i> (Linnaeus, 1763)	JF439070	C	15156	-0.025	80,813	-0,215	19,187	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Argynnis paphia</i> (Linnaeus, 1758)	KM592975	P	15208	-0.033	81,003	-0,200	18,339	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Boloria selene</i> (Schiffmuller, 1775)	HG998571	C	15156	-0.011	80,984	-0,228	19,016	new annotation
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Cethosia biblis</i> (Drury, 1773)	KF990124	C	15211	-0.067	79,837	-0,221	20,163	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Cethosia biblis</i> (Drury, 1773)	KR066948	C	15286	-0.067	80,211	-0,200	19,776	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Cethosia cyane</i> (Drury, 1773)	MN012975	C	15259	-0.077	80,123	-0,212	19,837	new annotation
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Damora sagana</i> (Doubleday, 1847)	KY971464	C	15151	-0.030	81,011	-0,202	18,989	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Fabriciana adippe</i> (Schiffmuller, 1775)	FR990011	C	15118	-0.025	80,579	-0,223	19,421	new annotation
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Fabriciana nerippe</i> (Felder & Felder, 1862)	JF504707	C	15140	-0.027	80,905	-0,210	19,095	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Heliconius cydno</i> (Doubleday 1847)	KM208636	C	15367	-0.035	81,363	-0,184	18,637	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Heliconius hecale</i> (Fabricius, 1776)	KM068091	C	15338	-0.033	81,627	-0,188	18,373	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Heliconius ismenius</i> Latreille, [1817]	KP294327	C	15346	-0.029	81,200	-0,200	18,800	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Heliconius melpomene</i> (Linnaeus, 1758)	KP100653	C	15327	-0.037	81,660	-0,186	18,340	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Heliconius pacheus</i> Salvin, 1871	KM014809	C	15369	-0.035	81,222	-0,182	18,778	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Heliconius sara</i> (Fabricius, 1793)	KP281778	C	15372	-0.024	80,634	-0,196	19,366	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Issoria eugenia</i> (Eversmann, 1847)	MK598743	C	15206	-0.032	81,501	-0,197	18,499	
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Issoria lathonia</i> (Linnaeus, 1758)	HM243590	C	15172	-0.031	81,169	-0,188	18,824	
MIQGO	Papilionoidea	Nymphalidae	Libytheinae	<i>Libythea celtis</i> (Laicharting, 1782)	HQ378508	C	15164	-0.017	81,205	-0,184	18,795	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Abrota ganga</i> Moore, 1857	KF590536	C	15356	-0.048	81,206	-0,182	18,794	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Adelpha bredowii</i> Geyer, 1837	MG747642	C	15187	-0.035	79,937	-0,207	20,063	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Adelpha ethelda</i> (Hewitson, 1867)	MG747609	C	15350	-0.037	79,844	-0,203	20,130	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Adelpha iphicles</i> (Linnaeus, 1758)	MG747610	C	15263	-0.042	80,541	-0,180	19,459	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma asura</i> Moore, 1858	KF590542	C	15181	-0.033	81,082	-0,201	18,885	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma cama</i> Moore, 1858	KF590526	C	15269	-0.047	80,562	-0,198	18,425	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma disjuncta</i> Leech, 1890	MG747630	C	15198	-0.034	81,123	-0,209	18,877	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma fortuna</i> Leech, 1889	MG747635	C	15192	-0.037	80,299	-0,213	19,701	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma jina</i> Moore, 1858	MG747636	C	15202	-0.032	80,812	-0,219	19,169	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma kanwa</i> Moore, 1858	MG747619	C	15278	-0.048	80,848	-0,188	19,139	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma kasa</i> Moore, 1858	KF590524	C	15230	-0.041	80,289	-0,206	19,711	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma librites</i> (Hewitson, 1859)	MG747643	C	15216	-0.044	80,724	-0,197	19,223	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma nefte</i> (Cramer, 1780)	MG747634	C	15438	-0.049	80,762	-0,189	19,161	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma opalina</i> (Kollar, 1844)	KF590551	C	15240	-0.026	80,138	-0,225	19,856	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma perius</i> (Linnaeus, 1758)	KF590528	C	15277	-0.037	79,636	-0,206	19,840	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma prava</i> Moore, 1857	MG747623	C	15100	-0.049	80,974	-0,186	18,947	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma punctata</i> Leech, 1890	MG747626	C	15240	-0.044	80,131	-0,219	19,869	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma ranga</i> Moore, 1857	MG747640	C	15265	-0.039	80,832	-0,202	19,168	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma recurva</i> Leech, 1893	MG747627	C	15189	-0.033	80,980	-0,222	19,014	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma selenophora</i> (Kollar, 1844)	KF590525	C	15200	-0.035	81,309	-0,185	18,691	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma selenophora</i> (Kollar, 1844)	KF590529	C	15208	-0.035	81,293	-0,180	18,707	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Athyma sulphata</i> (Cramer, 1779)	JQ347260	C	15268	-0.047	81,949	-0,176	18,051	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Auzakia danava</i> (Moore, [1858])	MG747639	C	15367	-0.057	80,640	-0,202	19,334	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Bhagadatta austenia</i> (Moore, 1872)	KF590545	C	15615	-0.024	79,148	-0,228	20,781	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Chalinga pratti</i> (Leech, 1890)	MG747613	C	15290	-0.047	81,550	-0,195	18,443	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Dophla evelina</i> (Stoll, 1790)	KF590532	C	15320	-0.039	80,979	-0,211	19,014	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Euthalia irrucescens</i> Grose-Smith, 1893	KF590527	C	15365	-0.042	81,028	-0,184	18,633	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Lelecella limenitoides</i> Oberthür, 1890	MN922294	C	15203	-0.025	81,556	-0,184	18,444	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Lexias dirtea</i> Fabricius, 1793	KF590531	C	15250	-0.046	81,357	-0,181	18,643	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Limenitis amphysa</i> Ménétriés, 1859	KY569555	C	15164	-0.036	80,975	-0,201	19,025	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Limenitis archippus</i> (Cramer, 1776)	MG747621	C	15220	-				

GO	SUPERFAMILY	FAMILY	SUBFAMILY	SPECIES	GenBank	C/P	Size	AT-skew	AT%	GC-skew	GC%	NOTE
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Phaedyra columella</i> (Cramer, [1780])	KY593936	C	15197	-0.031	80.312	-0.204	19.688	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Sumalia zulema</i> (Doubleday, 1848)	MG747611	C	15297	-0.032	79.996	-0.237	20.004	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Tacola eulimene</i> (Godart, 1824)	MG747616	C	15669	-0.037	78.269	-0.231	21.731	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Tacola larymna</i> (Doubleday, [1848])	MG747644	C	15503	-0.045	79.978	-0.234	20.015	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Tanaecia julii</i> Lesson, 1837	KF590548	C	15316	-0.043	81.209	-0.178	18.686	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Tarattia gutama</i> Moore, 1858	MG747608	C	15266	-0.048	81.194	-0.177	18.748	
MIQGO	Papilionoidea	Nymphalidae	Limenitidinae	<i>Aglais ladakensis</i> (Moore, 1878)	MN732892	C	15222	-0.008	80.535	-0.221	19.465	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Aglais urticae</i> (Linnaeus, 1758)	LR990014	C	15196	-0.006	80.482	-0.213	19.518	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Anartia jatrophae saturata</i> Staudinger, 1885	MT712074	C	15297	-0.040	81.382	-0.192	18.618	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Araschnia levana</i> (Linnaeus, 1758)	MT712075	C	15207	-0.009	81.647	-0.190	18.353	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Baeotus beotus</i> (Doubleday, 1849)	MW566598	C	15131	-0.010	80.457	-0.215	19.543	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Doleschallia melana</i> Staudinger, 1886	MT704829	C	15269	0.006	80.018	-0.237	19.982	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Hypolimnas bolina</i> (Linnaeus, 1758)	KF990127	C	15260	0.018	79.672	-0.242	20.328	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Inachis io</i> (Linnaeus, 1758)	LR989926	C	15369	-0.003	80.688	-0.227	19.312	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Inachis io</i> (Linnaeus, 1758)	KM592970	C	15250	-0.004	80.525	-0.226	19.462	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia almana</i> (Linnaeus, 1758)	KF590539	C	15256	-0.010	80.257	-0.227	19.736	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia almana</i> (Linnaeus, 1758)	KX267580	C	15232	-0.010	80.213	-0.227	19.761	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia coenia grisea</i> Austin & J.F. Emmel, 1998	KX267578	C	15228	-0.017	80.667	-0.199	19.326	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia coenia</i> Hübner, [1822]	KT380025	C	15222	-0.018	80.699	-0.198	19.301	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia coenia</i> Hübner, [1822]	KX267572	C	15211	-0.016	79.456	-0.198	19.262	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia coenia</i> Hübner, [1822]	KX267579	C	15206	-0.018	80.633	-0.200	19.367	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia evarete flirtea</i> (Fabricius, 1793)	KX267569	C	15121	-0.017	80.597	-0.198	19.403	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia evarete zonalis</i> C. & R. Felder, [1867]	KX267573	C	15240	-0.019	80.709	-0.197	19.291	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia genoveva hilaris</i> C. & R. Felder, [1867]	KX267570	C	15224	-0.016	80.748	-0.201	19.233	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia genoveva hilaris</i> C. & R. Felder, [1867]	KX267571	C	15238	-0.017	80.883	-0.204	19.117	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia iphita</i> (Cramer, 1779)	KU577289	P	14957	-0.001	79.902	-0.215	20.004	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia iphita</i> (Cramer, 1779)	KU577290	C	15190	-0.019	80.619	-0.198	19.335	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia lemonias</i> (Linnaeus, 1758)	KP941756	C	15230	-0.009	80.374	-0.224	19.606	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia orithya</i> (Linnaeus, 1758)	KF199862	C	15214	-0.016	80.380	-0.180	19.620	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia orithya madagascariensis</i> Guenée, 1865	KX267581	C	15231	-0.018	80.664	-0.199	19.322	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia rhadama</i> (Boisduval, 1833)	KX423732	P	15208	-0.017	79.964	-0.213	19.509	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia stygia</i> (Aurivillius, 1894)	MN623383	C	15233	-0.001	79.354	-0.248	20.442	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia vestina</i> C. & R. Felder, [1867]	KX267577	C	15224	-0.019	81.102	-0.193	18.891	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Kallima inachus</i> (Doyère, 1840)	MN012995	C	15172	-0.013	80.616	-0.212	19.384	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Kallima inachus</i> (Doyère, 1840)	HM243591	C	15150	-0.012	80.601	-0.213	19.399	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Kallima inachus</i> (Doyère, 1840)	JN857943	C	15183	-0.013	80.300	-0.212	19.700	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Kallima paralekta</i> (Horsfield, [1829])	MW192438	C	15200	-0.002	79.533	-0.241	20.467	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Kallimoides rumia</i> (Westwood, 1850)	MT704827	C	15234	-0.007	80.898	-0.217	19.102	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Mallika jacksoni</i> (Sharpe, 1896)	MT704828	C	15193	-0.015	79.385	-0.227	20.615	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Melitaea athalia</i> (Rottemburg, 1775)	HG992208	C	15157	-0.020	80.326	-0.216	19.674	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Melitaea cinxia</i> (Linnaeus, 1758)	HG992240	C	15173	0.006	79.826	-0.251	20.174	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Melitaea cinxia</i> (Linnaeus, 1758)	CM002851	C	15171	0.004	79.810	-0.243	20.190	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Melitaea cinxia</i> (Linnaeus, 1758)	GQ398377	C	15170	0.007	80.013	-0.245	19.987	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Melitaea cinxia</i> (Linnaeus, 1758)	HM243592	C	15162	0.009	79.963	-0.247	20.037	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Melitaea yuenty</i> Oberthür, 1886	MN013001	C	15149	0.006	79.695	-0.246	20.305	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Mellicta ambigua</i> (Ménétriés, 1859)	MK252271	C	15205	-0.019	80.572	-0.219	19.428	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Nymphalis polychloros</i> (Linnaeus, 1758)	HG992273	C	15249	0.007	79.730	-0.245	20.270	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Polygonia c aureum</i> (Linnaeus, 1758)	MT654530	C	15202	-0.006	80.614	-0.231	19.386	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Polygonia c aureum</i> (Linnaeus, 1758)	KX096653	C	15208	-0.006	80.635	-0.231	19.366	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Polygonia c-aureum</i> (Linnaeus, 1758)	MF407452	C	15209	-0.006	80.604	-0.230	19.395	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Protogoniomorpha anacardii duprei</i> (Vinson, 1863)	MT702382	C	15220	0.002	80.033	-0.233	19.967	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Vanessa atalanta</i> (Linnaeus, 1758)	LR990581	C	15240	-0.001	80.400	-0.226	19.600	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Vanessa cardui</i> (Linnaeus, 1758)	LR999956	C	15303	-0.022	81.036	-0.192	18.964	new annotation
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Vanessa indica</i> (Herbst, 1794)	MG736927	C	15191	0.002	80.074	-0.234	19.926	
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Yoma sabina</i> (Cramer, 1780)	KF590535	C	15191	0.002	80.074	-0.188	18.801	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Callerebia suroia</i> Tylter, 1914	KF906483	C	15208	-0.042	79.484	-0.223	20.516	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Coenonympha amaryllis</i> (Stoll, 1782)	MN756798	C	15125	-0.039	79.425	-0.226	20.575	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Davidina armandi</i> Oberthür, 1879	KF881046	C	15214	-0.023	79.696	-0.241	20.304	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Elymnias hypermnestra</i> (Linnaeus, 1763)	KF906484	C	15167	-0.044	80.458	-0.200	19.542	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Faunis aerope</i> (Leech, 1890)	MN012985	C	16381	-0.056	80.838	-0.214	19.150	new annotation
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Hipparchia autonoe</i> (Esper, 1783)	MT090762	C	15300	-0.017	78.876	-0.239	21.124	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Hipparchia autonoe</i> (Esper, 1784)	GQ868707	C	15489	-0.016	79.088	-0.243	20.912	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lasiommata deidamia</i> (Eversmann, 1851)	MG880214	C	15244	-0.034	81.120	-0.194	18.880	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lasiommata majuscula</i> (Leech, 1892)	MN012997	C	15263	-0.038	81.629	-0.190	18.371	new annotation
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe albolineata</i> (Poujade, 1884)	KF881051	C	15248	-0.034	79.164	-0.240	20.836	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe baileyi</i> South, 1913	MN611526	C	15225	-0.023	79.074	-0.238	20.926	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe baucis</i> Leech, 1891	MN611527	C	15251	-0.031	78.736	-0.248	21.264	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe confusa</i> Aurivillius, 1897	MT654529	P	14945	-0.056	80.422	-0.201	19.578	new annotation
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe dura</i> Marshall 1882	KF906485	C	15259	-0.035	79.265	-0.243	20.735	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe hayashii</i> Koiwaya, 1993	MN611528	C	15246	-0.036	79.070	-0.235	20.930	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe helle</i> Leech, 1891	MN611529	C	15253	-0.019	78.588	-0.258	21.412	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe marginalis</i> Motschulsky, 1860	MN611530	C	15229	-0.042	80.189	-0.231	19.811	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe nigrifascia</i> Leech, 1890	MN611531	C	15239	-0.023	79.316	-0.244	20.684	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe oculatissima</i> (Poujade, 1885)	MN611532	C	15243	-0.037	79.276	-0.233	20.724	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe satyrina</i> Butler, 1871	MN611533	C	15271	-0.041	78.764	-0.218	21.236	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe syrcis</i> Hewitson, 1863	MN611534	C	15252	-0.038	79.144	-0.245	20.856	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe titania</i> Leech, 1891	MN611535	C	15257	-0.028	78.469	-0.244	21.531	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe uemurai</i> (Sugiyama, 1994)	MN611536	C	15272	-0.022	78.529	-0.250	21.471	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe uemurai</i> (Sugiyama, 1994)	MN611537	C	15262	-0.022	78.522	-0.251	21.478	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe verma</i> (Kollar, 1844)	MN611538	C	15239	-0.042	79.270	-0.227	20.730	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lethe verma</i> (Kollar, 1844)	MN611539	C	15238	-0.041	79.275	-0.226	20.725	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Lopinga achine</i> (Scopoli, 1763)	ON087695	C	15411	-0.028	79.781	-0.234	20.219	new mitogenome
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Maniola jurtina</i> (Linnaeus, 1758)	HG995237	C	15256	-0.038	79.719	-0.234	20.281	new annotation
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Melanargia asiatica</i> (Oberthür & Houlbert, 1922)	KF906486	C	15142	-0.036	79.045	-0.239	20.955	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Melanargia caof</i> Lang, 2018	MN012999	C	15469	-0.028	79.766	-0.251	20.234	new annotation
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Melanitis leda</i> (Linnaeus, 1758)	JF905446	C	15122	-0.037	79.804	-0.240	20.196	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Melanitis phedima</i> (Cramer, 1780)	KF590538	C	15142	-0.037	79.937	-0.242	20.063	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Minois dryas</i> Scopoli, 1763	MK521433	C	15195	-0.028	80.191	-0.222	19.809	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Mycalasis intermedia</i> Moore, 1892	MN610565	C	15386	-0.029	80.859	-0.235	19.141	
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Mycalasis mineus</i> (Linnaeus								

