The Complete Mitochondrial Genome of the Rice Moth, Corcyra cephalonica

Authors: Wu, Yu-Peng, Li, Jie, Zhao, Jin-Liang, Su, Tian-Juan, Luo, A-Rong, et al.

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The complete mitochondrial genome of the rice moth, *Corcyra cephalonica*

Yu-Peng Wu[1,2,4a], Jie Li[3b], Jin-Liang Zhao[2c], Tian-Juan Su[2,5d], A-Rong Luo[2e], Ren-Jun Fan[6f], Ming-Chang Chen[7g], Chun-Sheng Wu[2h], Chao-Dong Zhu[2i]*

[1]Institute of Loess Plateau, Shanxi University, Taiyuan 030006, China
[2]Key Laboratory of Zoological Systematics and Evolution (CAS), Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
[3]Institute of Fruit Trees, Shanxi Academy of Agricultural Sciences, 030031
[4]Plant Protection and Quarantine Station of Shanxi Province, Taiyuan 030001, China
[5]College of Life Sciences, Capital Normal University, Beijing, 100048, China
[6]Institute of Plant Protection, Shanxi Academy of Agricultural Sciences, 030031
[7]Shanxi Academy of Agricultural Sciences, 030031

Abstract

The complete mitochondrial genome (mitogenome) of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) was determined as a circular molecular of 15,273 bp in size. The mitogenome composition (37 genes) and gene order are the same as the other lepidopterans. Nucleotide composition of the *C. cephalonica* mitogenome is highly A+T biased (80.43%) like other insects. Twelve protein–coding genes start with a typical ATN codon, with the exception of *cox1* gene, which uses CGA as the initial codon. Nine protein–coding genes have the common stop codon TAA, and the *nad2*, *cox1*, *cox2*, and *nad4* have single T as the incomplete stop codon. 22 tRNA genes demonstrated cloverleaf secondary structure. The mitogenome has several large intergenic spacer regions, the spacer1 between *trnQ* gene and *nad2* gene, which is common in Lepidoptera. The spacer 3 between *trnE* and *trnF* includes microsatellite–like repeat regions $(AT)_{18}$ and $(TTAT)_3$. The spacer 4 (16 bp) between *trnS2* gene and *nad1* gene has a motif ATACTAT; another species, *Sesamia inferens* encodes ATCATAT at the same position, while other lepidopteran insects encode a similar ATACTAA motif. The spacer 6 is A+T rich region, include motif ATAGA and a 20-bp poly(T) stretch and two microsatellite $(AT)_9$, $(AT)_8$ elements.

Keywords: Galleriinae, mitogenome
Abbreviations: mitogenome, mitochondrial genome; **PCGs**, protein–coding genes
Correspondence: [a] wuyupeng007@163.com, [b] lijie_durham@hotmail.com, [c] liangzidaojian@163.com, [d] sutianjuan126@126.com, [e] luoar@ioz.ac.cn, [f] rjfan@163.com, [g] mcchensx@sina.com, [h] wucs@ioz.ac.cn, [i] zhucd@ioz.ac.cn, * Corresponding author
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Introduction

Animal mitogenomes are typically enclosed circular molecules of 14-20 kb in length with 37 genes, 13 protein–coding genes (PCGs), 22 transfer RNA (tRNA), and two ribosomal RNA (rRNA). It also contains an A+T rich non–coding area (also called control region) responsible for regulating transcription and replication of the mitogenome (Boore 1999; Taanman 1999). Mitogenomes have a simple structure, undergo fast evolution, are normally maternal inherited, and have been broadly applied in phylogenetic reconstruction, phylogeography, population structure and dynamics, and molecular evolution (Zhang et al. 1995; Nardi et al. 2003; Arunkumar et al. 2006). Recent advancements in sequencing technology have lead to rapid growth of mitogenome data in Genbank. To date, the complete mitogenome sequences of more than 140 species have been determined for insects, including 31 species of Lepidoptera that have been entirely or nearly entirely sequenced (Coates et al. 2005; Kim et al. 2006; Lee et al. 2006; Cameron et al. 2007; Cha et al. 2007; Cameron and Whiting 2008; Liu et al. 2008; Jiang et al. 2009; Hong et al. 2009; Pan et al. 2008; Salvato et al. 2008; Kim MI et al. 2009; Hu et al. 2010; Liao et al. 2010; Li et al. 2010; Zhao et al. 2010; Margam et al. 2011).

