Gene

First mitogenome of subfamily Langiinae (Lepidoptera: Sphingidae) with its phylogenetic implications

--Manuscript Draft--

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Abstract:	To date, a relatively complete classification has been generated, but the phylogeny of the family remains to be fully resolved. Among the outstanding issues is the taxonomic status of the subfamily Langiinae and its sole included genus and species, Langia zenzeroides. To begin to address this problem, we generated nine new complete mitochondrial genomes, including that of Langia, and together with that of Theretra oldenlandiae from our previous study and 25 other Sphingidae mitogenomes downloaded from GenBank, analyzed the phylogenetic relationships of Sphingidae and investigated the mitogenomic differences among members of the Langiinae, Sphinginae, Smerinthinae and Macroglossinae. The mitogenomes of Sphingidae varied from 14995 bp to 15669 bp in length. The gene order of all newly sequenced mitogenomes was identical, containing 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes and the A + T-rich region. Nucleotide composition was A + T biased, and all the protein-coding genes exhibited a positive AT-skew, which was reflected in the nucleotide composition, codon, and amino acid usage. The A + T-rich region was comprised of nonrepetitive sequences, which contained regulatory elements related to the control of replication and transcription. We analyzed concatenated gene sequences, with third codon positions of protein coding genes and rRNAs excluded, using Maximum Likelihood and Bayesian Inference techniques. All four currently recognized subfamilies were recovered as monophyletic but in contrast to the most recent studies, our preferred tree placed Langiinae as the first subfamily to diverge within Sphingidae rather as sister to Smerinthinae + Sphinginae. Our results also support the removal of the genus Barbourion from the smerinthine tribe Ambulycini to an unresolved position in "Smerinthinae incertae sedis".
Suggested Reviewers:	Ian Kitching, Doctor Natural History Museum i.kitching@nhm.ac.uk Ian Kitching is an expert in Sphingidae study
	Akito Kawahara, Doctor University of Florida kawahara@umd.edu Akito Kawahara made great contributions in Sphingidae research.
	Min Jee Kim, Doctor Korea Institute of Oriental Medicine minjeekim@chonnam.ac.kr Min Jee Kim has done a lot of research on Asian Sphingids.

Abstract: To date, a relatively complete classification has been generated, but the phylogeny of the family remains to be fully resolved. Among the outstanding issues is the taxonomic status of the subfamily Langiinae and its sole included genus and species, Langia zenzeroides.

Opposed Reviewers:	

January 16, 2021
Editor
Gene

Dear Xavier Carette,

On behalf of my co-authors, I would like to submit our revised manuscript entitled titled "First mitogenome of subfamily Langiinae (Lepidoptera: Sphingidae) with its phylogenetic implications" for publication in Gene. We have modified our manuscript according to the comments of reviews. Besides, we corrected two species which was misidentified before.

Sphingidae belongs to the Lepidoptera, which is a species-rich family contains more than 1460 represented species named sphinx or hawkmoth in English belonging to 206 genera. Due to its unique shape, strong flight ability and special phenomenon of hybridization, hawkmoth has been favored by many researchers in animal taxonomy, zoogeography, molecular biology, pollination biology, agricultural entomology and other aspects. However, the higher classification of Sphingidae is still a controversial issue. Further research remains to be done to clarify the phylogenetic relationships of different subfamilies and diverse species.

In this study, we generated nine new complete mitochondrial genomes, including that of Langia, and together with that of Theretra oldenlandiae from our previous study and 25 other Sphingidae mitogenomes downloaded from GenBank, analyzed the phylogenetic relationships of Sphingidae and investigated the mitogenomic differences among members of the Langiinae, Sphinginae, Smerinthinae and Macroglossinae. The mitogenomes of Sphingidae varied from 14995 bp to 15669 bp in length. The gene order of all newly sequenced mitogenomes was identical, containing 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes and the A + T-rich region. Nucleotide composition was A + T biased, and all the protein-coding genes exhibited a positive AT-skew, which was reflected in the nucleotide composition, codon, and amino acid usage. The A + T-rich region was comprised of nonrepetitive sequences, which contained regulatory elements related to the control of replication and transcription. We analyzed concatenated gene sequences, with third codon positions of protein coding genes and rRNAs excluded, using Maximum Likelihood and Bayesian Inference techniques. All four currently recognized subfamilies were recovered as monophyletic but in contrast to the most recent studies, our preferred tree placed Langiinae as the first subfamily to diverge within Sphingidae rather as sister to Smerinthinae + Sphinginae. Our results also support the removal of the genus Barbourion from the smerinthine tribe Ambulycini to an unresolved position in "Smerinthinae incertae sedis".