GO	SUPERFAMILY	FAMILY	SUBFAMILY	SPECIES	GenBank	C/P	Size	AT-skew	AT%	GC-skew	GC%	NOTE
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Graphium eurypylus</i> (Linnaeus, 1758)	MN012987	C	15193	-0.015	80.833	-0.203	19,167	new annotation
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Graphium leechi</i> (Rothschild, 1895)	KX011066	C	15836	-0.012	81.031	-0.212	18,969	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Graphium mullah chungianus</i> (Murayama, 1961)	MW549197	C	15240	-0.009	80.965	-0.201	19,035	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Graphium nomius</i> (Esper, 1793)	MN013014	C	15113	-0.037	80.097	-0.207	19,903	new annotation
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Graphium parus</i> (de Nicéville, 1886)	MT198821	C	15231	0.003	79.929	-0.218	20,071	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Graphium sarpedon</i> (Linnaeus, 1758)	MN012989	C	15306	-0.005	80.655	-0.219	19,228	new annotation
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Graphium timur</i> (Ney, 1911)	KJ472924	C	15226	-0.008	80.363	-0.197	19,637	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Iphiclydes podalirius</i> (Linnaeus, 1758)	MK507891	P	14914	0.013	80.636	-0.184	18,338	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Lamproptera curius</i> (Fabricius, 1787)	KJ141168	C	15277	-0.008	80.474	-0.193	19,526	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Lamproptera meges</i> (Zinken-Sommer, 1831)	LT999978	C	15113	0.006	80.811	-0.195	19,189	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Losaria neptunus</i> (Guérin-Méneville, 1840)	LT999979	P	14901	-0.041	80.632	-0.186	19,368	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Meandrusa payeni</i> (Boisduval, 1836)	MN013000	C	15249	0.004	80.156	-0.237	19,844	new annotation
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Meandrusa sciron</i> (Leech, 1890)	LS975123	P	14875	-0.006	79.408	-0.219	20,578	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Mimoides lysithous</i> (Hübner, [1821])	LT999982	C	15038	-0.008	80.842	-0.203	19,158	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Ornithoptera priamus</i> (Linnaeus, 1758)	LT999981	P	14804	-0.040	79.202	-0.254	20,798	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Ornithoptera richmondia</i> (Gray, [1853])	LT999980	P	14763	-0.042	79.293	-0.246	20,687	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Pachliopta aristolochiae</i> (Fabricius, 1775)	KU950357	C	15232	-0.037	81.624	-0.199	18,376	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio bianor</i> Cramer, 1777	MN013007	C	15339	-0.013	80.631	-0.213	19,369	new annotation
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio bianor</i> Cramer, 1777	JN019809	C	15340	-0.015	80.554	-0.212	19,446	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio bianor</i> Cramer, 1777	KC433409	C	15332	-0.014	80.648	-0.213	19,352	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio dardanus</i> Brown, 1776	KX033351	C	15349	-0.014	79.393	-0.249	20,451	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio dardanus</i> Brown, 1776	KX033352	C	15311	-0.013	80.511	-0.226	19,333	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio dardanus</i> Brown, 1776	KX033358	C	15346	-0.007	79.063	-0.265	20,937	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio dardanus polytrophus</i> Rothschild & Jordan, 1903	KX033353	P	15084	-0.005	78.275	-0.272	21,062	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio demoleus</i> Linnaeus, 1758	MN013008	C	15216	-0.031	80.935	-0.189	19,065	new annotation
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio demoleus</i> Linnaeus, 1758	KR024009	C	15249	-0.028	80.884	-0.186	19,116	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio glaucus</i> Linnaeus, 1758	KR822739	C	15306	-0.006	80.426	-0.221	19,574	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio helenus</i> Linnaeus, 1758	MN013010	C	15352	-0.004	80.113	-0.249	19,887	new annotation
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio helenus</i> Linnaeus, 1758	KM244656	C	15349	-0.005	80.096	-0.250	19,904	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio maackii</i> Ménétries, 1859	KC433408	C	15357	-0.014	80.693	-0.214	19,307	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio machaon</i> Linnaeus, 1758	LS975121	C	15179	-0.026	80.302	-0.205	19,685	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio maraho</i> (Shiraki & Sonan, 1934)	FJ810212	C	16094	0.006	80.496	-0.262	19,430	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio memnon</i> Linnaeus, 1758	LD700014	C	15296	0.007	79.812	-0.246	20,188	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio memnon</i> Linnaeus, 1758	MH981597	C	15262	-0.008	79.741	-0.246	20,259	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio paris</i> Linnaeus, 1758	MN629008	C	15363	-0.016	80.525	-0.228	19,417	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio polytes</i> Linnaeus, 1758	KM014701	C	15256	-0.023	81.142	-0.208	18,858	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio protenor</i> Cramer, 1775	MN013015	C	15257	-0.016	80.514	-0.233	19,486	new annotation
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio protenor</i> Cramer, 1775	KY272622	C	15268	-0.018	80.495	-0.228	19,505	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio rex</i> Oberthür, 1886	KX033354	C	15239	-0.021	79.526	-0.236	20,474	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio slateri</i> Hewitson, 1857	LT999985	P	14821	-0.010	80.595	-0.208	19,398	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio syfanius</i> Oberthür, 1886	KJ396621	C	15359	-0.020	80.604	-0.205	19,396	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio thoas</i> Linnaeus, 1771	MW548255	C	15258	-0.009	80.646	-0.225	19,347	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio xuthus</i> Linnaeus, 1767	KT922004	C	15359	-0.013	80.526	-0.228	19,474	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio xuthus</i> Linnaeus, 1767	KU356933	C	15359	-0.013	80.467	-0.226	19,533	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio xuthus</i> Linnaeus, 1767	MW548256	C	15350	-0.013	80.469	-0.229	19,466	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Parides ascanius</i> (Cramer, 1775)	LS974638	C	15212	-0.054	79.306	-0.213	19,005	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Pharmacophagus antenor</i> (Drury, 1775)	LS975119	C	15096	-0.048	80.975	-0.212	18,866	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Protesilaus protesilaus</i> (Linnaeus, 1758)	LT999984	C	14964	-0.004	81.917	-0.173	18,083	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Protographium marcellus</i> (Cramer, 1777)	MK507890	P	14784	-0.007	78.355	-0.179	18,676	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus aureus</i> Mell, 1923	HM563681	C	15242	-0.005	79.806	-0.238	20,194	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus aureus</i> Mell, 1923	KP941013	C	15234	-0.003	79.828	-0.241	20,172	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus aureus</i> Mell, 1923	KP941014	C	15234	-0.003	79.848	-0.241	20,146	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus aureus</i> Mell, 1923	KP941015	C	15234	-0.004	79.795	-0.240	20,205	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus aureus</i> Mell, 1923	KP941016	C	15234	-0.003	79.808	-0.240	20,192	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus aureus</i> Mell, 1923	KP941017	C	15234	-0.004	79.828	-0.240	20,172	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus aureus</i> Mell, 1923	KP941018	C	15235	-0.004	79.810	-0.241	20,190	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus aureus</i> Mell, 1923	KP941019	C	15234	-0.004	79.815	-0.240	20,185	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus imperialis</i> Hope, 1843	KR018842	C	15229	-0.005	79.592	-0.232	20,408	
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Teinopalpus imperialis</i> Hope, 1843	LS975139	C	15229	-0.004	79.565	-0.236	20,356	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Allanacstria cerisyi</i> Godart, 1824	LS974636	P	15279	-0.021	80.575	-0.205	18,738	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Archon apollinus</i> (Herbst, 1789)	LT999971	P	14971	-0.020	81.077	-0.175	18,229	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Bhutanitis mansfieldi</i> (Riley 1939)	LT999973	P	14994	-0.017	81.539	-0.213	18,447	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Luehdorfia chinensis</i> Leech, 1893	KM453725	C	16028	-0.008	81.963	-0.211	18,037	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Luehdorfia chinensis</i> Leech, 1893	KM453726	C	15580	-0.007	81.624	-0.208	18,376	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Luehdorfia chinensis</i> Leech, 1893	KM453727	C	15580	-0.007	81.605	-0.209	18,395	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Luehdorfia japonica</i> Leech, 1889	LT999996	P	15347	-0.011	81.449	-0.196	18,551	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Luehdorfia taibai</i> Chou, 1994	KC952673	C	15553	-0.009	81.463	-0.202	18,537	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius apollo</i> (Linnaeus, 1758)	ON087696	C	15352	-0.012	81.364	-0.194	18,636	new mitogenome
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius apollo</i> (Linnaeus, 1758)	KF746065	C	15404	-0.016	81.304	-0.190	18,696	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius apollonius</i> (Eversmann, 1847)	MG252290	C	15381	-0.014	81.737	-0.190	18,263	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius bremeri</i> Bremer, 1864	FJ871125	C	15389	-0.011	81.266	-0.194	18,734	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius bremeri</i> Bremer, 1864	HM243588	C	15390	-0.019	81.046	-0.194	18,954	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius cephalus</i> Grumm-Grshimailo, 1891	KP100655	C	15343	-0.012	81.399	-0.193	18,601	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius choui</i> Huang & Shi, 1994	KY072797	C	15367	-0.011	81.460	-0.198	18,540	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius epaphus</i> Oberthür, 1879	KM373898	C	15458	-0.018	81.395	-0.195	18,605	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius imperator</i> Oberthür, 1883	KM507326	C	15424	-0.013	81.088	-0.207	18,912	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius mercurius</i> Grumm-Grshimailo, 1891	MN728989	C	15372	-0.014	81.447	-0.194	18,553	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius nomion</i> von Waldheim, 1823	MF496134	C	15362	-0.016	81.324	-0.190	18,676	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Parnassius smintheus</i> Doubleday, [1847]	LT999983	C	15411	-0.012	80.618	-0.195	18,889	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Sericinus montela</i> Gray, 1852	HQ259122	C	15243	-0.008	80.955	-0.223	19,045	
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Zerynthia polyxena</i> (Denis & Schiffermüller, 1775)	MK507888</							

