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Complete mitogenome data from a European specimen of *Ostrinia scapulalis* (Walker, 1859) (Lepidoptera, Pyraloidea, Crambidae, Pyraustinae)

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Abstract

We present an assembly and annotation of the mitogenome of a European specimen of the Adzuki bean borer, *Ostrinia scapulalis* (Walker, 1859). The present data were obtained by combining WGS data issue of a *de novo* and a previously published sequence library [1]. We also provide the phylogenetic positioning of the mitogenome within the *Ostrinia* genus, the *Crambidae* family and with more distant Lepidoptera species.

Keywords

Annotation, assembly, Crambidae, Lepidoptera, mitogenome, phylogeny, Pyraloidea

Specifications Table

Subject	Insect Science
Specific subject area	Lepidoptera, Crambidae, Mitogenomics

Type of data	• FASTQ: DNA sequence reads • FASTA: mitochondrial genome assembly • TABLE: gene annotations, base composition, Lepidoptera species used for phylogenetic analysis • FIGURE: mitogenomic circular map, AT-rich region sequence, phylogenetic tree
How data were acquired	Whole-genome shotgun sequencing on Illumina NextSeq 500 v2 platform using the paired-end protocol (2 × 150 base pairs (bp)).
Data format	Raw sequence reads and analysed (assembled and annotated) mitogenome.
Parameters for data collection	Genomic DNA was extracted from one *Ostrinia scapulalis* larva using the DNeasy Tissue Kit from Qiagen (Hilden, Germany). A 2 × 150 bp shot-gun paired-end library with an insert size of 300 bp was generated and sequenced by LGC Genomics GmbH (Berlin, Germany) on an Illumina NextSeq 500 v2 platform.
Description of data collection	The *Ostrinia scapulalis* mitogenome was assembled with MITObim v1.9. Genomic regions were annotated with MITOS v2. The circular mitochondrial genome map was generated with OGDRAW. Multiple sequence alignments were generated separately for each protein-coding and rRNA gene with MAFFT v7.471. Phylogenetic trees were built on the concatenated gene alignments with PHYML v3.0 and MrBayes v3.2.7a and visualized with FigTree v1.4.4.
Data source location	Samples were collected in 2015 near Abbeville/France (50° 8'13.57"N; 1°50'23.62"E).
Data accessibility	Repository name: NCBI BioProject Data identification number: PRJNA637835 Direct URL to data: http://www.ncbi.nlm.nih.gov/bioproject/637835 Repository name: NCBI Genbank Data identification number: MT801073 Direct URL to data: http://www.ncbi.nlm.nih.gov/nuccore/MT801073

Value of the Data

- This data represents the mitogenome of a European specimen of *Ostrinia scapulalis*.

- The data can benefit to researchers working on Lepidoptera evolution and higher systematics.
- The present data can be used for phylogenetic studies of Lepidoptera mitogenomes especially for getting deeper insights in diversification dynamics of *Ostrinia* species.

1. **Data Description**

The *O. scapulalis* (hereafter named OSCA) mitogenome had a size of 15,305 bp and an A+T content of 80.9%. All 38 genetic regions commonly known for arthropod mitogenomes [2] were identified: 13 protein-coding genes, 22 tRNA and 2 rRNA (l-rRNA and s-rRNA) genes, and one A+T-rich control region. The gene order and the composition of start on stop codons of the protein-coding genes corresponded to those observed for other *Ostrinia* spp. [3,4] and Crambidae [5] mitogenomes. Fifteen intergenic spacer regions (see Table 1: positive intergenic nucleotide values) of a total length of 341 bp were identified which ranged from 1 to 92 bp with the longest being located between the trnQ and ND2 genes. Furthermore, region overlaps (see Table 1: negative intergenic nucleotide values) of 1 to 35 bp could be observed for fifteen gene pairs. More annotation details can be found in Table 1 and are also represented as mitogenome map in Figure 1. Base compositions, AT and GC skews to each genomic region type are shown in Table 2. The tRNA secondary structures showed the typical clover-leaf except for trnS1 in which the dihydrouridine (DHU) arm was replaced by an unstable loop (Supplementary Fig. 1). The characteristics of the A+T-rich region are shown in Figure 2. Finally, we built a phylogeny based on 63 lepidopteran mitogenomes (including the generated OSCA mitogenome, Table 3), with a focus on Pyraloidea (Fig. 3 and Supplementary Fig. 2).