Lepidoptera is the second largest order after Coleoptera within in Insecta and includes moths and butterflies. Most of them are agricultural and forestry pests, pollinators, and resources insects (Li et al. 2009). *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) is in a small subfamily of Galleriinae with 261 species of Pyralidae, which contains more than 330 species of 70 genera (Heppner 1991). The genus *Corcyra* contains only two species, *C. nidicolella* and *C. cephalonica*; the latter is known to be a stored product pest, and is controlled with botanical insecticides and trapped with sex pheromone (Türkera 1998; Allotey and Azalekor 2000; Coelho et al. 2007). *Corcyra cephalonica* is used as the host for cultivating *Trichogramma* and other parasitoid wasps (Muthukrishnan et al. 2003; Jalali et al. 2007). Moreover, it is lately being used as an experimental model insect. A group of the functional genes have been identified (Nagamanju et al. 2003; Chaitanya and Dutta-Gupta, 2010; Damara et al. 2010; Gullipalli et al. 2010), but information regarding the mitochondrial genome is lacking. The availability of the mitogenome sequence will definitely be beneficial in the basic and applied studies on *C. cephalonica*.

In this paper, the mitogenome of *C. cephalonica* was sequenced and analyzed. So far, there are four species within Pyraloidea with known mitogenome: *Diatraea saccharalis* (Li et al. 2010, *Ostrinia furnacalis* and *O. nubilalis* (Coates et al. 2005), and *Chilo suppressalis* [unpublished, JF339041].

Materials and Methods

DNA samples extraction

Corcyra eggs were collected from Guangdong Province of China and raised in the laboratory in Beijing. The hatched adults were collected, preserved in 100% ethanol, and stored at −20 °C. Total DNA was extracted and isolated from single specimens using the DNeasy Tissue kit (QIAGEN, www.qiagen.com) according to manufacturer instructions.

Primer design, PCR, and sequencing

The short fragment amplifications were performed using the universal PCR primers from Simon et al. (1994). The degenerate and specific primer pairs were designed based on the known mitochondrial sequences in Lepidoptera, or designed by Primer 5.0 software on the fragments that we previously sequenced (Table 1). All the primers were synthesized by Shanghai Sangon Biotechnology Co., Ltd, www.sangon.com. For fragments of length less than 2 kb, PCR conditions were as follows: 95 °C for five min, 34 cycles of 94 °C for 30 sec, 50-55 °C (depending on primer combinations), 1-3 min (depending on putative length of the fragments) at 68 °C, and a final extension step of 72 °C for 10 min. For fragments of length longer than 2 kb, PCR conditions were as follows: 92 °C for two min, 40 cycles of 92 °C for 30 sec, 50-55 °C for 30 sec (depending on primer combinations), 60 °C for 12 min, and a final extension step of 60 °C for 20 min.

The entire mitogenome of the *Corcyra* was amplified in 17 fragments. For most fragments, 2×Taq PCR MasterMix (Tiangen Biotech Co., Ltd., www.tiangen.com) was used in the amplification; fragments longer than 2 kb (e.g., *rrnL*-*rrnS* and *nad4*-*cob*) and with higher AT contents (e.g., *rrnS*-*nad2* and *cox3*-*nad5*) were amplified using Takara LA Taq (Takara Co. www.takara-bio.com). All amplifications were performed on an Eppendorf Mastercycler and Mastercycler gradient in 50 µL reaction volumes. The reaction volume of 2×Taq PCR MasterMix contained 22 µL sterilized distilled water, 25 µL 2×Master Mix, 1 µL of each primer (10 uM), and 1 µL of DNA template; the reaction volume of Takara LA Taq consisted of 26.5 µL sterilized distilled water, 5 µL 10×LA PCR Buffer II (Takara), 5 µL 25 mM $MgCl_2$, 8 µL of dNTPs Mixture, 2 µL of each primer (10 µM), 1 µL of DNA template, and 0.5 µL (1.25 U) of Takara LA Taq polymerase (Takara).

The PCR products were detected via electrophoresis in 1% agarose gel, purified using the 3S Spin PCR Product Purification Kit, and sequenced directly with ABI-377 automatic DNA sequencer. All fragments were sequenced from both strands. Short amplified products were sequenced directly by internal primers, and long amplified products were sequenced completely by primer walking. The *rrnS*-*nad2* region was sequenced after cloning. The purified PCR products were ligated to the *pEASY*-T3 Cloning Vector (Beijing TransGen Biotech Co., Ltd., transgen.com.cn) and then sequenced by M13-F and M13-R primers and walking. Sequencing was performed using ABI BigDye ver 3.1 dye terminator sequencing technology and run on ABI PRISM 3730x1 capillary sequencers.