GO	SUPERFAMILY	FAMILY	SUBFAMILY	SPECIES	GenBank	C/P	Size	AT-skew	AT%	GC-skew	GC%	NOTE
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Baltia butleri</i> Alphéraky, 1889	MH380204	C	15124	0,013	79,708	-0,251	20,292	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Cepora nadina</i> (H. Lucas, 1852)	MN012977	C	15229	-0,014	79,526	-0,236	20,474	new annotation
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Delias hyparete</i> (Linnaeus, 1758)	JX094279	C	15186	-0,017	79,843	-0,229	20,157	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Delias pasithoe</i> (Linnaeus, 1767)	MN012983	C	15204	0,000	79,203	-0,264	20,797	new annotation
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Delias pasithoe</i> (Linnaeus, 1767)	MK252291	C	15203	-0,001	79,195	-0,263	20,805	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Hebomoia glaucippe</i> (Linnaeus, 1758)	MN012992	C	15138	-0,034	79,647	-0,235	20,353	new annotation
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Mesapia peloria</i> (Hewitson, 1853)	KX495168	P	15159	-0,018	81,107	-0,229	18,893	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris brassicae</i> (Linnaeus, 1758)	LR989948	C	15324	0,016	79,340	-0,226	20,660	new annotation
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris canidia</i> (Sparman, 1768)	KP162184	C	15153	0,013	79,661	-0,226	20,339	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris melete</i> Menetries, 1857	EU597124	C	15140	0,012	79,782	-0,222	20,218	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris napi</i> (Linnaeus, 1758)	HG993187	C	15095	0,011	80,139	-0,227	19,861	new annotation
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris napi</i> (Linnaeus, 1758)	MT576638	C	15178	0,011	80,076	-0,224	19,924	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris rapae</i> (Linnaeus, 1758)	LR990608	C	15161	0,014	79,665	-0,233	20,335	new annotation
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris rapae</i> Linnaeus, 1758	GQ398376	C	15164	0,014	79,662	-0,232	20,338	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris rapae</i> Linnaeus, 1758	HM156697	C	15157	0,014	79,739	-0,232	20,261	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pontia callidice</i> Hübner, 1799-1800	MH380206	C	15109	-0,003	79,866	-0,229	20,134	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pontia callidice</i> (Linnaeus, 1758)	MH380207	C	15124	-0,001	79,926	-0,218	20,074	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pontia edusa</i> Fabricius, 1777	MN013009	C	15127	-0,001	79,903	-0,219	20,097	new annotation
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Pontia edusa</i> Fabricius, 1777	MK252290	C	15125	-0,001	79,914	-0,218	20,086	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Prioneris clemathe</i> Doubleday, 1846	MK263225	C	15131	-0,022	80,761	-0,233	19,239	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Talbotia nagana</i> (Moore 1884)	MH380205	C	15155	0,002	79,815	-0,221	20,185	
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Talbotia nagana</i> (Moore 1884)	MW013541	C	15155	0,002	79,729	-0,220	20,271	
MIQGO	Papilionoidea	Riodinidae	Nemeobiinae	<i>Abisara fylloides</i> Moore, 1902	HQ259069	C	15301	-0,028	81,171	-0,203	18,829	
MIQGO	Papilionoidea	Riodinidae	Nemeobiinae	<i>Dodona durga</i> (Kollar, 1844)	MN012981	C	15349	-0,025	82,018	-0,149	17,982	new annotation
MIQGO	Papilionoidea	Riodinidae	Nemeobiinae	<i>Dodona eugenes</i> Bates, 1868	MT890732	C	15680	-0,019	81,014	-0,167	18,986	
MIQGO	Papilionoidea	Riodinidae	Nemeobiinae	<i>Zemeros fleggyas</i> (Cramer, 1780)	MK521434	C	15219	-0,035	81,792	-0,160	18,208	
MIQGO	Papilionoidea	Riodinidae	Riodininae	<i>Apodemia morio</i> (Felder & Felder, 1859)	KJ647171	C	15262	-0,047	81,523	-0,192	18,477	

C/P	Complete/Partial genome
Size	Genome Size
AT-skew	
AT%	percentage of A+T
GC-skew	
GC%	percentage of G+C

Table S2. Mitogenomes of Papilionoidea excluded from the final data set.

GO	SUPERFAMILY	FAMILY	SUBFAMILY	SPECIES	GenBank	C/P	Size	NOTE
MIQGO	Papilionoidea	Hesperiidae	Hesperiinae	<i>Ampittia dioscorides</i> (Fabricius, 1793)	KM102732	C	15313	bad <i>atp6</i> and <i>cox3</i> ; excluded form final data set
2S1GO	Papilionoidea	Hesperiidae	Pyrginae	<i>Ctenoptilum vasava</i> (Moore, [1866])	JF713818	C	15468	bad <i>cox1</i> and <i>trnK</i> ; excluded form final data set
IGO	Papilionoidea	Hesperiidae	Pyrginae	<i>Pyrgus armicanus</i> (Oberthür, 1910)	MT628570	P	9120	Incomplete gene order iGO; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Apaturinae	<i>Apatura illia</i> (Denis & Schiffermüller, 1775)	JF437925	C	15245	bad <i>atp6</i> ; excluded form final data set
IGO	Papilionoidea	Nymphalidae	Charaxinae	<i>Polyura schreiber</i> (Godart, [1824])	KT232257	C	15420	iGO no <i>trnF</i> ; excluded form final data set
IGO	Papilionoidea	Nymphalidae	Cyrestinae	<i>Dichorragia nesimachus</i> (Doyere, 1840)	KF990126	P	14367	iGO, some genes lacking; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Tirumala limniace</i> (Cramer, [1775])	KJ784473	C	15285	bad <i>trnH</i> ; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Tirumala limniace</i> (Cramer, [1775])	KM593191	C	15275	bad sequence, multiple framshifts; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Danainae	<i>Tirumala limniace</i> (Cramer, [1775])	KX889069	C	15278	bad sequence, multiple framshifts; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Acraea (Pareba) issoria</i> (Hübner, 1819)	GQ376195	C	15245	bad <i>nad5</i> ; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Heliconiinae	<i>Heliconius clysonymus</i> Latreille, [1817]	KP784455	C	15302	bad <i>rrnS</i> and <i>CoRe</i> ; excluded form final data set
IGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia genoveva</i> (Cramer, [1780])	KX267576	P	15206	iGO; excluded form final data set
IGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia genoveva genoveva</i> Cramer, [1780]	KX423729	C	15233	iGO; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia genoveva infuscata</i> C. & R. Felder, [1867]	KX423730	P	15145	multiple unresolved bases; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia litoralis</i> Brevignon, 2009	KX267568	P	15214	multiple unresolved bases; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia litoralis</i> Brevignon, 2010	KX267574	C	15177	multiple unresolved bases and bad <i>trnF</i> ; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Junonia vestina vestina</i> C. Felder & R. Felder, 1867	KX423728	C	15191	bad <i>nad3</i> and <i>trnE</i> ; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Precis andremiaja</i> (Boisduval, 1833)	MH917706	C	15239	bad <i>trnL1</i> and <i>trnC</i> ; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Nymphalinae	<i>Salamis anteva</i> (Ward, 1870)	MH917707	C	15201	bad <i>trnA</i> and <i>trnC</i> ; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Stichopthalma louisiana</i> Wood-Mason, 1877	KP247523	C	15721	bad <i>nad3</i> and <i>trnH</i> ; excluded form final data set
MIQGO	Papilionoidea	Nymphalidae	Satyrinae	<i>Triphysa phryne</i> (Pallas, 1771)	KF906487	C	15143	bad <i>nad2</i> ; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Graphium sarpedon</i> (Linnaeus, 1758)	LT999975	P	15098	bad sequence; excluded form final data set
IGO	Papilionoidea	Papilionidae	Papilioninae	<i>Pachliopta kotzebuea</i> (Eschscholtz, 1821)	LS975120	C	15045	iGO, <i>trnE</i> lacking; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio bianor</i> Cramer, 1777	KF859738	C	15357	different than conspecific sequences; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio dardanus</i> Brown, 1776	JX313686	C	15337	multiple unresolved bases; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio dardanus</i> Brown, 1776	KX033355	C	15338	multiple unresolved bases; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio helenus</i> Linnaeus, 1758	KP247522	C	15607	bad sequence; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio machaon</i> Linnaeus, 1758	HM243594	C	15185	bad <i>na5</i> , <i>rrnS</i> , and <i>CoRe</i> ; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio polytes</i> Linnaeus, 1758	KM215138	C	15260	bad <i>nad1</i> , <i>trnV</i> and <i>nad2</i> ; excluded form final data set
IGO	Papilionoidea	Papilionidae	Papilioninae	<i>Papilio xuthus</i> Linnaeus, 1767	EF621724	P	13964	iGO; excluded form final data set
IGO	Papilionoidea	Papilionidae	Papilioninae	<i>Trogonoptera brookiana</i> Wallace, 1855	LT999986	C	15005	iGO; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Hypermnestra helios</i> (Nickerl, 1846)	LS975127	P	14919	bad sequence; excluded form final data set
MIQGO	Papilionoidea	Papilionidae	Parnassiinae	<i>Luehdorfia chinensis</i> Leech, 1893	KU360130	C	15550	bad sequence; excluded form final data set
MIQGO	Papilionoidea	Pieridae	Coliadinae	<i>Eurema hecabe</i> (Linnaeus, 1758)	KC257480	C	15160	<i>nad2</i> very different than conspecific taxa; excluded form final data set
IGO	Papilionoidea	Pieridae	Pierinae	<i>Delias pasithoe parthenope</i> Wallace, 1867	KM244698	P	13155	iGO; excluded form final data set
MIQGO	Papilionoidea	Pieridae	Pierinae	<i>Hebomoia glaucippe</i> (Linnaeus, 1758)	KC489093	C	15701	bad <i>trnL1</i> ; excluded form final data set
IGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris brassicae</i> (Linnaeus, 1758)	KM592974	C	12887	iGO; excluded form final data set
IGO	Papilionoidea	Pieridae	Pierinae	<i>Pieris rapae</i> Linnaeus, 1758	KM592973	P	9751	iGO; excluded form final data set

IGO incomplete gene order

Table S3. Mitogenomes of Outgroups included in the final data set.