We sincerely hope to publish our article in your journal. Please don't hesitate to contact us if we can do anything to assist you in evaluating our manuscript. Thank you very much for your consideration.

Yours sincerely,
Yi-Xin Huang, PhD
Collaborative Innovation Center of Recovery and Reconstruction of Degraded

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197
198   Dear reviewer,
199
200   Thanks for your time reviewing our paper. Now we have tried our best to
201   correct these problems.
202
203   Reviewer #1: This is a good study to determine the first complete mitogenomes
204   of subfamily Langiinae and demonstrate the phylogenetic relationships of high
205   level in the family Sphingidae. This study used Bayesian and Maximum
206   Likelihood methods to reconstruct the phylogeny within four subfamilies. The
207   text is well arranged and in good writing. There are only following some minor
208   revisions needed which are marked in the attachment.
209   1.In the line 270, what options were used during genome assembled. Even if all
210   options were standard, they must be specified.
211   Response: This part has been revised as "The assembly of the mitochondrial
212   (mt) genome was accomplished with Novoplasy. Mito was chosen as the type
213   and genome range was set as 12000 to 20000 with the K-mer of 23. The read
214   length was adjusted to 150 and insert size was set to 300. The insert range was
215   modified to 1.8."in line 243-246.
216
217   2.In the line 430, the word "3CP" first exit, need to note the complete
218   information.
219   Response: This part has been revised as " Removing the third codon positions
220   (3CP) of PCGs is the most commonly used strategy to reduce compositional
221   heterogeneity in this type of gene, and removal of RNAs (rRNAs and tRNAs) is
222   another common such method in mitogenomic studies investigating
223   phylogenetic relationships in Lepidoptera". Please refer to line 404.
224
225   3.In the line 473, this sentence "According to the latest Sphingidae Taxonomic
226   Inventory, there are four subfamilies of Sphingidae, Langiinae, Sphinginae,
227   Smerinthinae and Macroglossinae." should be modified "According to the latest
228   Sphingidae Taxonomic Inventory, there are four subfamilies as Langiinae,
229   Sphinginae, Smerinthinae and Macroglossinae."
230   Response: This part has been delete and rewrote from line 416 to 448.
231
232   4.In the line 474, this sentence "Both the The phylogenetic tree reconstructed by
233   PCG12 and PCG123 were in favor of the latest classification with four
234   subfamilies." should be modified "Both the phylogenetic tree reconstructed by
235   PCG12 and PCG123 were in favor of the latest classification with four
236   subfamilies."
237   Response: This part has been delete and rewrote from line 416 to 448.
238
239   5.The position of gene code (Strand) in Tab. 7 should be consistent with article.