2. **Experimental Design, Materials and Methods**

DNA sampling and sequencing

Ostrinia scapulalis larval samples were collected in 2015 from a stem of mugwort in North of France (50° 8'13.57"N; 1°50'23.62"E). Genomic DNA was extracted from one larva using the DNeasy Tissue Kit from Qiagen (Hilden, Germany) according to the manufacturer's protocol. Subsequently, a 2 × 150 bp shot-gun paired-end library with an insert size of 300 bp, was generated using the DNA extract and sequenced by LGC Genomics GmbH (Berlin, Germany) on an Illumina NextSeq 500 v2 platform. In total, 372,260,856 genomic reads were generated of

which 306,203,188 remained after quality trimming with Trimmomatic [6, v0.36] (specific parameters set were: PE -pred33, ILLUMINACLIP:$ADAPTERF:2:26:10 HEADCROP:5 LEADING:26 TRAILING:26 SLIDINGWINDOW:5:28 MINLEN:30, with $ADAPTERF containing all Illumina adapters and their reverse complement, respectively).

Mitogenome assembly and feature analyses

The OSCA mitogenome was assembled in two steps as follows. First, the scaffolds of a previously published *O. scapulalis* genome draft [1], OSCA v1.2 (BioProject PRJNA390510), were searched for putative mitochondrion sequences by using *blastn* [3, v2.2.28] against the mitochondrial genome sequence of the closely related Asian corn borer *Ostrinia furnacalis* (Guenée) (NCBI accession number NC_003368). Genome scaffolds with high quality mitochondrion hits were selected and assembled by LGC with CAP3 [3, v2013] into a mitogenome sequence of 14,864 bp. In a second step, this draft mitogenome was refined using MITObim [4, v1.9, using the default k-mer size of 31 bp and the --pair parameter] and the Trimmomatic-cleaned paired-end Illumina reads. Gene identification as well as tRNA structure prediction were carried out with MITOS [3, v2], using as reference set 'RefSeq 81 Metazoa' and the genetic code '5' for invertebrates. Nucleic base contents were determined with the *wordcount* program of the EMBOSS toolkit [3, v6.6.0.0]. AT/GC skewness indices describing the genomic compositional asymmetry of a mitogenome or specific sequence region were calculated as: AT skew = [A-T] / [A+T] and GC skew = [G-C] / [G+C], where each base letter represents the respective base count. The circular map of the OSCA mitogenome was generated with the program OGDRAW [12, v09/08/2020, https://chlorobox.mpimp-golm.mpg.de/OGDraw.html].

Phylogenetic analyses

In order to perform comparative analyses of *O. scapulalis* with other Lepidoptera the nucleotide sequences of the 13 PCGs and 2 rRNA genes of 63 mitogenomes - comprising 59 Lepidoptera species - (Table 3) were downloaded from the NCBI 'nucleotide' (https://www.ncbi.nlm.nih.gov/nuccore, May 2020) database. Our taxon sampling was focused on the superfamily Pyraloidea, encompassing 34 species belonging to the Crambidae (including *O. scapulalis*) and 13 species belonging to the Pyralidae. Regarding *Ostrinia* spp., we included in total nine mitogenomes belonging to five *Ostrinia* species. As outgroups we used 12 representative species of the lepidopteran superfamilies Bombycoidea, Geometroidea, Noctuoidea and Papilionoidea; based on the comprehensive study of Wahlberg et al. [13], we

used the three Papilionoidea representatives to root our trees. The PCG and rRNA nucleotide sequences of all species were grouped into 15 gene-specific sequence sets. Each gene set was in separate globally aligned with the program MAFFT [7, v7.471] by applying the Needleman-Wunsch algorithm (mafft-ginsi for PCGs and mafft-qinsi for rRNA sequences) with a maximum of 1,000 refinement iterations (--maxiterate 1000), leaving the other parameters set to their default value. Subsequently, for each species all gene-specific alignments were concatenated following the same gene order using in-house Python and Perl scripts.