GO	SUPERFAMILY	FAMILY	SUBFAMILY	SPECIES	GenBank	C/P	Size	AT-skew	AT%	GC-skew	GC%
MIQGO	Alucitoidea	Alucitidae		<i>Alucita montana</i> Barnes & Lindsey, 1921	KJ508059	P	15272	0,001	79,970	-0,198	19,880
MIQGO	Bombycoidea	Bombycidae	Bombycinae	<i>Bombyx mandarina</i> (Moore, 1872)	AB070263	C	15928	0,055	81,680	-0,213	18,320
MIQGO	Bombycoidea	Bombycidae	Bombycinae	<i>Emolatia moorei</i> (Moore, 1879)	MF100139	C	15377	0,016	80,757	-0,227	19,243
MIQGO	Bombycoidea	Bombycidae	Bombycinae	<i>Ocinara albicollis</i> (Walker, 1862)	MF100144	C	15439	0,004	81,378	-0,200	18,622
MIQGO	Bombycoidea	Bombycidae	Bombycinae	<i>Rondotia menciana</i> Moore, 1885	KC881286	C	15301	0,050	78,864	-0,260	21,136
MIQGO	Bombycoidea	Bombycidae	Oberthuerinae	<i>Andraca olivacea</i> Matsumura, 1927	MF100135	C	15880	0,033	79,509	-0,258	20,491
MIQGO	Bombycoidea	Bombycidae	Oberthuerinae	<i>Mustilia undulosa</i> Yang & Mao, 1995	MF100142	C	15720	0,030	80,134	-0,242	19,866
MIQGO	Bombycoidea	Bombycidae	Oberthuerinae	<i>Mustilizans hepatica</i> (Moore, 1879)	MF100141	C	15745	0,017	80,210	-0,214	19,790
MIQGO	Bombycoidea	Bombycidae	Oberthuerinae	<i>Oberthueria jiatongae</i> Zolotuhin & Xing Wang, 2013	MF100143	C	15673	0,014	79,678	-0,224	20,322
MIQGO	Bombycoidea	Brahmaeidae		<i>Brahmaea hearseyi</i> (White, 1862)	KU884326	C	15442	-0,007	80,806	-0,221	19,194
MIQGO	Bombycoidea	Endromidae		<i>Prismosticta fenestrata</i> Butler, 1880	MF100145	C	15772	0,016	81,176	-0,189	18,824
MIQGO	Bombycoidea	Eupterotidae		<i>Ganisa cyanogrisea</i> Mell, 1929	MF100140	C	15250	-0,001	79,121	-0,246	20,380
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Actias artemis aliena</i> (Butler, 1879)	KF927042	C	15243	-0,013	78,620	-0,251	21,380
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Antheraea assamensis</i> Helfer, 1837	KU301792	C	15312	-0,018	80,179	-0,222	19,821
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Attacus atlas</i> (Linnaeus, 1758)	KF006326	C	15282	0,004	79,302	-0,241	20,698
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Cricula trifenestrata</i> Helfer, 1837	KY644697	C	15425	0,011	77,679	-0,277	22,314
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Eriogyna pyretorum</i> Westwood, 1847	FJ685653	C	15327	-0,031	80,818	-0,205	19,182
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Neoris haraldi</i> Schawerda, 1922	MF664471	C	15383	-0,011	79,510	-0,219	20,230
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Samia canningsi</i> (Hutton, 1860)	KJ159909	C	15384	-0,007	79,875	-0,227	20,125
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Saturnia boisduvalii</i> Eversmann, 1847	EF622227	C	15360	-0,024	80,625	-0,217	19,375
MIQGO	Bombycoidea	Saturniidae	Saturniinae	<i>Saturnia jonasii</i> (Butler, 1877)	MF346379	C	15261	-0,018	79,484	-0,214	20,516
MIQGO	Bombycoidea	Sphingidae	Macroglossinae	<i>Ampelophaga rubiginosa</i> Bremer & Grey, 1853	KT153024	C	15282	0,017	81,534	-0,189	18,466
MIQGO	Bombycoidea	Sphingidae	Macroglossinae	<i>Macroglossum stellatarum</i> Linnaeus, 1758	MG747645	C	15290	0,002	81,164	-0,193	18,836
MIQGO	Bombycoidea	Sphingidae	Macroglossinae	<i>Theretra japonica</i> (Boisduval, 1869)	MG655620	C	15399	0,023	80,356	-0,245	19,644
MIQGO	Bombycoidea	Sphingidae	Smerinthinae	<i>Parum colligata</i> (Walker, 1856)	MG888667	C	15288	0,005	81,057	-0,192	18,943
MIQGO	Bombycoidea	Sphingidae	Sphinginae	<i>Manduca sexta</i> (Linnaeus, 1763)	EJ286785	C	15516	-0,005	81,787	-0,181	18,213
MIQGO	Bombycoidea	Sphingidae	Sphinginae	<i>Notonagemia analis scribae</i> (Austaut, 1911)	KU934302	C	15303	0,025	80,246	-0,212	19,754
MIQGO	Bombycoidea	Sphingidae	Sphinginae	<i>Psilogramma increta</i> (Walker 1865)	MF974243	C	15252	0,025	80,330	-0,221	19,670
MIQGO	Bombycoidea	Sphingidae	Sphinginae	<i>Sphinx morio</i> (Rothschild & Jordan, 1903)	KC470083	C	15299	0,001	81,169	-0,195	18,831
MIQGO	Copromorphoidea	Carposinidae		<i>Carposina sasakii</i> Matsumura, 1900	HQ840719	C	15611	0,031	81,500	-0,162	18,500
MIQGO	Cossoidea	Cossidae	Cossinae	<i>Eogystia hippolophaecolus</i> (Hua, Chou, Fang & Chen, 1990)	KC831443	C	15431	0,030	78,433	-0,269	21,567
MIQGO	Drepanoidea	Drepanidae	Drepaninae	<i>Drepana arcuata</i> Walker, 1855	KJ508053	P	15302	0,005	81,127	-0,180	18,723
MIQGO	Gelechioidea	Autostichidae	Symmocinae	<i>Oegoconia novimundi</i> (Busck, 1915)	KJ508036	P	15408	-0,032	77,603	-0,329	22,209
MIQGO	Gelechioidea	Cosmopterigidae	Chrysopelinae	<i>Perimede</i> sp.	KJ508041	P	15131	-0,057	80,424	-0,229	19,397
MIQGO	Gelechioidea	Elachistidae	Ethmiinae	<i>Ethmia eupostica</i> Powell, 1985	KJ508047	P	15347	-0,014	79,553	-0,237	20,317
MIQGO	Gelechioidea	Elachistidae	Stenomatininae	<i>Promalactis suzukiella</i> (Matsumura, 1931)	KM875542	C	15507	-0,025	81,486	-0,183	18,514
MIQGO	Gelechioidea	Gelechiidae	Anacampsininae	<i>Mesophleps albiinella</i> (Park, 1990)	KU366707	C	15274	-0,061	80,536	-0,229	19,464
MIQGO	Gelechioidea	Gelechiidae	Dichomeridinae	<i>Dichomeris ustalella</i> (Fabricius, 1794)	KU366706	C	15410	-0,037	81,116	-0,179	18,884
MIQGO	Gelechioidea	Gelechiidae	Gelechiinae	<i>Tecia solanivora</i> (Povolný, 1973)	KT326187	C	15251	-0,013	78,264	-0,224	21,736
MIQGO	Gelechioidea	Gelechiidae	Pexicopiinae	<i>Pectinophora gossypiella</i> Saunders, 1843	KM225795	C	15202	-0,007	80,687	-0,204	19,313
MIQGO	Gelechioidea	Oecophoridae	Oecophorinae	<i>Endrosia sarcitrella</i> (Linnaeus, 1758)	KJ508037	P	15317	-0,010	79,611	-0,226	20,232
MIQGO	Gelechioidea	Oecophoridae	Oecophorinae	<i>Stathmopoda auriferella</i> (Walker, 1864)	KX138529	C	15456	-0,059	81,496	-0,197	18,504
MIQGO	Gelechioidea	Stathmopodidae		<i>Atrijuglans hetaohei</i> Yang, 1977	KT581634	C	15379	-0,034	81,319	-0,186	18,681
MIQGO	Gelechioidea	Stathmopodidae		<i>Hieromantis kurokoi</i> Yasuda, 1988	KU605775	C	15208	-0,045	80,260	-0,203	19,740
MIQGO	Geometroidea	Geometridae	Ennominae	<i>Abraxas suspecta</i> (Warren, 1894)	KY095828	C	15537	0,021	80,871	-0,191	19,129
MIQGO	Geometroidea	Geometridae	Ennominae	<i>Apracheia cinerarius</i> Erschoff, 1874	KR478686	C	15661	0,029	80,538	-0,192	19,462
MIQGO	Geometroidea	Geometridae	Ennominae	<i>Biston panterinaria</i> (Bremer & Grey, 1853)	JX406146	C	15517	0,064	79,551	-0,260	20,442
MIQGO	Geometroidea	Geometridae	Ennominae	<i>Celerena</i> sp.	KM244697	C	15403	0,037	80,751	-0,211	19,249
MIQGO	Geometroidea	Geometridae	Ennominae	<i>Ectropis obliqua</i> Prout, 1915	MF417804	C	15475	0,006	80,931	-0,178	19,069
MIQGO	Geometroidea	Geometridae	Ennominae	<i>Janakowskia athleta</i> Oberthür, 1884	KR822683	C	15534	0,042	79,529	-0,217	20,471
MIQGO	Geometroidea	Geometridae	Ennominae	<i>Phthonandria atrilineata</i> (Butler, 1881)	EU569764	C	15499	0,007	81,025	-0,192	18,975
MIQGO	Geometroidea	Geometridae	Larentiinae	<i>Dysstroma truncata</i> (Hufnagel, 1767)	KJ508061	P	15828	-0,008	79,953	-0,201	19,901
MIQGO	Geometroidea	Geometridae	Larentiinae	<i>Operophtera brumata</i> (Linnaeus, 1758)	KP027400	C	15748	0,005	79,966	-0,209	20,034
MIQGO	Gracillarioidea	Gracillariidae		<i>Cameraria ohridella</i> Deschka & Dimic, 1986	KJ508042	P	15513	0,019	81,113	-0,178	18,739
MIQGO	Gracillarioidea	Gracillariidae		<i>Phyllonorycter froelichiella</i> (Zeller, 1839)	KJ508048	P	15538	0,010	82,025	-0,185	17,827
MIQGO	Lasiocampoidea	Lasiocampidae		<i>Apatelopteryx phenax</i> De Lajonquière, 1968	KJ508055	P	15552	0,027	80,331	-0,207	19,515
MIQGO	Lasiocampoidea	Lasiocampidae		<i>Dendrolimus houi</i> De Lajonquière, 1979	KY000409	C	15381	0,030	79,930	-0,243	20,070
MIQGO	Lasiocampoidea	Lasiocampidae		<i>Euthrix laeta</i> (Walker, 1855)	KU870700	C	15368	0,019	80,193	-0,213	19,807
LacoGO	Mimallonoidea	Mimallonidae		<i>Lacosoma valva</i> Schaus, 1905	KJ508050	P	16108	0,022	80,873	-0,196	18,922
MIQGO	Noctuoidea	Noctuidae		<i>Doa</i> sp.	KJ508058	P	15228	0,005	80,437	-0,200	19,412
MIQGO	Noctuoidea	Erebidae	Aganinae	<i>Asota plana lacteata</i> Butler, 1881	KJ173908	C	15416	-0,002	80,345	-0,238	19,655
MIQGO	Noctuoidea	Erebidae	Arctiinae	<i>Aglaomorpha histrio</i> (Walker, 1855)	KY800518	C	15472	-0,003	79,899	-0,254	20,088
MIQGO	Noctuoidea	Erebidae	Arctiinae	<i>Amata formosae</i> Butler, 1876	KC513737	C	15463	-0,027	79,493	-0,266	20,507
MIQGO	Noctuoidea	Erebidae	Arctiinae	<i>Callimorpha dominula</i> (Linnaeus, 1758)	KP973953	C	15496	-0,011	81,021	-0,201	18,979
MIQGO	Noctuoidea	Erebidae	Arctiinae	<i>Cyana</i> sp.	KM244679	C	15494	-0,014	81,199	-0,223	18,801
MIQGO	Noctuoidea	Erebidae	Arctiinae	<i>Hyphantria cunea</i> (Drury, 1773)	GU592049	C	15481	0,010	80,389	-0,230	19,611
MIQGO	Noctuoidea	Erebidae	Arctiinae	<i>Lemyra melli</i> (Daniel, 1943)	KP307017	C	15418	0,001	78,668	-0,225	21,332
MIQGO	Noctuoidea	Erebidae	Arctiinae	<i>Paraona staudingeri</i> Alphéraky, 1897	KY827330	C	15427	0,009	80,191	-0,251	19,809
MIQGO	Noctuoidea	Erebidae	Arctiinae	<i>Vamuna virilis</i> (Rothschild, 1913)	KJ364659	C	15417	0,000	80,392	-0,229	19,608
MIQGO	Noctuoidea	Erebidae	Hermiinae	<i>Hydrilodes lentalis</i> Guenee, 1854	MH013484	C	15570	-0,020	81,079	-0,207	18,921
MIQGO	Noctuoidea	Erebidae	Hypeninae	<i>Paragabara curvicornuta</i> Kononenko & Matov, 2010	KT362742	C	15532	-0,008	80,440	-0,226	19,553
MIQGO	Noctuoidea	Lymantriidae		<i>Euproctis cryptosticta</i> Collenette, 1934	KY996558	C	15462	0,011	79,860	-0,245	20,140
MIQGO	Noctuoidea	Lymantriidae		<i>Gynaephora alpherakii</i> (Grum-Grzhimailo, 1891)	KJ957168	C	15755	0,004	81,441	-0,263	18,559
MIQGO	Noctuoidea	Lymantriidae		<i>Lymantria dispar</i> (Linnaeus, 1758)	FJ617240	C	15569	0,016	79,877	-0,247	20,123
MIQGO	Noctuoidea	Lymantriidae		<i>Somena scintillans</i> (Walker, 1856)	MH051839	C	15410	-0,009	80,818	-0,248	19,182
MIQGO	Noctuoidea	Noctuidae	Amphipyriinae	<i>Sesamia inferens</i> (Walker, 1856)	JN039362	C	15413	-0,001	80,237	-0,230	19,763
MIQGO	Noctuoidea	Noctuidae	Amphipyriinae	<i>Spodoptera exigua</i> (Hübner, 1808)	JX316220	C	15365	0,010	80,931	-0,195	19,069
MIQGO	Noctuoidea	Noctuidae	Catocalinae	<i>Eudocima phalonia</i> (Linnaeus, 1763)	KY196412	C	15575	-0,012	80,687	-0,219	19,313
MIQGO	Noctuoidea	Noctuidae	Hadeninae	<i>Mythimna separata</i> Walker, 1865	KF730242	C	15332	-0,011	80,987	-0,196	19,013
MIQGO	Noctuoidea	Noctuidae	Heliethinae	<i>Australothlis rubescens</i> Walker, 1858	KF977797	C	15382	0,000	81,355	-0,187	18,645
MIQGO	Noctuoidea	Noctuidae	Heliethinae	<i>Helicoverpa armigera</i> (Hübner, [1808])	GU188273	C	15347	0,001	80,973	-0,192	19,027
MIQGO	Noctuoidea	Noctuidae	Noctuinae	<i>Agrotis ipsilon</i> (Hufnagel, 1766)	KF163965	C	15377	-0,006	81,251	-0,177	18,749
MIQGO	Noctuoidea	Noctuidae	Noctuinae	<i>Striacosta albicosta</i> (Smith, 1888)	KM488268	C	15553	0,012	79,329	-0,238	20,671
MIQGO	Noctuoidea	Noctuidae	Plusiinae	<i>Ctenoplosia limbire</i>							

GO	SUPERFAMILY	FAMILY	SUBFAMILY	SPECIES	GenBank	C/P	Size	AT-skew	AT%	GC-skew	GC%
MIQGO	Pyraloidea	Pyralidae	Epipaschiinae	<i>Lista haraldusalis</i> (Walker, 1859)	KF709449	C	15213	-0,007	81,516	-0,171	18,484
MIQGO	Pyraloidea	Pyralidae	Galleriinae	<i>Galleria mellonella</i> (Linnaeus, 1758)	KT750964	C	15320	-0,039	80,418	-0,237	19,582
MIQGO	Pyraloidea	Pyralidae	Phycitinae	<i>Amyelois transitella</i> (Walker, 1863)	KT692987	C	15205	-0,048	79,638	-0,237	20,362
MIQGO	Pyraloidea	Pyralidae	Phycitinae	<i>Ephestia elutella</i> (Hübner, 1796)	MG748858	C	15346	-0,043	80,653	-0,217	19,347
MIQGO	Pyraloidea	Pyralidae	Phycitinae	<i>Euzophera pyriella</i> Yang, 1994	KY825744	C	15184	-0,052	79,814	-0,242	20,179
MIQGO	Pyraloidea	Pyralidae	Phycitinae	<i>Meroptera pravella</i> (Grote, 1878)	MF073207	C	15260	-0,019	80,550	-0,199	19,332
MIQGO	Pyraloidea	Pyralidae	Phycitinae	<i>Plodia interpunctella</i> (Hübner, 1813)	KP729178	C	15287	-0,049	80,153	-0,231	19,847
MIQGO	Pyraloidea	Pyralidae	Pyralinae	<i>Hypsopygia regina</i> (Butler, 1879)	KP327714	C	15212	-0,037	78,655	-0,228	21,345
MIQGO	Thyridoidea	Thyrididae		<i>Pyrioides aurea</i> Butler, 1881	KT337662	C	15362	0,000	79,938	-0,216	20,062
MIQGO	Thyridoidea	Thyrididae		<i>Rhodoneura mellea</i> (Saalmüller, 1881)	KJ508038	P	15615	-0,023	80,666	-0,232	19,167
MIQGO	Tortricoidea	Tortricidae	Olethreutinae	<i>Cydia pomonella</i> (Linnaeus, 1758)	JX407107	C	15253	-0,004	80,128	-0,207	19,872
MIQGO	Tortricoidea	Tortricidae	Olethreutinae	<i>Grapholita dimorpha</i> Komai, 1979	KJ671625	C	15813	-0,011	80,845	-0,189	19,155
MIQGO	Tortricoidea	Tortricidae	Olethreutinae	<i>Retinia pseudotsugaicola</i> Liu & Wu, 2001	KF498969	C	15282	-0,005	79,774	-0,224	20,226
MIQGO	Tortricoidea	Tortricidae	Olethreutinae	<i>Spilonota lechriaspis</i> Meyrick, 1932	HM204705	C	15368	-0,018	81,195	-0,188	18,805
MIQGO	Tortricoidea	Tortricidae	Tortricinae	<i>Acleris fimbriana</i> (Thunberg & Becklin, 1791)	HQ662522	C	15933	-0,012	80,951	-0,169	19,049
MIQGO	Tortricoidea	Tortricidae	Tortricinae	<i>Adoxophyes orana</i> (Fischer von Röslerstamm, 1834)	JX872403	C	15343	-0,001	79,958	-0,211	20,042
MIQGO	Tortricoidea	Tortricidae	Tortricinae	<i>Choristoneura longicellana</i> (Walsingham, 1900)	HQ452340	C	15759	0,010	81,141	-0,193	18,859
MIQGO	Urodoidea	Urodidae		<i>Urodus decens</i> Meyrick, 1925	KJ508062	P	15279	-0,018	80,202	-0,205	19,668
MIQGO	Yponomeutoidea	Lyonetiidae		<i>Leucoptera malifoliella</i> Costa, 1836	JN790955	C	15646	0,016	82,564	-0,199	17,436
MIQGO	Yponomeutoidea	Lyonetiidae		<i>Lyonetia clerkei</i> Linnaeus, 1758	MF045483	C	15259	0,017	79,920	-0,180	20,080
MIQGO	Yponomeutoidea	Plutellidae		<i>Plutella australiana</i> Landry & Hebert, 2013	MG787473	C	15962	0,014	80,673	-0,185	19,327
MIQGO	Yponomeutoidea	Yponomeutidae	Praydinae	<i>Prays oleae</i> Bernard, 1788	KM874804	C	16499	0,019	81,817	-0,179	18,183
MIQGO	Zygaenoidea	Limacodidae		<i>Monema flavescens</i> Walker, 1855	KU946971	C	15396	0,013	80,475	-0,226	19,525
ParaGO	Zygaenoidea	Limacodidae		<i>Parasa consocia</i> Walker, 1865	KX108765	C	15296	-0,001	80,583	-0,238	19,417
ChalGO	Zygaenoidea	Zygaenidae	Chalcosiinae	<i>Eterusia aedea</i> Clerck, 1759	MH316560	C	15196	-0,005	79,606	-0,211	20,394
ChalGO	Zygaenoidea	Zygaenidae	Chalcosiinae	<i>Histia rhodope</i> (Cramer, 1775)	MF542357	C	15209	0,015	78,447	-0,243	21,553
ChalGO	Zygaenoidea	Zygaenidae	Chalcosiinae	<i>Pidorus atratus</i> Butler, 1877	MG882482	C	15383	0,010	79,861	-0,207	20,139
ChalGO	Zygaenoidea	Zygaenidae	Chalcosiinae	<i>Rhodopsona rubiginosa</i> (Leech, 1898)	KM244668	C	15248	0,010	78,922	-0,228	21,078