240 Response: This part has been revised as "J-strand and N-strand" in Tab.7-10.
241
242
243 Reviewer #2: The author describe the analysis of mitogenomes of a number of
244 Chinese sphingids.
245 The DNA analysis appears to be plus/minus ok; it follows routine protocols and
246 using established and adequate software.
247
248 However, the study has serious flaws:
249 1. there appears to be no design in the study. Just some sphingids were collected
250 and sequenced. In a scientific study, you need an hypothesis which you want to
251 test. In this case, you need a phylogenetic question, which you want to solve.
252 This would require that you optain enough and adequate samples to answer the
253 question. Here, it was just chance.
254 Response: We have redesigned our hypothesis and proposed some problems
255 needed to be illustrated in "Abstract" and "Instruction". Such as "the phylogeny
256 of the family remains to be fully resolved. Among the outstanding issues is the
257 taxonomic status of the subfamily Langiinae and its sole included genus and
258 species, Langia zenzeroides." , "In this study, nine new mitochondrial genomes
259 are sequenced to enrich the diversity of mitogenomes available in Sphingidae,
260 and a preliminary phylogenetic tree of Sphingidae generated to provide further
261 information on the relationships among taxa of the family. ", details please see
262 Abstract and line 228-231.
263
264 2. Who identified the sphingids? Which museum collection was used?
265 Response: We invited Ian Kitching, an expert of Sphingidae, to help us in
266 identifying the sphingids, *species Ambulyx liturata* was found to be
267 misidentified and revised as *A. ochracea*, species *Griseosphinx preechari* was
268 found to be misidentified and revised as *Acosmerycoides harterti*. The
269 collections were preserved in the entomology museum of Anhui Normal
270 University.
271
272 3. How many individuals/ taxon were samplöed?
273 Response: We checked more than 300 hundred of sphingidae specimens.
274
275 4. The introduction is very weak. The description of Sphingids, their biology
276 and systematics is very superficial and leaves the impression that the authors
277 lack a deep understanding of their subject.
278 Response: We have rewrote the instruction after reading and consulting more
279 articles and books about Sphingidae. Now it was arranged as
280   "Hawkmoths (Sphingidae) are a family of moths comprising more than 1460
281 species in 206 genera (van Nieukerken et al., 2011). Adult hawkmoths are
282 mostly medium to large insects that can fly at 40-50 kilometers per hour by

virtue of their streamlined bodies and long, blade-like wings (Akkuzu et al., 2007). Well-known as flower visitors and significant pollinators, most adult hawkmoths have well-developed probosces (Krpač et al., 2019). The larvae of hawkmoths are cylindrical, medium to large, generally with a single caudate scolus, and some species are significant agricultural pests (Nagamine et al., 2019).

The history of the first 250 years of the higher taxonomy of Sphingidae was summarized by Kitching and Cadiou (2000), who then proposed a new higher classification, recognizing three subfamilies: Sphinginae, Smerinthinae and Macroglossinae. The Sphinginae was then divided into two tribes, Sphingini and Acherontiini; the Smerinthinae into three tribes, Smerinthini, Sphingulini and Ambulycini, and the Macroglossinae into three tribes, Dilophonotini (with two subtribes, Dilophonotina and Hemarina), Philampelini and Macroglossini (comprising two subtribes, Macroglossina and Choerocampina). Building upon the molecular phylogenetic analyses of Kawahara et al. (2009) and Kawahara and Barber (2015), Kitching & Rougerie et al. (2018) proposed an updated higher classification of the family that was implemented on the Sphingidae Taxonomic Inventory website (Kitching, 2020)), in which four subfamilies were recognized with the phylogenetic relationship: (Macroglossinae (Langiinae (Smerinthinae, Sphingidae))). Rather unexpectedly, the genus *Langia* Moore, 1872, was recovered as the sister group of a clade comprising Smerinthinae and Sphinginae, rather than as a subordinate group within subfamily Smerinthinae (Kawahara et al., 2009), and thus required its own subfamily Langiinae. The genus *Langia* includes only a single species, *Langia zenzeroides* Moore, 1872, one of the largest species in the family, and is widely distributed in temperate and higher elevation tropical regions of east Asia, including China, Korea, India, Nepal, Vietnam, and Thailand.

Mitochondrial genome sequence analysis has proven to be an effective molecular tool to resolve issues relating to the phylogenetics of Lepidoptera (e.g., Timmermans et al., 2019). In this study, nine new mitochondrial genomes are sequenced to enrich the diversity of mitogenomes available in Sphingidae, and a preliminary phylogenetic tree of Sphingidae generated to provide further information on the relationships among taxa of the family."