Phylogenetic trees were generated with the web version (v3.0, http://www.atgc-montpellier.fr/phyml/) of the program PHYML [14] and a local installation of MrBayes [4, v3.2.7a]. PHYML was run using the Smart Model Selection [16] option with the Akaike Information Criterion and the tree searching method BIONJ being set and by applying 1,000 bootstraps. Concerning the MrBayes analysis, a separate partition was set for each gene. For each partition the evolutionary model was set to the GTR substitution model (nst=6) with gamma-distributed rate variation (rates=invgamma) across sites and a proportion of invariable sites (GTR +I +Γ). The standard nucleotide substitution model (nucmodel=4by4) was chosen and priors settings were left as by default. Trees were built using 100,000 samples (ngen=1000000, samplefreq=10). Further parameters were : printfreq=100, diagnfreq=1000, nchains=4, savebrlens=yes, starttree=random, startparams=reset, outgroup pamac (corresponding to *Papilio machaon*), sump burnin=25000, sumt burnin=25000. The phylogenetic tree was visualized using the program FigTree [11, v1.4.4].

Acknowledgments

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Author contributions

BG conceived the study. RS led the ANR research program on European *Ostrinia* genomics. RS, PA, SN collected the samples in the field. SN, PA prepared the samples and extracted the DNA. BG was responsible for the bioinformatics analyses, i.e improvement of the mitogenome assembly, annotation, characterization, comparative analyses. BG and GJK did the phylogenetic analyses. All authors wrote and approved the manuscript.

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Table 1: Gene features of the *O. scapulalis* mitogenome

The gene names, the coordinates, the orientation, the size, the gene overlaps/intergenic spacers (column 6) and start/stop codons of the *O. scapulalis* mitogenome are listed.

Gene (anticodon)	Start	Stop	Strand	Size	Intergenic nucleotides	Start/Stop codon
trnM(cat)	1	68	+	68	-1	
trnI(gat)	68	135	+	68	0	
trnQ(ttg)	136	204	-	69	92	
ND2	297	1,289	+	993	-15	ATT/TAA
trnW(tca)	1,275	1,341	+	67	-8	
trnC(gca)	1,334	1,398	-	65	0	
trnY(gta)	1,399	1,465	-	67	8	
COX1	1,474	3,009	+	1,536	-5	CGA/TAA
trnL2(taa)	3,005	3,071	+	67	48	
COX2	3,120	3,788	+	669	-35	ATA/TAA
trnK(ctt)	3,754	3,824	+	71	-1	
trnD(gtc)	3,824	3,892	+	69	0	
ATP8	3,893	4,054	+	162	-7	ATA/TAA
ATP6	4,048	4,722	+	675	-1	ATG/TAA
COX3	4,722	5,513	+	792	2	ATG/TAA
trnG(tcc)	5,516	5,582	+	67	0	
ND3	5,583	5,936	+	354	10	ATT/TAA
trnA(tgc)	5,947	6,013	+	67	-1	
trnR(tcg)	6,013	6,077	+	65	-1	
trnN(gtt)	6,077	6,143	+	67	2	
trnS1(gct)	6,146	6,211	+	66	1	
trnE(ttc)	6,213	6,279	+	67	-2	
trnF(gaa)	6,278	6,346	-	69	-17	
ND5	6,330	8,057	-	1,728	24	ATT/TAA
trnH(gtg)	8,082	8,148	-	67	-1	
ND4	8,148	9,488	-	1,341	7	ATG/TAA
ND4L	9,496	9,789	-	294	9	ATG/TAA
trnT(tgt)	9,799	9,865	+	67	0	
trnP(tgg)	9,866	9,930	-	65	32	
ND6	9,963	10,469	+	507	-1	ATT/TAA
CYTB	10,469	11,617	+	1,149	-1	ATG/TAA
trnS2(tga)	11,617	11,684	+	68	38	
ND1	11,723	12,661	-	939	1	ATG/TAG
trnL1(tag)	12,663	12,730	-	68	21	
l-rRNA	12,752	14,023	-	1,272	46	
trnV(tac)	14,070	14,135	-	66	0	
s-rRNA	14,136	14,914	-	779	0	
A+T-rich region	14,915	15,305	+	391	0	