C/P	Complete/Partial genome
Size	Genome Size
AT-skew	
AT%	percentage of A+T
GC-skew	
GC%	percentage of G+C

Alignment S1

nad2 vs ISP *trnQ-nad2* pairwise alignments in *Lopinga achine* (Nymphalidae, Satyrinae)

Segment (Starting nucleotide-ending nucleotide)

nad2 segment A (18-79)

```
                10      20      30      40      50      60
Lopinga achine new00000 nad2 segment A  TTCCTAAATAAAATATTTTTTTATTTTACTTATTTTITAGAACCA-TAATTTCAATT-TCCTCTA
Lopinga achine new00000 ISP trnQ-nad2  TTTTAAAAAAAATGA---TAATTTAAATCATCCCTAGAAATAAT-ATTTTC--TTAT-TTTTA
```

Alignment length: 64; identity: 40 (62.50%); different 24 (37.50 %).

nad2 segment B (233-288)

```
                10      20      30      40      50      60
Lopinga achine new00000 nad2 segment B  TCTTAAAGATTATTTTAAATAA--AAAT-TTTTATGG-ATAA-ATTCATTTCAATTTTA
Lopinga achine new00000 ISP trnQ-nad2  TTTTAAAAAAAATGATAATTAAATCATCCCTAAGAAATAATATT--TCCTATTTTTA
```

Alignment length: 61; identity: 35 (57.38%); different: 26 (42.62%)

nad2 segment C (684-738)

```
                10      20      30      40      50      60
Lopinga achine new00000 nad2 segment C  TTTTAAATTTAAATTTCTATT-AAAT--TTTCTATTATAATTAAT-TTTCTT-TCCTTA
Lopinga achine new00000 ISP trnQ-nad2  TTTTAAAAAAAATGATA-ATTAAATCATCCCTA--AGAAATAATTTCTTATTTTTA
```

Alignment length: 60; identity: 41 (68.33%); different: 19 (31.67%).

nad2 segment D (900-955)

```
                10      20      30      40      50      60
Lopinga achine new00000 nad2 segment D  TTTTAAATTTAAATGAT--TTAAATTTATAT-TAA-AAATAAT-TTT-TTAATTATAATTA
Lopinga achine new00000 ISP trnQ-nad2  TTTTAAAAAAAATGATAATTAAATC-ATCCCTAAGAAATAATAATTCCTA-TT-T--TTA
```

Alignment length: 62; identity: 44 (70.97%); different: 18 (29.03%)

Alignment S2

nad2 vs ISP *trnQ-nad2* pairwise alignments in *Parnassius apollo* (Papilionidae, Parnassiinae)

Segment (Starting nucleotide-ending nucleotide)

nad2 segment A (3-44)

```
                10      20      30      40
Parnassius apollo new00000 nad2 segment A TTTT TTT TAATTTAAAT TCAAA TAAA -TATTTT TATTTTAT
Parnassius apollo new00000 ISP trnQ-nad2 TTTATT GAATTTAAAT ---AA CAAAAC TAACCCCTA TATTTTATG
```

Alignment length: 43; identity: 29 (67.44%); different: 14 (32.56 %).

nad2 segment B (102-146)

```
                10      20      30      40
Parnassius apollo new00000 nad2 segment B TGGATT AGAAAT CAACTTATT TAA GATT TAT CCCCCTAA TTTCCAA
Parnassius apollo new00000 ISP trnQ-nad2 TTTATT GAATTTAAATAAC -AAAC -TAA CCCCCTA -TTT-AG
```

Alignment length: 45; identity: 28 is (62.22%); different: 17 (37.78 %).

nad2 segment C (249-289)

```
                10      20      30      40
Parnassius apollo new00000 nad2 segment C ATTAAAAAATT TGA AATTA AAT -TAA TTT -CTATTTTAA
Parnassius apollo new00000 ISP trnQ-nad2 TTTATTGAATTT --AAAT -AA CAAAAC TAA CCCCCTATTTTATG
```

Alignment length: 43; Identity: 27 (62.79%); Different: 16 (37.21%).

nad2 segment D (585-626)

```
                10      20      30      40
Parnassius apollo new00000 nad2 segment D TTTATT -GAATTT TTTATT TAA TAA T -TA -TTGATT -TTAATTAG
Parnassius apollo new00000 ISP trnQ-nad2 TTTATTGAATTT ---AA TAA CAAAAC TAACCC -CCTATT -TTAG
```

Alignment length: 46; Identity: 28 (60.87%); Different: 18 (39.13%).

nad2 segment E (718-760)

```
                10      20      30      40
Parnassius apollo new00000 nad2 segment E TTAATT -AATTT TTTAT CCCTT AGG AGGAT TAC CCCCCT -TTTTAG
Parnassius apollo new00000 ISP trnQ-nad2 TTTATTGAATTTAA -AT ---AAC AAAAC TAA CCCCCTA TTTTATG
```

Alignment length: 45; Identity: 29 (64.44%); Different: 16 (35.56%).

nad2 segment F (942-983)

```
                10      20      30      40
Parnassius apollo new00000 nad2 segment F ATTATT -AATTA TAAATA TTT CAA GAA -TAA TTT -CT -TTAATTAG
Parnassius apollo new00000 ISP trnQ-nad2 TTTATTGAATTT -TAAATA A--CAA -AAC TAA CCCCCTA TT -TTAG
```

Alignment length: 45; identity: 31 (68.89%); different: 14 (31.11%).

Alignment S3

nad2 vs ISP *trnQ-nad2* pairwise alignments in *Choaspes benjaminii* (KJ629164) (Hesperiidae, Coliadinae)

Segment (Starting nucleotide-ending nucleotide)

nad2 segment A (9-78)

```
Choaspes benjaminii KJ629164 nad2 segment A
Choaspes benjaminii KJ629164 ISP trnQ-nad2 6 x (TA) +
      10      20      30      40      50      60      70      80
TAAATATTAATTCATAATAAA-A-ATTTTTT-|---TTT-|---TTTATTTT|TAATTA|TAA|AA|CCCTAAT|TTC|TATTT|CTT|CA
TAAAAATAAATTTTATAAATAA|TATTTTTTAA|AAA|TTT|TAA|TTTATTT|---ATTT|TAA|TAA|---AAT|---TATTT|TT|A
```

Alignment length: 80; Identity: 53 (66.25%); Different: 27 (33.75%).

nad2 segment B (236-303)

```
Choaspes benjaminii KJ629164 nad2 segment B
Choaspes benjaminii KJ629164 ISP trnQ-nad2 6 x (TA) +
      10      20      30      40      50      60      70
TAAAAATAATTTTATAAATAA|ATTTT|---G|AAATT|ATAAATTTATTT|TCA|ATTTTAAT|AATT|CTT|CC|TTA
TAAAAATAATTTTATAAATAA|TATTTTTTAAAT|AAATTTATAAATTTATTT|---ATTTTAAT|AAAT|TATTT|TTA
```

Alignment length: 73; identity: 57 (78.08%); different: 16 (21.92%).

nad2 segment C (572-646)

```
Choaspes benjaminii KJ629164 nad2 segment C
Choaspes benjaminii KJ629164 ISP trnQ-nad2 6 x (TA) +
      10      20      30      40      50      60      70      80
TAATTAGAGAAATTTA|TTTATATTT|TATTTTTT|---T|AAATTTAT|CTTT|CTT|ATT|ATAATTA|TAT|GTT|TTATTTTTT|C
TAA--A-A-AT|AAT|---TTTATAAA|TATTTTTTAA|TAAATTTAT|AA|TTT|AT|TA|TT|TAATA|A-A-A--TTATTTTTA
```

Alignment length: 80; identity: 54 (67.50%); different: 26 (32.50%).

nad2 segment D (651-733) (Kim et al. 2014, segment)

```
Choaspes benjaminii KJ629164 nad2 segment D
Choaspes benjaminii KJ629164 ISP trnQ-nad2 6 x (TA) +
      10      20      30      40      50      60      70      80
TATAAATAATTTA|TTTTATAAAT|CAAT|TATTT|ATC|TATAAT|ATAAATTT|TTTA|AT|TAAATTTATTA|TATTT|AT|TAA|---TT|TT|CTT|---
TAAAAATAAT|---TTTATAAAT|---A|TATTT|---T|TA|---ATAAATTT|---AT|---AATTTATTT|TATTT|TATAAATAAT|TT|TTA
```

Alignment length: 88; identity: 61 (69.32%); different: 27 (30.68%).

nad2 segment E (917-994)

```
Choaspes benjaminii KJ629164 nad2 segment E
Choaspes benjaminii KJ629164 ISP trnQ-nad2 6 x (TA) +
      10      20      30      40      50      60      70      80
TTAAAAAT|---TTT|CA|TTTAAAAATA|ATT|AC|TTTATG|TTT|ATTTA|TAT|TATTTAA|TTTAA|TT|CC|TTAATA|GG|AATA|ATTTTTTA
TAAAAATAATTTT|---TTTATAAAT|TATT|---TTTA-A|TAAATTT|TAT|AATTTA|TTTA|TT|---TTAATA|A-AATTTTTTTA
```

Alignment length: 81; identity: 58 (71.60%); different: 23 (28.40%).

6 x (TA), repeated dinucleotide not considered in the alignment

Kim, J.M., Whang, A.R., Park, J.S., Kim, I. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperiidae) and phylogenetic reconstruction of Lepidoptera. *Gene* 2014, 549, 97-112.

Alignment S4

nad2 vs ISP *trnQ-nad2* pairwise alignments in *Lobocla bifasciatus* (KJ629166) (Hesperiidae, Eudaminae)

Segment (Starting nucleotide-ending nucleotide)

nad2 segment A (230-302)

```
                10      20      30      40      50      60      70
Lobocla bifasciatus KJ629166 nad2 segment A      TTTTATTAAAAATAATTTTATTAATAAAATTTTTCGAAATAAATAATTTT--TTTAT--CAATTTTAAATTAATTCCCTCATT
Lobocla bifasciatus KJ629166 ISP trnQ-nad2 11 x (TA) + TATTAAATAAAATAATTTAATTT---ATTTT---AAATAAATTTTAAATTTATTCCTATTAACTA--TT--TT--TT
```

Alignment length: 76; identity: 52 (68.42%); different: 24 (31.58%).

nad2 segment B (641-717) (Kim et al. 2014, segment)

```
                10      20      30      40      50      60      70
Lobocla bifasciatus KJ629166 nad2 segment B      TATTTTATATTTTTTAATA--TTTATTTTATTAA--TCAATTTATTTATTTTAAATAATAAATTCCCTATTAAAAATTT--TTTAAAT
Lobocla bifasciatus KJ629166 ISP trnQ-nad2 11 x (TA) + TATTAAATAAA---AATAATTTAATTTATTATTA--AATAA---ATTTTAAATTT--ATT--CTATTTAAACTAATTTT--TT
```

Alignment length: 79; identity: 53 (67.09%); different: 26 (32.91%).

nad2 segment C (901-972)

```
                10      20      30      40      50      60      70
Lobocla bifasciatus KJ629166 nad2 segment C      TATTTTAAATTTAAAAATGATTTTAAATATTTTATTAA--AAATAAATAATTTCTCTTTTATTTTATTTAA--TATTTTAAAT
Lobocla bifasciatus KJ629166 ISP trnQ-nad2 11 x (TA) + TATTAAATAAA---AAAAATATTTA---ATTTATTT--AAATAAATTTT--TAAATTTATTCTATTTAAACTATTTT--TT
```

Alignment length: 77; identity: 58 (75.32%); different: 19 (24.68%).

11 x (TA), repeated dinucleotide not considered in the alignment

Kim, J.M., Whang, A.R., Park, J.S., Kim, I. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperiidae) and phylogenetic reconstruction of Lepidoptera. *Gene* 2014, 549, 97-112.