5. The phylogeny part misses several studies
Response: We searched the development history of the phylogeny of Sphingidae and refered to some landmark event in the process of research on Sphingidae. Now it has been revised as " The history of the first 250 years of the higher taxonomy of Sphingidae was summarized by Kitching and Cadiou (2000), who then proposed a new higher classification, recognizing three subfamilies: Sphinginae, Smerinthinae and Macroglossinae. The Sphinginae was then divided into two tribes, Sphingini and Acherontiini; the Smerinthinae into three tribes, Smerinthini, Sphingulini and Ambulycini, and the

326 Macroglossinae into three tribes, Dilophonotini (with two subtribes,
327 Dilophonotina and Hemarina), Philampelini and Macroglossini (comprising two
328 subtribes, Macroglossina and Choerocampina). Building upon the molecular
329 phylogenetic analyses of Kawahara et al. (2009) and Kawahara and Barber
330 (2015), Kitching & Rougerie et al. (2018) proposed an updated higher
331 classification of the family that was implemented on the Sphingidae Taxonomic
332 Inventory website (Kitching, 2020)), in which four subfamilies were recognized
333 with the phylogenetic relationship: (Macroglossinae (Langiinae (Smerinthinae,
334 Sphingidae))). Rather unexpectedly, the genus *Langia* Moore, 1872, was
335 recovered as the sister group of a clade comprising Smerinthinae and
336 Sphinginae, rather than as a subordinate group within subfamily Smerinthinae
337 (Kawahara et al., 2009), and thus required its own subfamily Langiinae. The
338 genus *Langia* includes only a single species, *Langia zenzeroides* Moore, 1872,
339 one of the largest species in the family, and is widely distributed in temperate
340 and higher elevation tropical regions of east Asia, including China, Korea,
341 India, Nepal, Vietnam, and Thailand." Please see line 206-225.
342
343 6. The ms is written in a very sloppy way, with many typos, and mistakes in
344 grammar and language.
345 Response: We apology for our carelessness. Mistakes of our grammar and
346 language had been checked and revised.
347
348 7. The results contain a lot of descriptive data which can relegated to a
349 supplement
350 Response: We had delete these descriptive data in results part.

Abstract

To date, a relatively complete classification has been generated, but the phylogeny of the family remains to be fully resolved. Among the outstanding issues is the taxonomic status of the subfamily Langiinae and its sole included genus and species, Langia zenzeroides. To begin to address this problem, we generated nine new complete mitochondrial genomes, including that of Langia, and together with that of Theretra oldenlandiae from our previous study and 25 other Sphingidae mitogenomes downloaded from GenBank, analyzed the phylogenetic relationships of Sphingidae and investigated the mitogenomic differences among members of the Langiinae, Sphinginae, Smerinthinae and Macroglossinae. The mitogenomes of Sphingidae varied from 14995 bp to 15669 bp in length. The gene order of all newly sequenced mitogenomes was identical, containing 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes and the A + T-rich region. Nucleotide composition was A + T biased, and all the protein-coding genes exhibited a positive AT-skew, which was reflected in the nucleotide composition, codon, and amino acid usage. The A + T-rich region was comprised of nonrepetitive sequences, which contained regulatory elements related to the control of replication and transcription. We analyzed concatenated gene sequences, with third codon positions of protein coding genes and rRNAs excluded, using Maximum Likelihood and Bayesian Inference techniques. All four currently recognized subfamilies were recovered as monophyletic but in contrast to the most recent studies, our preferred tree placed Langiinae as the first subfamily to diverge within Sphingidae rather as sister to Smerinthinae + Sphinginae. Our results also support the removal of the genus Barbourion from the smerinthine tribe Ambulycini to an unresolved position in "Smerinthinae incertae sedis".

1. Introduction

Hawkmoths (Sphingidae) are a family of moths comprising more than 1460 species in 206 genera (van Nieukerken et al., 2011). Adult hawkmoths are mostly medium to large insects that can fly at 40-50 kilometers per hour by virtue of their streamlined bodies and long, blade-like wings (Akkuzu et al., 2007). Well-known as flower visitors and significant pollinators, most adult hawkmoths have well-developed probosces (Krpač et al., 2019). The larvae of hawkmoths are cylindrical, medium to large, generally with a single caudate scolus, and some species are significant agricultural pests (Nagamine et al., 2019).