Analysis and annotation

Sequence annotation was performed using the DNAStar package (DNAStar Inc., www.dnastar.com). The sequence was checked manually for consistency by alignment, and tRNA genes were found using tRNAscan-SE software v.1.21 (Lowe and Eddy 1997) with manual editing. The undermined putative tRNAs were identified by sequence alignment with other insects of Pyralidae (Diatraea, *O. furnacalis*, and *O. nubilalis*) using Bioedit (Hall 1999). Secondary structure was inferred using DNASIS v.2.5. The *trnS1(AGN)* secondary structure was developed as proposed by Steinberg and Cedergren (1994). PCGs and rRNAs were identified by similarity to other lepidopteran sequences. The nucleotide sequences of the PCGs were translated based on the invertebrate mtDNA genetic code. Since the *Corcyra* does not utilize the AGG codon, use of the variant arthropod genetic code (Abascal et al. 2006) was unnecessary.

Nucleotide composition and codon usage were calculated using MEGA4.0 (Tamura et al. 2007).

Results

Genome structure and organization

The *Corcyra* mitogenome is a circular molecule 15,273 bp in length; data were uploaded to Genbank (HQ897685). The *Corcyra* mitogenome showed the standard gene complement containing 13 PCGs, 2 rRNAs, 22 tRNAs, and non–coding regions typical for lepidopterans. The *trnM* is coded between the A+T rich region and tRNA-Ile (order is A+T region-*trnM*-*trnI*-*trnQ*), which was different from the ancestral gene order of insects (A+T region-*trnI*-*trnQ*-*trnM*). Since the *trnS2(UCN)* was not found by tRNA-Scan-SE, it was later determined by sequence comparison with other lepidopteran insects.

The *Corcyra* mitogenome was biased toward A+T content (80.43%) with the value falling into the lepidopteran range of 77.84% in *Ochrogaster lunifer* (Salvato et al. 2008) to 82.66% in *Coreana raphaelis* (Kim et al. 2006). Additionally, the A+T content was 78.96% in PCGs, 82.95%, in *rrnL* genes, and 85.86% in *rrns* genes. These values were also well within the range reported for other lepidopterans. The A+T content (96.58%) of A+T rich region was the highest value among the known lepidoteran MtDNA sequences (Table 3).

Protein–coding genes

The initial and termination codons of thirteen PCGs are shown in Table 2. Twelve PCGs started with a typical ATN codon (ATT for *nad2, cox2, atp8, nad3, nad6*; ATA for *nad5, cob, nad1*; ATG for *atp6,cox3, nad4, nad4l*). One exception is *cox1*gene, which used CGA as a start codon.

The putative start codon CGA is common across insects (Fenn et al. 2007) such as *Bombyx mori* (Yukuhiro et al. 2002), *O. nubilalis and O. furnacalis* (Coates et al. 2005), *Adoxophyes honmai* (Lee et al. 2006), *Coreana* (Kim et al. 2006), *Antheraea pernyi* (Liu et al. 2008), *B. mandarina* (Pan et al. 2008), *Ochrogaster* (Salvato et al. 2008), *Artogeia melete* (Hong et al. 2009), *Eriogyna pyretorum* (Jiang et al. 2009), and *Hyphantria cunea* (Liao et al. 2010).

Nine PCGs had the common stop codon TAA, while the *nad2, cox1, cox2, nad4* have single T as an incomplete stop codon, also found in other animal mitochondrial genes (Clary and Wolstenholme 1985). The common interpretation of this phenomenon is that the TAA terminator is created via post–transcriptional polyadenylation (Ojala et al. 1981).

Transfer and ribosomal RNA genes

The 22 tRNA genes ranging from 64 to 73 nucleotides were spread over the mitogenome. Fourteen tRNAs were coded on the J–strand and eight on the N–strand, which is the same organization observed in other lepidopteran mitogenomes. Complete cloverleaf secondary structures could be inferred for 21 of the 22 tRNAs with the exception of *trnS1(AGN)*, which lacks the DHU arm (Figure 1). A total of 43 unmatched base pairs were scattered in 20 tRNA genes, including 20 pairs in the DHU stems, eight pairs in the amino acid acceptor stems, nine pairs in the TΨC stems, and six pairs in the anticodon stems. 24 of them are G-U pairs, which form a weak bond. The remaining were A-A, C-A, C-U, G-A, G-G, and U-U mismatches.

As in the other insect mitogenome sequences, two rRNA genes were present in *Corcyra*.

The *rrnL* were found between *trnL(CUN)* and *trnV*, and the *rrnS* between *trnV* and the A+T rich region, respectively.