Alignment S5

nad2 vs ISP *trnQ-nad2* pairwise alignments in *Euschemon rafflesia* (KY513288) (Hesperiidae, Euschemoninae)

Segment (Starting nucleotide-ending nucleotide)

nad2 segment A (7-85)

```
          10      20      30      40      50      60      70
Euschemon rafflesia KY513288 nad2 segment A  TTA CTTAAT ATTAATT CTAAC AAAA TAT TTTT ATTT TTAT TTTT TTTT TGAACCC TTAT TTCAT CTCAGC TAAAT
Euschemon rafflesia KY513288 ISP trnQ-nad2      CTA-TTAAAATAAATT-----AAATTAATTTTTTTCATTATATAAAAATTTT-AAA---TTTATATTTATTTTAAATTTT
```

Alignment length: 79; identity: 48 (60.76%); different: 31 (39.24%).

nad2 segment B (179-250)

```
          10      20      30      40      50      60      70
Euschemon rafflesia KY513288 nad2 segment B  CT-TTAAAATATTTT TTAAC TCAAT CAA TGGC TCCAT TAA TTTT TAT TTT CTAAT TTA TGGAA AAT AATT
Euschemon rafflesia KY513288 ISP trnQ-nad2      CTA-TTAAAATAAA--TTAAATTAATTTT-TT-TCCAT-TATA TAAATTTTAATTTAATTATTT-TTT--AATTTATTT
```

Alignment length: 76; identity: 52 (68.42%); different: 24 (31.58%).

nad2 segment C (391-466)

```
          10      20      30      40      50      60      70      80
Euschemon rafflesia KY513288 nad2 segment C  -TCTTGACAA AAAATTA CCCCCTA TAATTTTATTTGTCAT ATTATATAAAT AA-AAATTTT TT-TTATT ATTAATTAT AATTAA
Euschemon rafflesia KY513288 ISP trnQ-nad2      CTAATTAA-AA TAAATTA AAT---TAATTTT-TT---CATTATATAAATTTTAAATTTTATAATTATTTTTAATTAA--TT-
```

Alignment length: 80; identity: 54 (67.50%); different: 26 (32.50%).

nad2 segment D (572-645)

```
          10      20      30      40      50      60      70
Euschemon rafflesia KY513288 nad2 segment D  TAATTA GAGA AAAATTT TGTATTATATA TTTTATT TTATATCTTTTTT TAAATAGAG TTATA GCTTATTATTTT
Euschemon rafflesia KY513288 ISP trnQ-nad2      CTATTA AATA AAAATTAA--ATTA--AT-TTTT TCA TTATAT AAAATTTTAAATTTATA TTAT-TTTAATTATTT
```

Alignment length: 74; identity: 48 (64.86%); different: 26 (35.14%).

nad2 segment E (650-723)

```
          10      20      30      40      50      60      70
Euschemon rafflesia KY513288 nad2 segment E  TTATAAATATTTT TTATTAAT CAAATTA TTTATT TTTA ATATAAAATTTTATATTAAAATTATTTTTAAT-ATTT
Euschemon rafflesia KY513288 ISP trnQ-nad2      CTATTAAAATAAA TTAAATTAAT---TTTTCATT---ATATAAAATTTTAAATTTAATTATTATTATTTTTAATATTT
```

Alignment length: 75; identity: 54 (72.00%); different: 21 (28.00%).

nad2 segment F (830-902)

```
          10      20      30      40      50      60      70
Euschemon rafflesia KY513288 nad2 segment E  TTATTAATTATAAGCTTAAATCACATTATTTT TTATATTCG AATTA TTTA CTCTTGATCATATTTAATTATTT
Euschemon rafflesia KY513288 ISP trnQ-nad2      CTATTAATAAATTAATTA-ATTTTTTCA TTATAT--A AAT--TTTA AATTTAATTATTTTTAATTATTT
```

Alignment length: 73; identity: 51 (69.86%); Different: 22 (30.14%).

Alignment S6

nad2 vs ISP *trnQ-nad2* pairwise alignments in *Potanthus flavus* (KJ629167) (Hesperiidae, Hesperinae)

Segment (Starting nucleotide-ending nucleotide)

nad2 segment A (227-288)

```
          10      20      30      40      50      60
Potanthus flavus KJ629167 nad2 segment A  CTATTTTAAATTAATTTATTATTTATAAAA--AAATTTGAAAATT--AAATTTT--TAATAATCAATCTTA
Potanthus flavus KJ629167 ISP trnQ-nad2    TTATAAAAATTAATTAATTAT--ATAAAATTAATTT--AAAATTTTACTTTTATAAAT--TTATTTTA
```

Alignment length: 68; identity: 47 (69.12%); different: 21 (30.88 %).

nad2 segment B (559-622)

```
          10      20      30      40      50      60      70
Potanthus flavus KJ629167 nad2 segment B  TTATCTTCAATTATAAATTAG--ACA--AAATTTATTTTAA--TTTATTTTATAATTATA--TTCT--TTAATTA
Potanthus flavus KJ629167 ISP trnQ-nad2    TTATAA--AAATA--AATTAATTATTAATAAATTAATTTAAATTTTACTTTA--TTATAACT--TATT--TTA
```

Alignment length: 71; identity: 48 (67.61%); different: 23 (32.39 %).

nad2 segment C (665-738) (Kim et al. 2014, segment)

```
          10      20      30      40      50      60      70
Potanthus flavus KJ629167 nad2 segment C  TTATTAATCAATTAATTTTATTAATATAAATTAATTAATAAATAAATTT--TTTATTGATTAATTTCTTTCTTA
Potanthus flavus KJ629167 ISP trnQ-nad2    TTATAA--AATAAATTA--TT--ATATAAATTAAT--TTAAATTTTAC--TTTATT--A--TAATTA--TTT--TTA
```

Alignment length: 76; identity: 55 (72.37%); different: 21 (27.63 %).

nad2 segment D (897-976)

```
          10      20      30      40      50      60      70      80
Potanthus flavus KJ629167 nad2 segment D  TTATATTAAATTAATAATGATTTAATTTATTAAATAAATAAATTTTCTCCTTATTATAAATTTTAGAATAATTTCTA
Potanthus flavus KJ629167 ISP trnQ-nad2    TTATA--AAAATAAATTAATT--ATAFA--AATTAATTTAA--ATTTT--ACTTTATTATAAATTT--AT--TTT--TA
```

Alignment length: 80; identity: 54 (67.50%); different: 26 (32.50%).

Kim, J.M, Whang, A.R., Park, J.S., Kim, I. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperidae) and phylogenetic reconstruction of Lepidoptera. *Gene* 2014, 549, 97-112.

Alignment S7

nad2 vs ISP *trnQ-nad2* pairwise alignments in *Carterocephalus silvicola* (KJ629163) (Hesperiidae, Heteroptera)

Segment (Starting nucleotide-ending nucleotide)

nad2 segment A (17-85)

```

                10      20      30      40      50      60      70
Carterocephalus silvicola KJ629163 nad2 segment A  A C T T A A T A A A A T A T T T T T T T T T T T A C T T A A T T T T A G T T T A A T T C T C A A T C T C A G C T A A T T C T T
Carterocephalus silvicola KJ629163 ISP trnQ-nad2    G C C C A A A A A A T A T T T T T T T T T T T A C T T T A G T T A A A T T T T A T T A A A T T T A A A T T A A A T T A A T T A T T T T T T

```

Alignment length: 72; identity: 47 (65.28%); different: 25 (34.72%).

nad2 segment B (139-208)

```

                10      20      30      40      50      60      70
Carterocephalus silvicola KJ629163 nad2 segment B  A T T A A T A A T T G T A A T A A T T T A T T A A C T T C A G A A A G C A T C T T T A A A A T A T T T C T A G T T C A A T C T A T T G C T T
Carterocephalus silvicola KJ629163 ISP trnQ-nad2    G C C C A A A A A A T T T T A A T T A A C T T T A C T A T A A A T A T T A A A T T A A A T T T A A A T T T A A A T T A T T T T T T

```

Alignment length: 76; identity: 47 (61.84%); different: 29 (38.16%).

nad2 segment C (233-298)

```

                10      20      30      40      50      60      70
Carterocephalus silvicola KJ629163 nad2 segment C  T C A T T A A A A A T A A T T T T T A T T A A A A A T T T G A A A T G A A T T T T T T A C T A T C A A T T T A A T C A A T T C C T
Carterocephalus silvicola KJ629163 ISP trnQ-nad2    G C C C A A A A A A T A A T T T A T T A C T T T A G T A T A A A T T T A T A A T T T A A A T T T A A A T T A A A T T A A T T T T T

```

Alignment length: 77; identity: 47 (61.04%); different: 30 (38.96%).

nad2 segment D (408-480) (Kim et al. 2014, segment)

```

                10      20      30      40      50      60      70
Carterocephalus silvicola KJ629163 nad2 segment D  - C C C A T A A T T T T A T T G T C C T A T T A C T T A A T A A A A A T T T T A A T A T T A A T T A T A A T A A C T A A T A A T T T
Carterocephalus silvicola KJ629163 ISP trnQ-nad2    G C C C A A A A A T A A T T T A T T T A C T T T A G T A T A A A T T T A T A A T T T A A A T T A A A T T A A A T T A A T T A T T T T T

```

Alignment length: 78; identity: 51 (65.38%); different: 27 (34.62%).

nad2 segment E (927-994)

```

                10      20      30      40      50      60      70
Carterocephalus silvicola KJ629163 nad2 segment E  T A T T A A A A A T A A T T T T C T T T G T A T T A T T T A A T A G C T T A A T T T C A A T T T A G G A A T A A T T C T T A
Carterocephalus silvicola KJ629163 ISP trnQ-nad2    - G C C A A A A A T A A T T T T A C T T A G T A T A A T T T A T A A T T T A A A T T A T A A T T A T T T T T

```

Alignment length: 72; Identity: 49 (68.06%); Different: 23 (31.94%).

Kim, J.M., Whang, A.R., Park, J.S., Kim, I. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperidae) and phylogenetic reconstruction of Lepidoptera. *Gene* 2014, 549, 97-112.

Alignment S8

nad2 vs ISP *trnQ-nad2* pairwise alignments in *Daimio tethys* (KJ629165) (Hesperiidae, Tagiadinae)

Segment (Starting nucleotide-ending nucleotide)

nad2 segment A (17-86)

```
                10      20      30      40      50      60      70
Daimio tethys KJ629165 nad2 segment-A      A CTCAAAT AAAATATTTTTT TT TTAT ATTAACCTT TAGTACTTTT ATCTCTA TTTCAACAAA --- TTCAATG
Daimio tethys KJ629165 ISP trnQ-nad2 7 x (TA) + TCTCATA AAAATATTTTTT TAGTTAAAAA TAAAGTTT -A-TAAATTT -AT-T-TTTTAT TAAAAATA TTTTA
```

Alignment length: 75; identity: 49 (65.33%); different: 26 (34.67%).

nad2 segment B (173-250)

```
                10      20      30      40      50      60      70      80
Daimio tethys KJ629165 nad2 segment B      C TCTCTT AAAATATTTTTT AACTCAATCAAT T-GCTTCAAT TAAATTTCTATTATTTTCTA TCTATTAAAAATA TTTTCA
Daimio tethys KJ629165 ISP trnQ-nad2 7 x (TA) + TCTCATA AAAATATTTTTT TAGTTAAAAA TAAAGTTT -A-TAAATTT -TTATTTT ---T-TTATTAAAAATA TTTTAA
```

Alignment length: 80; identity: 56 (70.00%); different: 24 (30.00%).

nad2 segment C (408-478)

```
                10      20      30      40      50      60      70
Daimio tethys KJ629165 nad2 segment C      C C CATAATTT TATTGTCCTA TTATTTAAATA CA-AATTTT TAAATT-ATTATTA TAAATTATTAA ---TACTAGCA
Daimio tethys KJ629165 ISP trnQ-nad2 7 x (TA) + TCTCATA AAAA TATT-T-TT-TTAGTTAAAAA -ATAAGTTT TAAATT TATT-TT-T-TTATTAA AAA TATTTTTA
```

Alignment length: 76; identity: 50 (65.79%); different: 26 (34.21%).

nad2 segment D (681-759) (Kim et al. 2014, segment)

```
                10      20      30      40      50      60      70      80
Daimio tethys KJ629165 nad2 segment D      TAT-ATT TAAATA TAA-ATTTCTT -A-TTCAAAATTT -TTCTAT TAAATAAATTTT TATCTTTGGGAGGAT TACCCCTCTTTTA
Daimio tethys KJ629165 ISP trnQ-nad2 7 x (TA) + TCTCATA --TAA-A-AATATTTT TTAGTTAAAAA TAAAGTTT -TA-TAATTTATTTT TATT ---AAAAAT -A-----T-TTTTA
```

Alignment length: 85; identity: 50 (58.82%) different: 35 (41.18%).

nad2 segment E (808-880)

```
                10      20      30      40      50      60      70
Daimio tethys KJ629165 nad2 segment E      TTTTC-TAA T--TACTCTTTTATTT TTTATTAAATAAGTTTAAAT TACTTTATTTT TTTATATTCGAAT TATTTAATTC
Daimio tethys KJ629165 ISP trnQ-nad2 7 x (TA) + TCTCATA AAAA TATTT-TTTTAG--TTAA-AAAAAAGTTA--TAAATTTATTTT TAT--TAAAAA TATTTT-TTA
```

Alignment length: 77; identity: 54 (70.13%); different: 23 (29.87%).

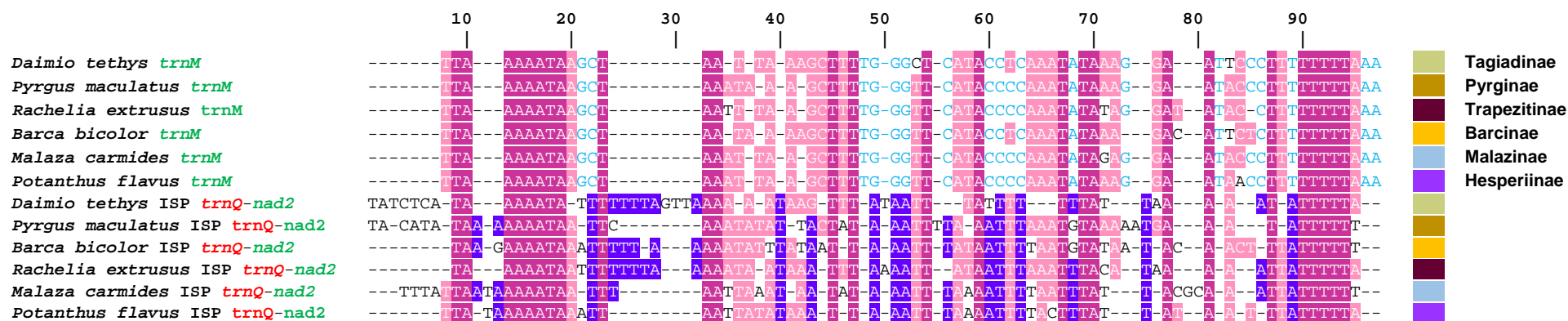
The repeated element (TA)₇ was not included in the alignment

Kim, J.M, Whang, A.R., Park, J.S., Kim, I. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperiidae) and phylogenetic reconstruction of Lepidoptera. *Gene* 2014, 549, 97-112.