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Mitochondrial genome sequence analysis has proven to be an effective molecular tool to resolve issues relating to the phylogenetics of Lepidoptera (e.g., Timmermans et al., 2019). In this study, nine new mitochondrial genomes are sequenced to enrich the diversity of mitogenomes available in Sphingidae, and a preliminary phylogenetic tree of

230 Sphingidae generated to provide further information on the relationships among taxa of
231 the family.

2. Materials and DNA Extraction

2.1 Sampling and DNA Extraction

234 Specimens of Sphingidae were collected by light trap at Anqing, Chizhou, Huangshan
235 and Lu'an, Anhui province, and Chengde, Hebei province, China (Table 1). Legs were
236 immediately preserved in absolute ethanol and stored at -20°C before DNA extraction
237 following the cetyltrimethyl ammonium bromide (CTAB) method (Shahjahan et al.,
238 1995).

2.2 Sequencing and assembly

240 A whole genome shotgun (WGS) strategy was used with sequencing on an Illumina
241 Miseq platform. The quality of the data was checked using FastQC (**Andrews**, Available
242 online:http://www.bioinformatics.babraham.ac.uk/projects/fastqc (accessed on 10 July
243 2020)). The assembly of the mitochondrial (mt) genome was accomplished with
244 Novoplasy. Mito was chosen as the type and genome range was set as 12000 to 20000
245 with the K-mer of 23. The read length was adjusted to 150 and insert size was set to 300.
246 The insert range was modified to 1.8.

2.3 Mitochondrial genome annotation

248 Twenty-two tRNA genes were identified with the use of MITOS WebServer, setting the
249 parameters with the Invertebrate Mito genetic code (Bernt et al., 2013). Their secondary
250 structures were plotted manually from the MITOS predictions using Adobe Illustrator.
251 Every sequence of tRNA genes was manually checked separately. Protein-coding genes
252 (PCGs) were identified as open reading frames corresponding to the 13 PCGs in the
253 metazoan mt genome. The rRNA genes and control region were identified by the
254 boundaries of the tRNA genes. Mitogenome maps were produced using Organellar
255 Genome DRAW (OGDRAW)(Lohse et al., 2013).

2.4 Comparative analysis

257 Base composition and relative synonymous codon usage (RSCU) were calculated using
258 MEGA X (Kumar et al., 2018). The relative composition of different bases was measured
259 in terms of GC and AT skews according to the formulae suggested by Hassanin et al.
260 (2005): GC-skew = (G-C)/(G+C) and AT-skew = (A-T)/(A+T). The number of

261 synonymous substitutions per synonymous site (Ks) and the non-synonymous

262 substitutions per non-synonymous site (Ka) for each of the concatenated 13 PCGs of the

263 Sphingidae mitogenome were calculated by DnaSP 5 (Rozas et al., 2003).

264 *2.5 Phylogenetic analysis*

265 Nine newly generated mitogenomes, one (*Theretra oldenlandiae*) previously published

266 by us (Wang et al., 2020) and 25 from GenBank were analyzed in this study, of which

267 two Geometridae, *Biston panterinaria* and *Phthonandria atrilineata*, were selected as

268 outgroups. Alignment of PCGs was conducted with MAFFT 7.3.1 using G-INS-I

269 algorithms (Katoh and Standley, 2016). Two rRNA segments were aligned with the R-

270 Coffee web server (Moretti et al., 2008). Subsequently, all alignments were concatenated

271 into a single matrix with DAMBE (Xia, 2013). PartitionFinder 1.1.1 was used to infer the

272 optimal partitioning strategy (Lanfear et al., 2012). The best fitting model was then

273 selected for each partition based on the BIC (Bayesian Information Criterion).