Codon usage

Relative synonymous codon usage values of *Corcyra* mitogenome are summarized in Table 4. The codons CTG, CCG, and AGG were not represented in the coding sequences. Leucine (14.42%), isoleucine (12.14%), phenylalanine (9.74%), and serine (9.23%) were the most common amino acids in *Corcyra* mitochondrial proteins (45.53%). These amino acids are abundant in other insects, averaging 45.08% (Lessinger et al. 2000).

Non–coding and overlapping region

The *Corcyra* mitogenome harbored 15 non–coding regions, from 1 to 351 bp to 512 bp. Intergenic spacer sequences covered four major regions of length more than 10 bp. The remaining intergenic spacer were less than 5 bp.

Spacer 1 (61 bp), located between *trnQ* gene and *nad2* gene, is a common intergenic spacer rich in AT nucleotides (96.72%). The location of this spacer is fixed in lepidopterans, but varied in length from 40 bp (*Parnassius bremeri*) (Kim MI 2009) to 88 bp (*Sasakia charonda*) (Unpublished, AP011824). This spacer can be taken as a lepidopteran mitogenome marker not found in other insect mitogenomes. Kim MI (2009) found that the intergenic spacer sequences and the *nad2* gene had higher sequence identity than other fragments of the mitogenome. There were 29 species with more than 60% identity of 32 total lepidopteran species sequenced (Table 5), suggesting that this spacer sequence originated from a partial duplication of the *nad2* gene.

Spacer 2 (49 bp) was found between *trnE* and *trnF* genes, including two microsatellite–like regions, (TA)18 and (TTAT)3, similar to other lepidopterans. The spacer in *Adoxophyes* (Lee et al. 2006) is 222 bp and contains a different motif (TATTA)31. The spacer in *Ochrogaster* (Salvato et al. 2008) is 70 bp, contains a microsatellite (TA)23, and shows triplication of a 10–nucleotide motif with some changes. In other lepidoptera insects it is shorter than 10 bp.

Spacer 3 (16 bp) was between the *trnS2(UCN)* and *nad1* genes, commonly detectable in lepidopteran insects, and measured 16-38 bp. This intergenic spacer sequence of most lepidopterans harbored the motif

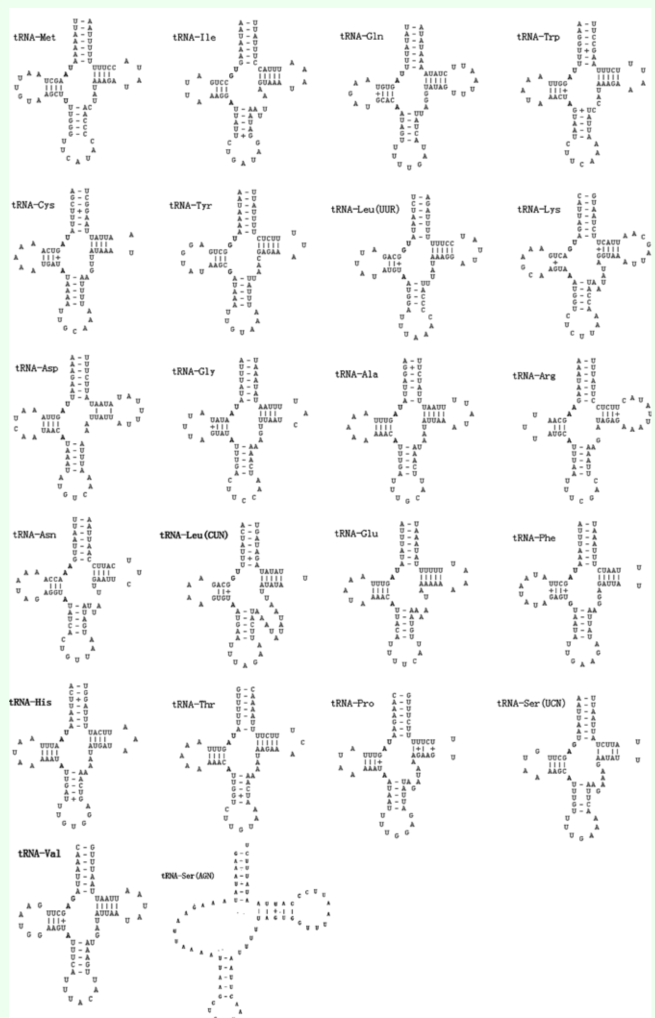

Figure 1. Putative secondary structures for the tRNA genes of the *Corcyra cephalonica* mitogenome. High quality figures are available online.