Table 2: Base composition and skewness indices for each specific gene region of the *O. scapulalis* mitogenome.

Type	Size [bp]	A%	G%	T%	C%	A+T%	AT skew	GC skew
Entire mitogenome	15,305	41.7	7.7	39.2	11.4	80.9	0.031	-0.197
Protein-coding genes	11,139	34.5	10.8	44.9	9.9	79.4	-0.131	0.045
tRNA	1,480	41.7	10.8	39.9	7.6	81.6	0.022	0.172
rRNA	2,051	41.7	9.9	43.4	4.9	85.1	-0.021	0.338
A+T-rich region	391	42.7	2.0	49.6	5.6	92.3	-0.075	-0.467

Table 3: Lepidoptera mitogenomes which were used to build the phylogenetic trees.

Super-family	Family	Subfamily	Species	Genbank no.
Bombycoidea	Bombycidae	Bombycinae	*Bombyx mori*	NC_002355
Bombycoidea	Saturniidae	Saturniinae	*Actias selene*	NC_018133
Bombycoidea	Sphingidae	Sphinginae	*Manduca sexta*	NC_010266
Geometroidea	Geometridae	Ennominae	*Biston panterinaria*	NC_020004
Geometroidea	Geometridae	Ennominae	*Phthonandria atrilineata*	NC_010522
Geometroidea	Geometridae	Larentiinae	*Operophtera brumata*	NC_027723
Noctuoidea	Erebidae	Lymantriinae	*Lymantria dispar*	NC_012893
Noctuoidea	Noctuidae	Noctuinae	*Spodoptera frugiperda*	NC_027836
Noctuoidea	Notodontidae	Thaumetopoeina	*Ochrogaster lunifer*	NC_011128
Papilionoidea	Nymphalidae	Danainae	*Danaus plexippus*	NC_021452
Papilionoidea	Nymphalidae	Nymphalinae	*Melitaea cinxia*	NC_018029
Papilionoidea	Papilionidae	Papilioninae	*Papilio machaon*	NC_018047
Pyraloidea	Crambidae	Crambinae	*Chilo auricilius*	NC_024644
Pyraloidea	Crambidae	Crambinae	*Chilo sacchariphagus*	NC_029716
Pyraloidea	Crambidae	Crambinae	*Chilo suppressalis*	NC_015612
Pyraloidea	Crambidae	Crambinae	*Diatraea saccharalis*	NC_013274
Pyraloidea	Crambidae	Crambinae	*Pseudargyria interruptella*	NC_029751
Pyraloidea	Crambidae	Evergestinae	*Evergestis junctalis*	NC_030509
Pyraloidea	Crambidae	Nymphulinae	*Elophila interruptalis*	NC_021756
Pyraloidea	Crambidae	Nymphulinae	*Paracymoriza distinctalis*	NC_023471
Pyraloidea	Crambidae	Nymphulinae	*Paracymoriza prodigalis*	NC_020094
Pyraloidea	Crambidae	Nymphulinae	*Parapoynx crisonalis*	NC_031151
Pyraloidea	Crambidae	Pyraustinae	*Loxostege sticticalis*	NC_027174
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia furnacalis (2005)*	NC_003368
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia furnacalis (2020)*	MN793323
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia furnacalis (2020)*	MN747041