274 Alignments of individual genes were concatenated to generate four 33-taxa data sets:

275 1) the PCG matrix, including all three codon positions of protein-coding genes; 2) the

276 PCG12 matrix, including only the first and second codon positions of protein-coding

277 genes; 3) the PCGR matrix, including all three codon positions of protein-coding genes

278 and two rRNA genes; 4) the PCG12R matrix, including only the first and second codon

279 positions of protein-coding genes and two rRNA genes. Both ML (maximum-likelihood)

280 and BI (Bayesian inference) analyses were conducted on the concatenated dataset for

281 phylogeny reconstruction. Maximum likelihood analysis was conducted in IQtree v1.4.1

282 using the best-fit substitution model (Nguyen et al., 2015). An ultrafast bootstrap (UFB)

283 of 1000 replications (Bui et al., 2013) and the SH-aLRT test were used in this analysis to

284 assess branch supports (Guindon et al., 2010).

285 MrBayes 3.2 was used to conduct the analysis of Bayesian inference (Ronquist et al.,

286 2012) . Two simultaneous runs of one million generations were conducted and trees

287 sampled every 100 generations. Stationarity was considered to be reached when the

288 average standard deviation of split frequencies fell below 0.01. The first 25% of samples

289 were discarded as burn-in and the remaining samples were used to generate a 50%

290 majority rule consensus tree. FigTree v.1.3.1 was used to view the resulting trees

291 (Rambaut, Available online: http://tree.bio.ed.ac.uk/software/figtree (Accessed on 10

292 July 2020)).

3. Results and discussion

3.1 Genome structure and organization

The Sphingidae mitogenomes contained the complete set of 37 genes common to the mitogenomes of insects, including 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes and two ribosomal RNA (rRNA) genes (Cameron et al., 2012). In *Langia*, 23 genes (14 tRNAs and nine PCGs) were encoded by the majority-strand (J-strand) and 14 genes (4 PCGs, two rRNAs and eight tRNAs) were encoded by the minority-strand (N-strand), making the whole genome a typical double-chain circular molecular structure (Fig. 1).

The mitogenome lengths of the nine new sequences (together with the previously sequenced *Theretra oldenlandiae*; Wang et al. (2020)) ranged from 14995 (*Acosmerycoides harterti*) to 15669 bp (*Marumba sperchius*), within which *Langia zenzeroides* was 15366 bp. Compared with other Sphingidae mitogenomes, which range in length from 14 kb to 20 kb, these new sequences are thus of medium size (Zheng et al., 2018). In Sphinginae, the mitogenome of *N. analis analis* is 150099 bp in length. Among Macroglossinae, the mitogenomes ranged in length from 14995 to 15410 bp, whereas among Smerinthinae, they ranged from 15346 to 15669 bp. The nucleotide composition of all the mitogenomes had a high A+T content, with an average of 80.65%, showing a strong A/T bias (Table 2). Among the newly generated mitogenomes, *Ampelophaga rubiginosa* had the highest AT content and *Clanis undulosa gigantea* had the lowest, respectively 81.46% and 79.25%. *Ambulyx ochracea* had the highest A content, whereas the lowest A content was found in *Theretra oldenlandiae*. In terms of the full genomes, all the AT-skews were positive and GC-skews were negative, ranging from 0.1% to 3.8% and -23.6% to -19.4% respectively, indicating that the mitogenomes favor A and C in Sphingidae.

3.2 Protein-coding genes (PCGs)

The 13 PCGs of all the nine new mitogenomes and that of the previously published *Theretra oldenlandiae* contained three cytochrome c oxidase subunits, seven NADH dehydrogenase subunits, two ATPase subunits and one cytochrome b gene, which is similar to other Lepidoptera.

The concatenated length of the 13 PCGs of *Langia zenzeroides* is 11193 bp, which encodes 3732 amino acid residues. In Sphinginae, the concatenated length of the 13 PCGs of *Notonagemia analis analis* is 11193 bp, which encodes 3731 amino acid residues. Among Smerinthinae, the concatenated lengths of the 13 PCGs of *Ambulyx ochracea*, *Clanis undulosa gigantea*, *Marumba gaschkewitschii* and *Marumba sperchius* are 11193 bp, 11219 bp, 11214 bp and 11211 bp, encoding 3731, 3739, 3738 and 3737 amino acid

326 residues, respectively. Among Macroglossinae, the concatenated lengths of the 13 PCGs

327 of *Ampelophaga rubiginosa* (Anhui), *Cephonodes hylas*, *Acosmerycoides harterti* and

328 *Theretra oldenlandiae* are 11192 bp, 11190 bp, 11192bp and 11193 bp, encoding 3730,

329 3730, 3730 and 3731 amino acid residues, respectively.