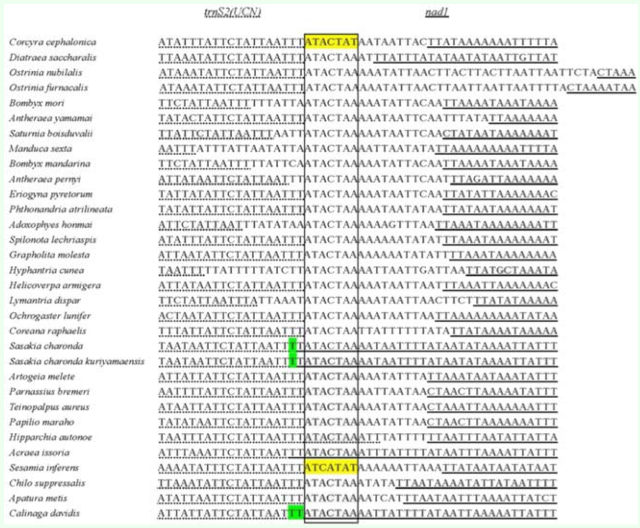

Figure 2. Alignment result of Spacer 4 tRNA-Ser(UCR)-NAD1 in 28 lepidopteran species. High quality figures are available online.

(ATACTAA), except for ATACTAT in *Corcyra* and ATCATAT in *Sesamia* (Figure 2). Similarly, in Hymenoptera there is a 6 bp conserved motif (THACWW) (Wei et al. 2010), and in Coleoptera the motif is 5 bp (TACTA). Such conservation suggests that the motif is functional in Lepidoptera. This motif is possibly fundamental to site recognition by the transcription termination peptide (Taanman 1999).

Spacer 4 (10 bp) was between *nad1* and *trnL(CUN)*. *Ostrinia furnacalis* and *O. nubilalis* also showed 10 bp spacers, while other lepidopteran spacers measured 1-6 bp.

Spacer 5 (351 bp) was A+T rich and found between *rrnS* and *trnM* with AT nucleotides (96.58%). There was a motif ATAGA followed by a 20 bp poly-T stretch downstream of *rrnS*, and two microsatellite–like regions (TA)9 and (TA)8. Finally, a 10 bp poly-A was present upstream of *trnM*. The feature was found to be common for other lepidopterans sequenced to date.

Overlapping sequences had a total of 35 bp spread over eight regions. Like other insect species (*Adoxophyes*) (35), *atp8* and *atp6* had a seven–nucleotide overlap (ATGATAA), known to be translated from the same cistronic mRNAs. The longest overlapping sequence (8 bp) was between *trnW* gene and *trnC* genes. The remaining overlapping sequences were all less than 6 bp.

Discussion

The *Corcyra* mitogenome is shorter than most lepidoteran mitogenomes previously reported. The shortest mitogenome is 15,140 bp (*Artogeia*) (Hong et al. 2009), and the longest is 15,928 bp (*B. mandarina*) (Pan et al. 2008). The *Corcyra* mitogenome had gene content and organization similar to other lepidopterans, which suggests that the mitochondrial gene arrangement in lepidopterans evolved independently after splitting from its stem lineage (Kim et al. 2006).

The most frequent amino acids in the *Corcyra* mitochondrial proteins were leucine, isoleucine, phenylalanine, and serine, all with high AT mutational bias that is a seemingly common feature in lepidopterans. Abascal et al. (2006) indicated that several arthropods have a new genetic code that translates the codon AGG as lysine instead of serineor arginine, these AGG reassignments may be events of parallel and correlated evolution between the arthropod genetic codes and the *trnK*/*trnS*. However, the variant codon, AGG, was not used by *Corcyra*.

The putative start codons of PCGs of the *Corcyra* mitogenome are ATNs, except for the CGA start codon of the *cox1* gene. Although tetranucleotides TTAG and hexanucleotide TATTAG have also been proposed as start codons for the *cox1* gene (Yukuhiro et al. 2002; Kim et al. 2006; Liu et al. 2008; Salvato et al. 2008; Kim SR et al.

2009), the TTAG lacks absolute conservation and may be of alternative function, not as an initiation codon (Margam et al. 2011). There are studies using ESTs (expressed sequence tags) to determine the *cox1* start codon. For example, some dipterans have an unorthodox UCG serine initiation codon, which was confirmed through mitogenome EST data (Morlais and Severson 2002; Krzywinski et al. 2006; Stewart and Beckenbach 2009). Mitogenome ESTs and alignment of the mitogenome sequence from all lepidopterans had shown that arginine (CGR) functions as the start codon of the *cox1* gene (Margam et al. 2011). These observations suggest that the use of EST data is valuable for the annotation of mitogenomes. The success of mitogenome sequencing will serve as the basis of the mating of EST and functional mitochondrial genome annotations.

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Table 1. Region, primers and sequences for PCRs in this study.