Alignment S9

Multiple alignment of *trnM*s and ISPs *trnQ-nad2* in selected species of the family Hesperidae.

Hesperidae

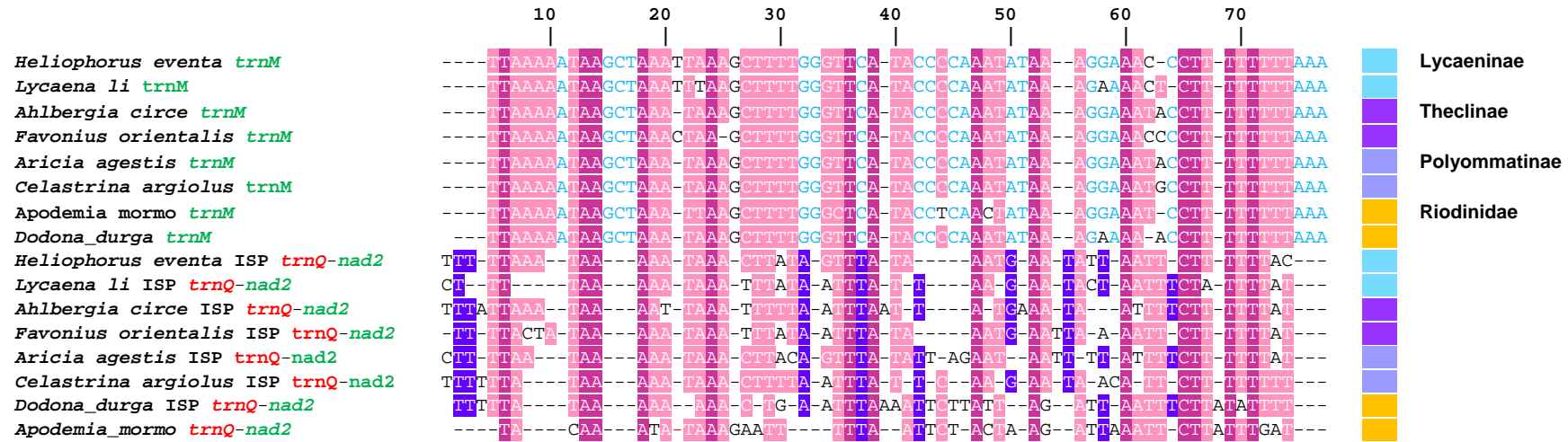


■, fully conserved nucleotide; ■, partly conserved nucleotide;
 ■, nucleotide peculiar for *trnM*; ■, nucleotide peculiar for ISP *trnQ-nad2*
 ■, position where the alignment cannot be done with certainty.

Alignment S10

Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the families Lycaenidae and Riodinidae.

Lycaenidae + Riodinidae



█, fully conserved nucleotide; █, partly conserved nucleotide;
 █, nucleotide peculiar for *trnM*; █, nucleotide peculiar for ISP *trnQ-nad2*
 █, position where the alignment cannot be done with certainty.

Alignment S11

Multiple alignment of *trnM*s and ISPs *trnQ-nad2* in selected species of the subfamily Pierinae.

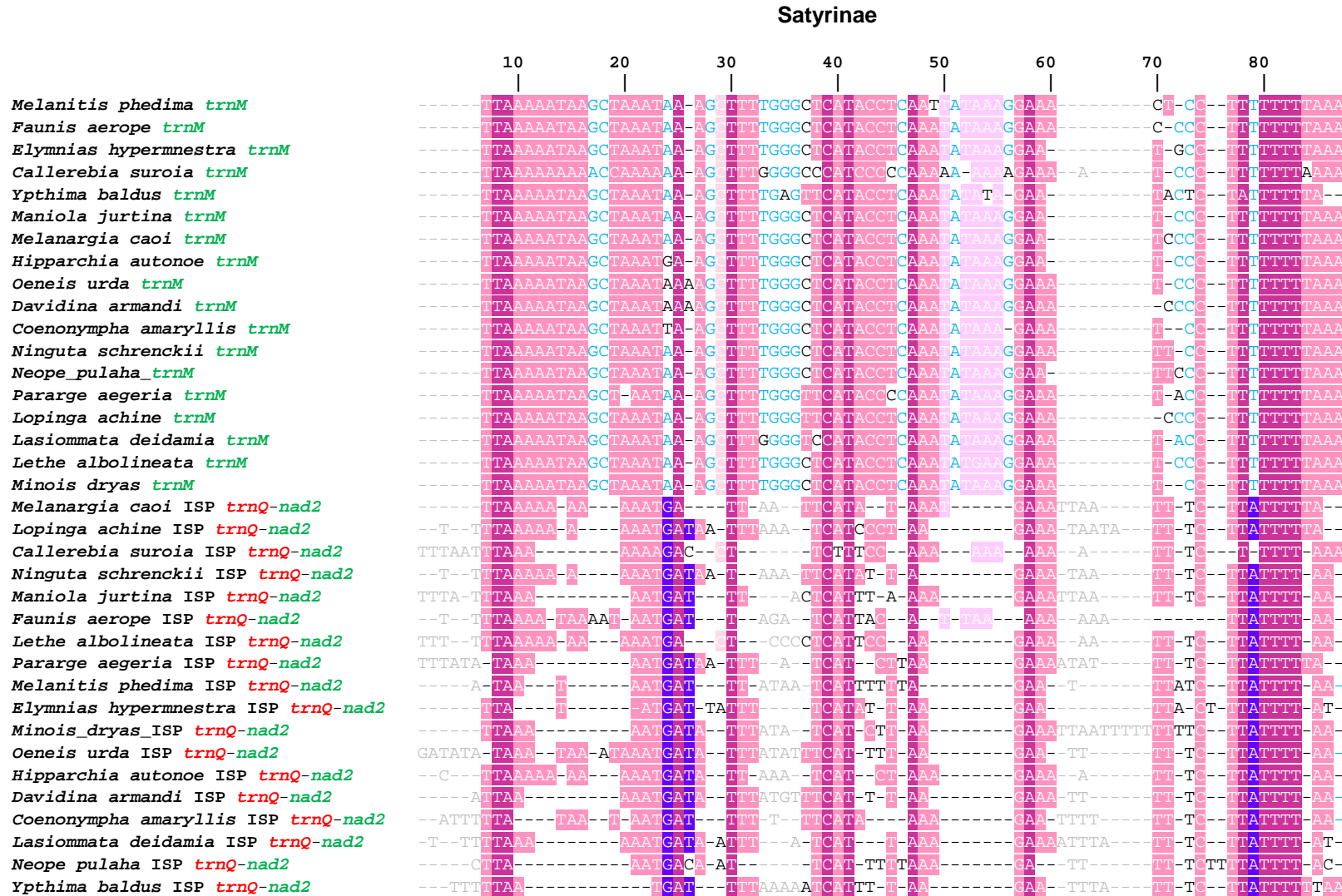
Pierinae

	10	20	30	40	50	60	70												
<i>Anthocharis cardamines trnM</i>	TTAA	GAATA	AGCTAAA	TTAAGCT	TTTGGGCT	CATACCT	CAAATATAAA	GA-TAT	TTC	TTTTT	CTTAAT								
<i>Baltia butleri trnM</i>	TTAA	GAATA	AGCTAAG	TTAAGCT	TTTGGGT	CATACCT	CAAATATAAA	GA-TAT	C	TTTTT	CTTAAT								
<i>Pontia callidice trnM</i>	TTAA	GAATA	AGCTAAG	TTAAGCT	TTTGGGCT	CATACCT	CAAATATAAA	GA-TAT	C	TTTTT	CTTAAT								
<i>Talbotia nagana trnM</i>	TTAA	GAATA	AGCTAAA	TTAAGCT	TTTGGGCT	CATACCT	CAAATATAAA	GAATA	-CC	TTTTT	CTTAAT								
<i>Appias lyncida trnM</i>	TTAA	GAATA	AGCTAAG	TTAAGCT	TTTGGGCT	CATACCT	CAAATATAAA	GA-TAT	C	TTTTT	CTTAAT								
<i>Pieris brassicae trnM</i>	TTAA	GAATA	AGCTAAA	TTAAGCT	TTTGGGCT	CATACCT	CAAATATAAA	GA-TAT	-AC	TTTTT	CTTAAT								
<i>Aporia crataegi trnM</i>	TTAA	GAATA	AGCTAAA	TTAAGCT	TTTGGGCT	CATACCT	CAAATATAAA	GA-T	-CCT	TTTTT	CTTAAT								
<i>Delias hyparete trnM</i>	TTAA	GAATA	AGCTAAG	TTAAGCT	TTTGGGCT	CATACCT	CAAATATAAA	GA-T	-C	TTTTT	CTTAAT								
<i>Anthocharis cardamines ISP trnQ-nad2</i>	TT	TTA	AAAA	ATG	AA	TTAAA	TTT	CT	TA	AT	AA	TATA	TTA	TA	TTT	TTTTT	TTA		
<i>Baltia butleri ISP trnQ-nad2</i>	TT	TTTTAA	AA	CTG	AA	TTAAA	TTT	CT	T	T	AA	TA	TT	T	TT	TAT	TTTTA	T	
<i>Pontia callidice ISP trnQ-nad2</i>	TT	TTC	AAAA	CTG	AA	TTAAA	TTT	CT	T	T	AT	TA	TT	T	TT	TAT	TTTTA	T	
<i>Talbotia nagana ISP trnQ-nad2</i>	TT	TT	AAAA	TA	G	AA	TTA	ATT	CT	TAC	AA	TA	C	A	T	TT	TAT	TTTTA	T
<i>Appias lyncida ISP trnQ-nad2</i>	TT	TT	AAAA	TA	G	AA	TTAAA	TTT	CT	TT	AA	TA	TT	T	TT	TAT	TTTTA	T	
<i>Aporia crataegi ISP trnQ-nad2</i>	TT	TT	AAAA	TA	G	AA	TTAAA	TTT	CT	TT	AA	TA	TT	T	TT	TAT	TTTTA	T	
<i>Delias hyparete ISP trnQ-nad2</i>	TT	TT	AAAA	TA	G	AA	TTAAA	TTT	CT	TT	AA	TA	TT	T	TT	TAT	TTTTA	T	
<i>Pieris brassicae ISP trnQ-nad2</i>	TT	TT	AAAA	TA	G	AA	TTAAA	TTT	CT	TT	AA	TA	TT	T	TT	TAT	TTTTA	T	

N, fully conserved nucleotide; N, partly conserved nucleotide;
N, nucleotide peculiar for *trnM*; N, nucleotide peculiar for ISP *trnQ-nad2*
 N, position where the alignment cannot be done with certainty.

Alignment S12

Multiple alignment of *trnMs* and ISPs *trnQ-nad2* in selected species of the subfamily Satyrinae (Nymphalidae).

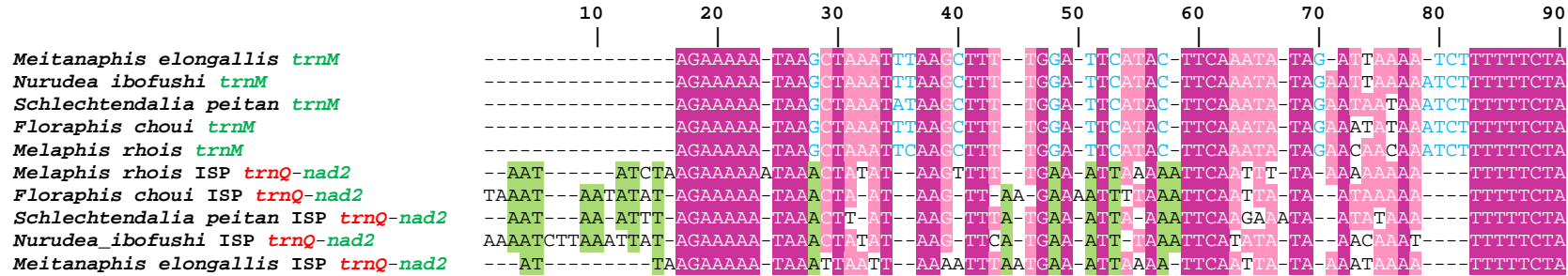


N, fully conserved nucleotide; N, partly conserved nucleotide;
N, nucleotide peculiar for *trnM*; N, nucleotide peculiar for ISP *trnQ-nad2*
 N, position where the alignment cannot be done with certainty.