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia nubilalis (2005)*	NC_003367
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia nubilalis (2020)*	MN793322
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia palustralis*	MH574940
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia scapulalis*	MT801073
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia scapulalis*	NC_048887
Pyraloidea	Crambidae	Pyraustinae	*Ostrinia zealis*	NC_048888
Pyraloidea	Crambidae	Pyraustinae	*Pyrausta despicata*	NC_046050
Pyraloidea	Crambidae	Schoenobiinae	*Scirpophaga incertulas*	NC_031329
Pyraloidea	Crambidae	Spilomelinae	*Cnaphalocrocis medinalis*	NC_015985
Pyraloidea	Crambidae	Spilomelinae	*Cydalima perspectalis*	NC_042150
Pyraloidea	Crambidae	Spilomelinae	*Dichocrocis punctiferalis*	NC_021389
Pyraloidea	Crambidae	Spilomelinae	*Glyphodes pyloalis*	NC_025933
Pyraloidea	Crambidae	Spilomelinae	*Glyphodes quadrimaculali*	NC_022699
Pyraloidea	Crambidae	Spilomelinae	*Haritalodes derogata*	NC_029202
Pyraloidea	Crambidae	Spilomelinae	*Maruca testulalis*	NC_024283
Pyraloidea	Crambidae	Spilomelinae	*Maruca vitrata*	NC_024099
Pyraloidea	Crambidae	Spilomelinae	*Nomophila noctuella*	NC_025764
Pyraloidea	Crambidae	Spilomelinae	*Omiodes indicata*	NC_039177
Pyraloidea	Crambidae	Spilomelinae	*Palpita hypohomalia*	NC_039632
Pyraloidea	Crambidae	Spilomelinae	*Patania inferior*	NC_040973
Pyraloidea	Crambidae	Spilomelinae	*Pycnarmon lactiferalis*	NC_033540
Pyraloidea	Crambidae	Spilomelinae	*Spoladea recurvalis*	NC_027443
Pyraloidea	Crambidae	Spilomelinae	*Tyspanodes hypsalis*	NC_025569
Pyraloidea	Crambidae	Spilomelinae	*Tyspanodes striata*	NC_030510
Pyraloidea	Pyralidae	Epipaschiinae	*Lista haraldusalis*	NC_024535
Pyraloidea	Pyralidae	Galleriinae	*Corcyra cephalonica*	NC_016866
Pyraloidea	Pyralidae	Galleriinae	*Galleria mellonella*	NC_028532
Pyraloidea	Pyralidae	Phycitinae	*Amyelois transitella*	NC_028443
Pyraloidea	Pyralidae	Phycitinae	*Ephestia elutella*	NC_039716
Pyraloidea	Pyralidae	Phycitinae	*Ephestia kuehniella*	NC_022476
Pyraloidea	Pyralidae	Phycitinae	*Euzophera pyriella*	NC_037175
Pyraloidea	Pyralidae	Phycitinae	*Meroptera pravella*	NC_035242
Pyraloidea	Pyralidae	Phycitinae	*Plodia interpunctella*	NC_027961
Pyraloidea	Pyralidae	Pyralinae	*Endotricha consocia*	NC_037501

Fig. 1. Map of the *O. scapulalis* mitogenome generated with OGDRAW. Annotated genes are highlighted.