Region	Upstream primer	Upstream primers sequences(5′→3′)	Downstream primer	Downstream primers sequences(5′→3′)	Size (bp)
trnQ-nad2	Gln10486[a]	TAAACTATATCTAATAATATCAAAAATTATTGTGC	N1-N-784[c]	TTTAATCCTCCGATAGCTCCAAT	600
nad2	N2-J-437[d]	TTTTAACTCAATCTATTGCATC	N2-N-794[d]	GAAATTAACATTCACCCTAAAT	400
nad2-cox1	N2-J-720[d]	TACTATTATTGGTGCTATTGG	C1-N-1858[d]	AATCTACTGAACTTCCACCAT	1000
cox1	LCO1490[d]	GGTCAACAAATCATAAAGATATTGG	HCO2198[d]	TAAACTTCAGGGTGACCAAAAAATCA	650
trnL2-trnK	Leu-J-3029[c]	CTAATATGGCAGACTATATGTAATGGA	Lys14111Re[a]	GACCATTACTTGCTTTCAGTCATCTAATG	750
cox2-*cox3*	C2-J-3277[d]	TATTGCTCTTCCCTCTTTACG	C3-N-4724[d]	TGGAAAGGATGATTGTGAAAA	1500
trnK-cox3	Lys14111[a]	CATTAGATGACTGAAAGCAAGTAATGGTC	C3-N-5460[b]	TCAACAAAGTGTCAATATCA	700
cox3-nad5	C3-J-5010[d]	TTTTGAGCATTTTTCCATAGA	N5-N-7756[d]	ATTAGGTTGAGATGGGTTAGGATT	2800
nad5-nad4	N5-2183[a]	AGATAAAGCAGTTAATATGCCAGCA	N4-N-8718[b]	GCCTATTCATCWGTTGCTCA	1000
nad5-cob	N5-J-7326[d]	ATTAGCAGAAATCCCAGCCAT	CB-N-10733[d]	TCCATTTGCATGTAATGTACG	3000
nad4-cob	N4-J-8583[d]	TATTCCTGATGAACATAAACC	CB-N-11102[d]	AAGGGTTATTTGATCCTGTTA	1600
cob	CB-J-10933[d]	TATGTTTTTCCTTGAGGACAAATATC	CB-N-11367[b]	TAACTCCTCCTAATTTATTGGGA	460
cob-rrnL	CB-5971[a]	CAAACAGGATCTAATAACCCTTTAGG	LR-N-12866[c]	ACATGATCTGAGTTCAAACCGG	1800
nad1-rrnL	CB-J-11520[d]	TCCAGTTGAAGACCCCTTACAT	N1-N-12212[d]	TTACGGTCAGTTGCTCAAACA	700
rrnL-rrnS	N1-J-12215[d]	TTGAGCAACTGACCGTAAACCCGGTA	SR-N-14511[d]	GAAACTTAAATAATTTGG	2300
rrnS	SR-J-14233[b]	GAAAGCGACGGGCAATATG	SR-N-14588[c]	AAACTAGGATTAGATACCCTATTAT	350
rrnS-nad2	SR-J-14737[d]	AACCGCAACTGCTGGCACAAA	C1-N-1757[d]	AAAGTAAGAGAAGGGGGGAGT	2500

[a] Primers from Lee et al. (2006), [b] Primers from Simon et al. (1994), [c] Primers from Zhao et al. (2010), [d] Primers newly designed for this genome.

Table 2. Summary of the mitogenome of the *Corcyra.*

Gene	Direction	Location	Size (bp)	Anticodon	Start codon	Stop codon
trnM	F	Jan-68	68	CAT		
trnI	F	69-134	66	GAT		
trnQ	R	132-200	69	TTG		
Spacer 1	N/A	201-261	61			
nad2	F	262-1261	1002		ATT	T
trnW	F	1262-1327	66	TCA		
trnC	R	1320-1383	64	GCA		
trnY	R	1384-1449	66	GTA		
cox1	F	1455-2985	1536		CGA	T
trnL2(UUR)	F	2986-3052	67	TAA		
cox2	F	3053-3734	682		ATT	T
trnK	F	3735-3807	73	CTT		
trnD	F	3810-3877	68	GTC		
atp8	F	3878-4042	165		ATT	TAA
atp6	F	4036-4716	681		ATG	TAA
cox3	F	4716-5504	789		ATG	TAA
trnG	F	5508-5572	65	TCC		
nad3	F	5573-5926	354		ATT	TAA
trnA	F	5930-5996	67	TGC		
trnR	F	5997-6064	68	TCG		
trnN	F	6065-6131	67	GTT		
trnS1(AGN)	F	6134-6199	66	GCT		
trnE	F	6204-6270	67	TTC		
Spacer 2	N/A	6271-6319	49			
trnF	R	6320-6386	67	GAA		
nad5	R	6381-8120	1740		ATA	TAA
trnH	R	8121-8185	65	GTG		
nad4	R	8186-9524	1339		ATG	T
nad4L	R	9525-9815	291		ATG	TAA
trnT	F	9818-9882	65	TGT		
trnP	R	9883-9947	65	TGG		
nad6	F	9950-10486	537		ATT	TAA
cob	F	10489-11631	1143		ATA	TAA
trnS2(UCN)	F	11634-11698	65	TGA		
Spacer 3	N/A	11699-11714	16			
nad1	R	11715-12641	927		ATA	TAA
Spacer 4	N/A	12642-12651	10			
trnL1(CUN)	R	12652-12721	70	TAG		
rrnL	R	12722-14076	1355			
trnV	R	14077-14144	68	TAC		
rrnS	R	14145-14922	778			
Spacer 5	N/A	14923-15273	351			