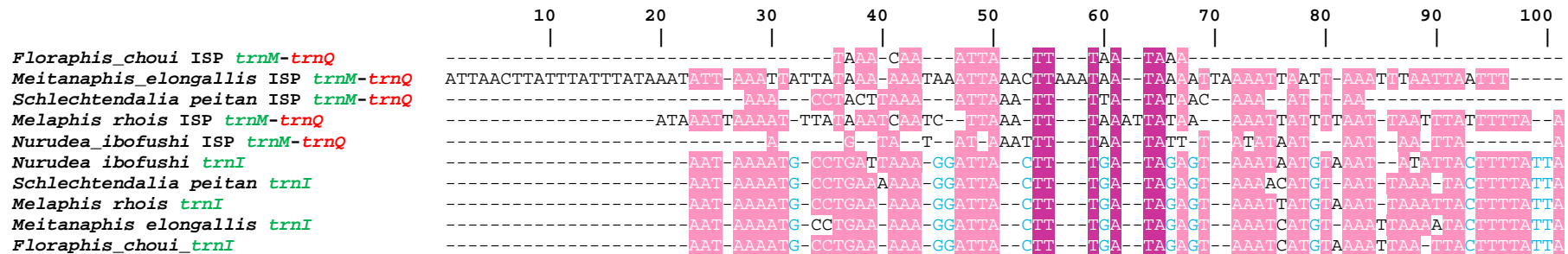
Alignment S13

Pemphigidae (Hemiptera)

trnM vs ISP *trnQ-nad2* alignment



trnI vs ISP *trnM-trnQ* alignment



Alignment S14

Curculionidae (Coleoptera)

trnM vs ISP *trnQ-nad2* alignment

	10	20	30	40	50	60	70	80
<i>Trypodendron domesticum</i> ISP <i>trnQ-nad2</i> 3'p	-TTATAAAA-TTATCCCTCATCTACCT--CTATTCAAGTTTATAGTTCAATCTA-AAA-----							
<i>Trypodendron signatum</i> ISP <i>trnQ-nad2</i>	AAATTAAGA-AAGG-T-AGCTACCTTTCTCTTGAAATATATAATTCAA-CTA-AAAAAT-T-----TTTAAATAATTAAT							
<i>Trypodendron signatum trnM</i>	--AA-AAAGATAAG-CT-AA-TAAG--CTTTGGTTCATACCCCAA-CCACAAAGGTCACCTCC-TTTCCTTTTA--							
<i>Trypodendron domesticum trnM</i>	--AA-AAAAGTAAG-CT-AA-TAAG--CTTTGGTTCATACCCCAA-CCATAGAGTTCACCCCTT-CTTTTA--							

CoRe 3'p + *trnI* vs ISP *trnM-trnQ* 5'p alignment

	10	20	30	40	50	60
<i>Trypodendron signatum</i> CoRe 3'p + <i>trnI</i>	ATTTAAAAATTAAATATTATAATTTA-TACAAAATAATAATTTTACCAAA-ACTA-AAAA					
<i>Trypodendron signatum</i> ISP <i>trnM-trnQ</i> 5'p	ATTTAAAACCAAATCTTTTACCCTTAATTTT-AAAAATAA-TTTTA--AATTA-TATAAAA					
	70	80	90	100	110	120
<i>Trypodendron signatum</i> CoRe 3'p + <i>trnI</i>	C--TCAATAAAATTTAATAAAAATAAATTTCAATTTAAAAC-TACAATTAATATC-TTTAAAAAA					
<i>Trypodendron signatum</i> ISP <i>trnM-trnQ</i> 5'p	CCTCATAAAA----AATAAACTA-TTT-ATTTAAAAC-GTGAA--AT-C-TTTAAAAAA					
	130	140	150	160	170	180
<i>Trypodendron signatum</i> CoRe 3'p + <i>trnI</i>	TT-TTACC-TTTTAAAAA-AAATTTACA--A--A-AT-A--ATTC-TAATTTAAT--TT					
<i>Trypodendron signatum</i> ISP <i>trnM-trnQ</i> 5'p	TTGTT---TTTAAAGTGCAACC-ACACCAACGTTATTATTTCAGCAATTT-AATCCCTT					
	190	200	210	220	230	240
<i>Trypodendron signatum</i> CoRe 3'p + <i>trnI</i>	AAAAATTAATAATATGATGCCTGATAAAA-GGAATATTTTGATAGAAATATTACATGG					
<i>Trypodendron signatum</i> ISP <i>trnM-trnQ</i> 5'p	ACAAAT-AAGTT--TATTATA-CTAA-AAAATGG---ATTTTGA---AA-ATT--AT--					
	250	260				
<i>Trypodendron signatum</i> CoRe 3'p + <i>trnI</i>	AAATCCTAATTTTCCTCATA-TTA					
<i>Trypodendron signatum</i> ISP <i>trnM-trnQ</i> 5'p	---T--TATTTTCCTTTTAGCTA					

Trypodendron signatum: pairwise alignment of a genomic portion (3' end of Control Region + *trnI*) and the first 221 bases of the ISP *trnM-trnQ*

Alignment S15

Multiple alignment of *trnS1* sequences of selected species of Lepidoptera



Legend

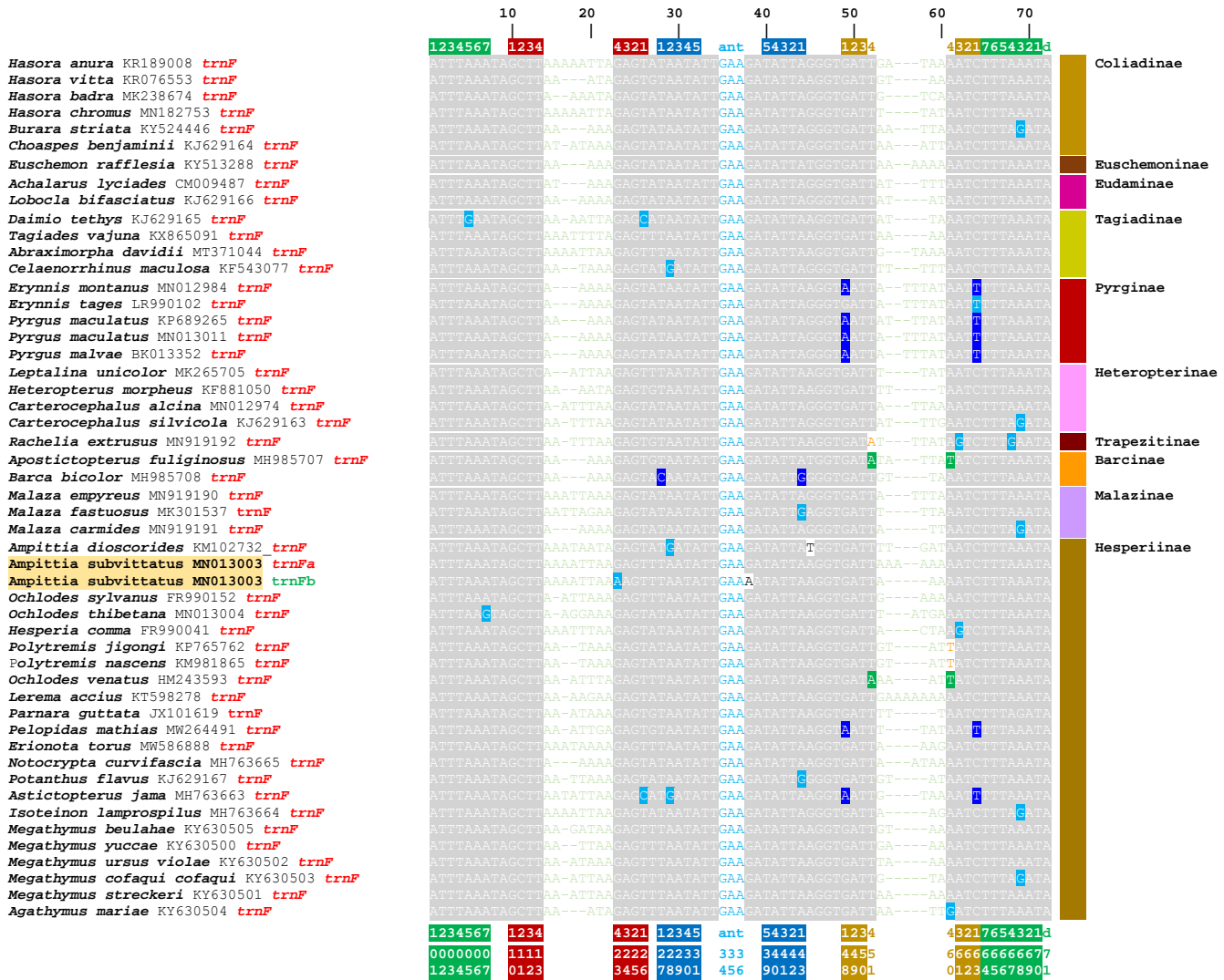
., the background base for the position.
 N, half-compensatory base change in the stem pair (e.g. T - G vs C - G; A - T vs G - T).
 N, half-compensatory base change in the stem pair exhibiting a mismatch (e.g. T - A vs A - A).
 N, fully compensatory base change in the stem pair exhibiting a mismatch (e.g. C - G vs T - T).
 N, type I fully compensatory base change in the stem pair (i.e. purine - pyrimidine vs purine - pyrimidine, e.g. G - C vs A - T).
 N, type II fully compensatory base change in the stem pair (i.e. purine - pyrimidine vs pyrimidine - purine, e.g. A - T vs T - A).
 Different colors are used to better differentiate the changes.
 N, a mismatch in the in the stem pair; N, a mismatch in a pair not always fully consistent (e.g. 5 5).
 N, substitution pattern not modelled.
 *, position in which a mismatch is prominent.
 N, N, molecular signature for a taxon.
 X, position 1-7 in the acceptor stem; X, position 1-4 in the DHU stem; X, position 1-5 in the anticodon stem; X, position 1-5 in the T_ΨC stem; ant, anticodon; d, discriminator nucleotide
 A compensatory base change implies the substitution of a nucleotide with a different base that does not disrupt the pairing in the stem.
 Only *trnS1s* different from plesiomorphic condition *trnS1^{TCT}*, are labelled with their anticodon i.e. *trnS1^{ACT}* and *trnS1^{TCT}*.

Reference

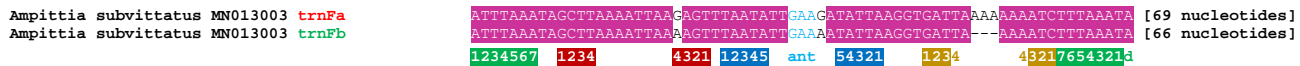
Montelli, S., Peruffo, A., Patarnello, T., Cozzi, B., Negrisolo, E. Back to water: signature of adaptive evolution in cetacean mitochondrial tRNAs. *PLoS ONE* 2016, 11, e0158129.

Alignment S16

Multiple alignment of *trnF* sequences in Hesperidae



Pairwise alignment of *trnFa* and *trnFb* in *Ampittia subvittatus*



Alignment length: 69; identity: 64 (92.75%); different: 5 (7.25%).

Legend

- ⋅, the background base for the position.
- N, half-compensatory base change in the stem pair (e.g. T - G vs C - G; A-T vs G-T).
- N, half-compensatory base change in the stem pair exhibiting a mismatch (e.g. T-A vs A-A).
- N, fully compensatory base change in the stem pair exhibiting a mismatch (e.g. C-G vs T-T).
- N, type I fully compensatory base change in the stem pair (i.e. purine - pyrimidine vs purine - pyrimidine, e.g. G - C vs A - T).
- N, type II fully compensatory base change in the stem pair (i.e. purine - pyrimidine vs pyrimidine - purine, e.g. A - T vs T - A).
- Different colors are used to better differentiate the changes.
- N, a mismatch in the in the stem pair; N, a mismatch in a pair not always fully consistent (e.g. 5 5).
- N, substitution pattern not modelled.
- *, position in which a mismatch is prominent.
- N, molecular signature for a taxon.
- N, position 1-7 in the acceptor stem; N position 1-4 in the DHU stem; N position 1-5 in the anticodon stem; N position 1-5 in the TWC stem; ant, anticodon; d, discriminator nucleotide
- A compensatory base change implies the substitution of a nucleotide with a different base that does not disrupt the pairing in the stem. Only *trnS1s* different from plesiomorphic condition *trnS1^{scT}*, are labelled with their anticodon i.e. *trnS1^{scT}* and *trnS1^{scT}*.

Reference

Montelli, S., Peruffo, A., Patarnello, T., Cozzi, B., Negrisolo, E. Back to water: signature of adaptive evolution in cetacean mitochondrial tRNAs. *PLoS ONE* 2016, 11, e0158129.

Alignment S17

The intergenic spacers: ISP *trnQa-trnQb*, ISP *trnQb-trnQc* and ISP *trnQc-trnQd*

Bhutanitis thaidina MN012973 ISP *trnQa-trnQb* (segments: A, B, C, D)

ATTACCCCTATTATATTGAATTTATATTTCAAAAAAAAAAAATCCCCCTGATTGATTTTTTAATATTAATTCAAATAAAATATTTTTTA
 CCCTTTTTTTTAAATAAAATTGCCTG
 ATCAAAGGATTATTTAATAAATAAAAAATGTAATATAACTACCTTTA

Pairwise alignments of portions of ISP *trnQa-trnQb* with ISP *trnQd-nad2*, CoRe 3'-end, *trnM* an *trnI*

<i>Bhutanitis thaidina</i> MN012973 ISP <i>trnQa-trnQb</i>	(segment A)	ATTACCCCTATTATATTGAATTTATATTTCAAAAAAAAAAAATCCCCCT-----
<i>Bhutanitis thaidina</i> MN012973 ISP <i>trnQd-nad2</i>		ATTACCCCTATTATATTGAATTTATATTTCAAAAAAAAAAAATCCCCCTATTTTAA
<i>Bhutanitis thaidina</i> MN012973 ISP <i>trnQa-trnQb</i>	(segment B)	GA-TTTGATT-TTTAATATTAAT--TCAA-A-TAAAAATTTTTTTA
<i>Bhutanitis thaidina</i> MN012973 CoRe (3' end)		GAATACGATTATTAAATATTAATTTAATAAATAATCTTTA
<i>Bhutanitis thaidina</i> MN012973 ISP <i>trnQa-trnQb</i>	(segment C)	-----CCCTTTTTTTAA
<i>Bhutanitis thaidina</i> MN012973 <i>trnM</i>		TTAAAAATAAGCTAAATTTAAGCTTTTGGGTTCAATACCCCAATATAAAGGAAACCCCTTTTTTTAA
<i>Bhutanitis thaidina</i> MN012973 ISP <i>trnQa-trnQb</i>	(segment D)	-A-AAATTCCTGATCAAAAGGATTATTTAAT-ATAAAAAATGTAATATACTACCTTTA---
<i>Bhutanitis thaidina</i> MN012973 <i>trnI</i>		ATTAAGTCCTGATCAAAAGGATTATTTGATGATAAAAAATGTAATATAATACCTTTATTAA

Pairwise alignment of ISPs *trnQ-nad2* in species of *Bhutanitis*

<i>Bhutanitis thaidina</i> MN012973 ISP <i>trnQd-nad2</i>	ATTACCCCTATTATATTGAATTTATATTTCAAAAAAAAAAAATCCCCCTATTTTAA
<i>Bhutanitis mansfieldi</i> LT999973 ISP <i>trnQ-nad2</i>	AT-TTTTATTATATTGAATTTATATTTCAAAAAAAAAAAATCCCCCTATTTTAA