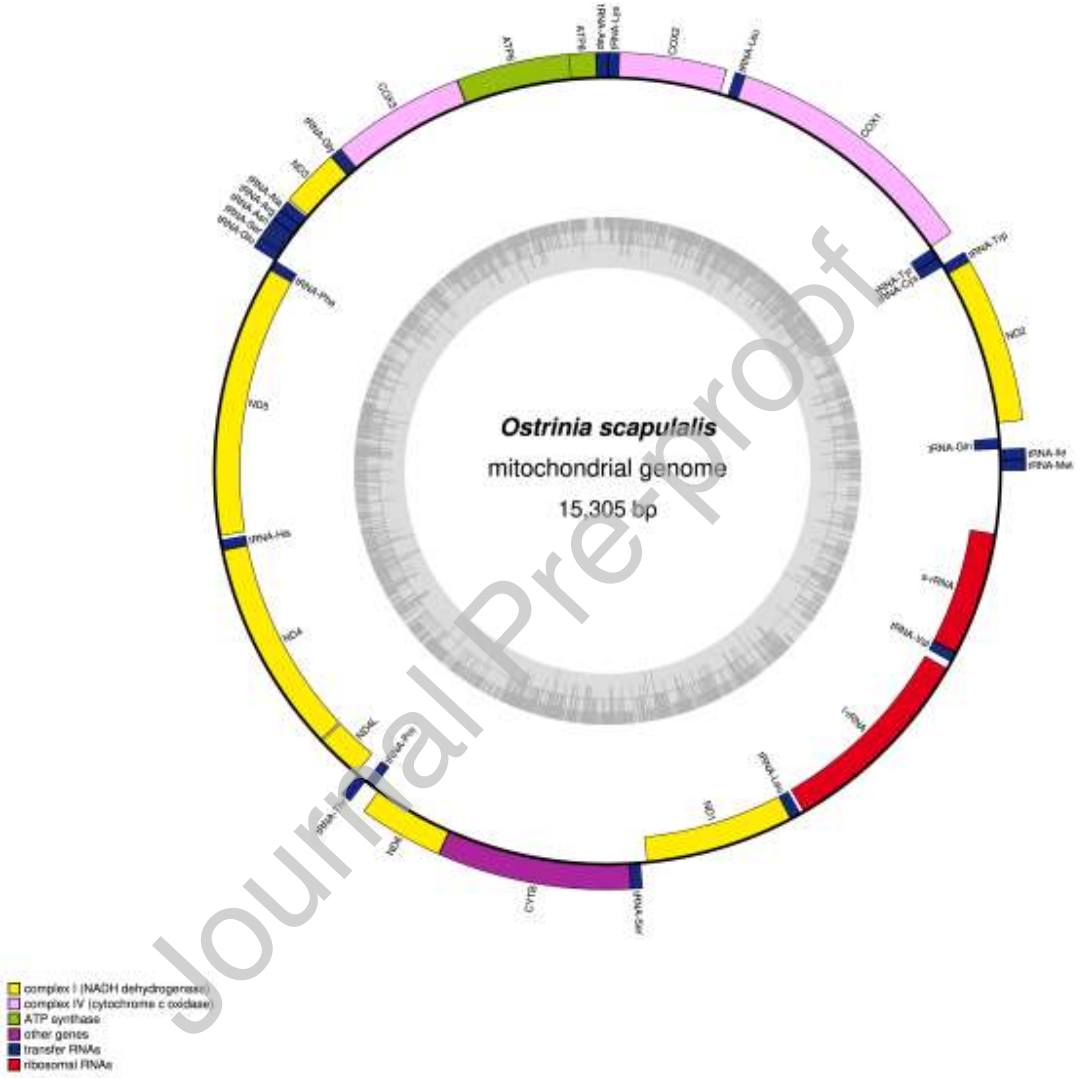

Fig. 2. Characteristics of the A+T-rich region of the *O. scapulalis* mitogenome. The AT-rich region consists of a TTAGA (red) motif which is followed by a 18 bp poly-T stretch (brown). Subsequently, it contains five ATTTA motifs (blue) of which the last one is followed by a (AT)10 microsatellite stretch (purple) and terminates with a polyT/polyA region (green).

Fig. 3. Phylogenetic tree representing 63 Lepidoptera mitogenomes which was built on concatenated alignments of the nuclear sequences of the 13 protein-coding and both rRNA genes applying a Maximum Likelihood approach with PHYML. For *Ostrinia* spp. the countries are also given in which the samples were taken.