Table 3. Characteristics of the lepidopteran mitogenomes.

Superfamily	Species	Size (bp)	A+T content (%)	PCG A+T content(%)	rrnL A+T content (%) 16S		rrnS A+T content (%) 12S		A+T-rich region A+T content (%)		GenBank accession #
					Size (bp)	A+T (%)	Size (bp)	A+T (%)	Size (bp)	A+T (%)	
Pyraloidea	*Corcyra cephalonica*	15273	80.43	78.96	1355	82.95	778	85.86	351	96.58	HQ897685
	Chilo suppressalis	15395	80.67	78.9	1383	84.24	788	86.17	348	95.4	JF339041
	Diatraea saccharalis	15490	80.02	77.9	1412	84.77	781	85.53	335	94.43	FJ240227
	Ostrinia nubilalis	14535	80.17	79.16	1339	84.91	434	82.03	—	—	AF442957
	Ostrinia furnacalis	14536	80.37	79.42	1339	84.99	435	82.76	—	—	AF467260
	Sesamia inferens	15413	80.24	78.62	1385	83.39	784	85.33	311	95.82	JN039362
Bombycoidea	*Antheraea pernyi*	15566	80.16	78.53	1369	83.86	775	84.13	552	90.4	AY242996
	Antheraea yamamai	15338	80.29	78.94	1380	83.99	776	84.41	334	89.52	EU726630
	Bombyx mandarina	15928	81.68	79.64	1377	84.75	783	85.95	747	95.91	AB070263
	Bombyx mori	15643	81.32	79.57	1375	84.36	783	85.57	499	95.39	AF149768
	Eriogyna pyretorum	15327	80.82	79.41	1338	84.6	778	84.45	358	92.18	FJ685653
	Manduca sexta	15516	81.79	80.3	1391	85.26	777	85.71	324	95.37	EU286785
	Saturnia boisduvalii	15360	80.63	79.15	1391	84.76	774	84.11	330	91.52	EF622227
Geometroidea	*Phthonandria atrilineata*	15499	81.02	79.1	1400	85.71	803	86.03	457	98.25	EU569764
Tortricoidea	*Adoxophyes honmai*	15680	80.39	78.48	1387	83.56	779	85.37	490	94.29	DQ073916
	Grapholita molesta	15717	80.87	78.89	1377	84.75	772	85.36	771	95.85	HQ392511
	Spilonota lechriaspis	15368	81.19	79.72	1382	85.17	778	86.25	441	92.74	HM204705
Noctuoidea	*Helicoverpa armigera*	15347	80.97	79.43	1395	84.73	794	85.89	328	95.12	GU188273
	Hyphantria cunea	15481	80.39	78.95	1426	84.99	808	84.53	357	94.96	GU592049
	Lymantria dispar	15569	79.88	77.84	1351	84.23	799	85.23	435	96.09	FJ617240
	Ochrogaster lunifer	15593	77.84	75.73	1351	81.5	806	83.25	319	93.42	AM946601
Papilionoidea	*Acraea issoria*	15245	79.76	78.11	1331	83.85	788	83.76	430	96.05	GQ376195
	Apatura metis	15236	80.44	78.96	1333	84.47	779	84.85	394	92.89	JF801742
	Artogeia melete	15140	79.78	78.52	1319	83.47	777	85.46	351	89.17	EU597124
	Calinaga davidis	15267	80.45	78.94	1337	83.84	773	85.9	389	92.03	HQ658143
	Coreana raphaelis	15314	82.66	81.51	1330	85.26	777	85.84	375	94.13	DQ102703
	Hipparchia autonoe	15489	79.09	76.91	1335	83.67	775	85.29	678	94.54	GQ868707
	Papilio maraho	16094	80.5	78.17	1333	83.72	779	85.49	1270	94.62	FJ810212
	Parnassius bremeri	15389	81.27	80.18	1344	83.93	773	85.12	504	93.65	FJ871125
	Sasakia charonda	15244	79.87	78.22	1323	84.35	775	85.03	380	91.84	AP011824
	Sasakia charonda kuriyamaensis	15222	79.89	78.3	1311	84.21	775	85.03	380	91.84	AP011825
	Teinopalpus aureus	15242	79.81	78.31	1320	82.42	781	85.66	395	93.16	HM563681