330 Most PCGs start with ATG or ATT and stop with TAA (see tables 7-10). However,

331 all the *cox1* genes in these mitogenomes use CGA as the start codon. Different taxonomic

332 groups may have different start codons for *cox 1*, and the use of non-canonical start

333 codons in this gene is known as a common phenomenon in insects (Fenn et al., 2007).

334 Some PCGs ,such as cox 3, nad 3 and nad 1 in the mitogenomes of Smerinthinae also use

335 TAG as the stop codon. Three genes of Sphinginae (*nad2, cox1, nad5*), four genes of

336 Smerinthinae (*nad2, cox1, cox2, nad5*) and four genes of Macroglossinae (*nad2, cox1,*

337 *cox2, nad5*) use the incomplete stop codon, T. Three genes (*cox3, nad3, nad1*) in the

338 smerinthines, *Ambulyx ochracea* and *Marumba sperchius*, stop with TAG. Further

339 research is needed to verify whether the two species have their own and a similar

340 mechanism for transcription termination.

341 The dominant high A+T content, with an average of 80.65%, is not unusual in lepidopteran

342 mitogenomes. To investigate further this high A and T content, and the frequency of synonymous

343 codon usage, we calculated relative synonymous codon usage (RSCU) values. The relative

344 synonymous codon usages (RSCU) of the four subfamilies are shown in Fig. 2. Taken together, the

345 most frequently used codons are UUA (Leu2), CGA (Arg), GUU (Val) and GCU (Ala), whereas

346 those ending in G or C, CUG, CUC, CAG, GGC, were the less frequently used codons. The codons

347 ending with A or T are predominant, with an average of 89.67% of all mitogenomes, which leads,

348 In part at least, to the bias towards A and T.

349 The non-synonymous/synonymous substitution ratio (Ka/Ks) can be used to estimate

350 whether a sequence is undergoing purifying (negative), neutral, or positive selection. The

351 rate of nonsynonymous substitutions (Ka), synonymous substitutions (Ks), and the ration of Ka/Ks

352 were calculated for the PCGs of each mitogenome, using *Manduca sexta* as the reference sequence

353 (Fig. 3). A value of Ka/Ks greater than 1 means positive selection exists, indicating that

354 non-synonymous mutations are more favored by Darwinian selection, and they will be

355 retained at a rate greater than synonymous mutations. All the values of Ka, Ks and the ratio of

356 Ka/Ks were below 1, which suggests the presence of purifying selection in these species.

357 *3.3 Transfer and ribosomal RNA genes*

358   In total, 22 transfer RNA genes were found, ranging in size from 36 bp (*trnR* of *M. sperchius*) to 80

359   bp (*trnE* of *A. rubiginosa* (Anhui)). In Langiinae, the length of the tRNAs ranged from 63 bp to 70

360   bp (Fig. 4). The average nucleotide composition of these tRNAs was A: 41.4%, T: 40.4%, C:

361   10.6% and G: 8.0%, with a total average A+T content of 80.8%. Most AT-skews were positive, and

362   all GC-skews were negative, which indicates a slight bias towards the use of A and C in tRNAs

363   (Tables 3-6). Identical to the situation in other hawk moths, the gene arrangement and

364   orientation of the *trnI*, *trnM* and *trnQ* tRNAs was *trnM-trnI-trnQ*, which is considered to

365   be derived from the ancestral gene order *trnI-trnQ-trnM* (Boore, 1999). The two rRNA

366   genes, the larger ribosomal gene (*rrnL*) and the smaller ribosomal gene (*rrnS*), were

367   located between *trnL1* and *trnV*, and *trnV* and the A+T-rich region respectively, which is

368   identical to other sequenced hawkmoths. The average of the total size of two rRNAs was

369   2143 bp and the average A+T content was 84.3%. Like the tRNAs, most AT-skews were

370   positive and all GC-skews were negative. In contrast, in Smerinthinae, most AT-skews

371   were negative, indicating that rRNAs favor T more than tRNAs in Smerinthinae and

372   supporting the separation of Langiinae from this subfamily.