Table 4. Codon usage in the *Corcyra* mitochondrial genome.

Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	328	1.81	AUU(I)	425	1.88
UUC(F)	34	0.19	AUC(I)	26	0.12
UUA(L)	462	5.17	AUA(M)	269	1.87
UUG(L)	7	0.08	AUG(M)	19	0.13
UCU(S)	121	2.82	ACU(T)	99	2.44
UCC(S)	23	0.54	ACC(T)	9	0.22
UCA(S)	79	1.84	ACA(T)	52	1.28
UCG(S)	2	0.05	ACG(T)	2	0.05
UAU(Y)	182	1.93	AAU(N)	229	1.8
UAC(Y)	7	0.07	AAC(N)	25	0.2
UAA(*)	-	-	AAA(K)	84	1.6
UAG(*)	-	-	AAG(K)	21	0.4
UGU(C)	29	1.81	AGU(S)	26	0.61
UGC(C)	3	0.19	AGC(S)	3	0.07
UGA(W)	94	1.94	AGA(S)	89	2.08
UGG(W)	3	0.06	AGG(S)	0	0
Codon	Count	RSCU	Codon	Count	RSCU
CUU(L)	42	0.47	GUU(V)	78	2.17
CUC(L)	6	0.07	GUC(V)	3	0.08
CUA(L)	19	0.21	GUA(V)	59	1.64
CUG(L)	0	0	GUG(V)	4	0.11
CCU(P)	82	2.65	GCU(A)	87	2.92
CCC(P)	11	0.35	GCC(A)	2	0.07
CCA(P)	31	1	GCA(A)	29	0.97
CCG(P)	0	0	GCG(A)	1	0.03
CAU(H)	60	1.74	GAU(D)	59	1.87
CAC(H)	9	0.26	GAC(D)	4	0.13
CAA(Q)	58	1.97	GAA(E)	65	1.86
CAG(Q)	1	0.03	GAG(E)	5	0.14
CGU(R)	13	1.02	GGU(G)	54	1.09
CGC(R)	1	0.08	GGC(G)	4	0.08
CGA(R)	34	2.67	GGA(G)	115	2.32
CGG(R)	3	0.24	GGG(G)	25	0.51

A total of 3716 codons were analyzed excluding the initiation and termination codons. TThe amino acids encoded by codons are labeled according to the IUPAC-IUB single letter amino acid codes. RSCU, relative synonymous codon usage.

Table 5. Sequence Identity of Spacer1 and *nad2* in 32 Lepidoptera species.

Species	*trnQ-nad2*(bp)	*nad2*(bp)	Sequence identity(%)
A. hon	64	999	57.8
A. mel	48	1014	68.8
A. met	60	1002	70
A. iss	51	1014	62.7
A. per	56	1014	66.1
A. yam	53	1014	63
B. man	47	1023	68.8
B. mor	65	1005	66.2
S. boi	53	1014	62.3
C. cep	61	1002	72.7
C. rap	56	1014	62.5
C. sup	52	1014	70
C. dav	46	1014	65.3
D. sac	55	1014	75.9
E. pyr	54	1014	64.9
G. mol	62	999	67.7
H. arm	45	1011	70
H. aut	50	1012	69.8
H. cun	50	1011	69.2
L. dis	42	1012	70.8
M. sex	54	1015	64.3
O. lun	72	1014	60.3
O. fur	62	1002	66.1
O. nub	62	1002	66.1
P. atr	63	1002	69.8
P. bre	40	1013	66.7
P. mar	43	1012	58.8
S. cha1	88	876	61.1
S. cha2	87	1017	63.8
S. lec	43	1009	63.8
S. inf	68	1017	68.1
T. aur	43	1014	57.4

Species names are abbreviated by using one letter from the genus name and three letters from the species name. *S.cha*1 = *Sasakia charonda*, *S.cha*2 = *Sasakia charonda kuriyamaensis*.