373   *3.4 Intergenic spacers and overlapping sequences*

374   We observed 133 gaps in total in the nine new mitochondrial genomes sequenced in this

375   study and that of the previously published *Theretra oldenlandiae*, with the sizes ranging

376   from 1-121 bp. The longest intergenic spacer (121 bp) was observed in *M. sperchius*,

377   Smerinthinae, between the *cox3* and *trnG* genes (Tables 7-10). Sphinginae and

378   Smerinthinae mitogenomes show some similar intergenic spacers, 16 or 17 intergenic

379   spacers ranging from 1 bp to 121 bp were identified with a total length of 172 bp to 438

380   bp. Compared to Sphinginae and Smerinthinae, intergenic spacers in Macroglossinae

381   fluctuate widely in lwngth. The number of intergenic spacers of *A. rubiginosa*, *C. hylas*,

382   *A. harterti* and *T. oldenlandiae* mitogenomes were 10, 17, 12 and 12, ranging from 1 bp

383   to 63 bp, respectively, with a total length from 103 bp to 239 bp.

384   There were 63 overlapping gene regions, ranging from 1 bp to 8 bp in length in the

385   nine mitogenomes and that of the previously published *Theretra oldenlandiae*. The

386   longest overlapping sequence in each genome was between *trnW* and *trnC*. The number

387   of overlapping gene regions ranged from five to nine, with a total length from 19 bp to 28

388   bp. In general, Macroglossinae mitogenomes have more intergenic spacers but fewer

389   overlapping gene regions than Langiinae, Sphinginae and Smerinthinae.

390   *3.5 A+T rich region*

391   The A+T rich region, also called the control region (Taylor et al., 1993) because it is

392   generally supposed to contain regulatory elements related to the control of replication and

393   transcription (Zhang et al., 1995), is the largest non-coding region and is located between

394   *rrnS* and *trnM* in these mitogenomes. It plays an important role in molecular evolution

395   research (Zhang and Hewitt, 1997). The size of control region varied from 54 bp in *A.*

396   *harterti* to 423 bp in *T. oldenlandiae* (Table 3-6), indicating that *A. harterti* possesses a

397   quite a short control region compared with the other eight mitogenomes. Compared with

398   the other three regions (PCGs, tRNAs and rRNAs), the control region has the highest

399   A+T content, ranging from 92.1% to 95.5%. The AT-skew of all nine mitogenomes and

400   that of the previously published *Theretra oldenlandiae* varied from slightly negative (-

401   3.0%) to moderately negative (-44.0%), whereas the GC-skew was highly variable, from

402   moderately negative (-57.9%) to moderately positive (50.0%)

403   *3.6 Phylogenetic analyses*

404   Phylogenetic analyses based on both ML and BI optimality criteria recovered similar

405   topologies (Fig. 5), which also generally agreed with those of previous studies. Removing

406   the third codon positions (3CP) of PCGs is the most commonly used strategy to reduce

407   compositional heterogeneity in this type of gene, and removal of RNAs (rRNAs and

408   tRNAs) is another common such method in mitogenomic studies investigating

409   phylogenetic relationships in Lepidoptera (Kim et al., 2011; Yang et al., 2015). The ML

410   and BI topologies based on PCGR dataset were not concordant with each other, and we

411   consider that the datasets that included the RNAs (PCG123R and PCG12R) have low

412   credibility. The third position of PCGs may also interfere significantly with phylogenetic

413   reconstruction in the present study. Consequently, we concluded that removal of both the

414   RNAs and the 3CP of PCGs was likely to produce results that are more consistent, and

415   thus that dataset PCG12 is the most reliable. As both methods (BI and ML) produced the

416   same topology for each of the PCG12 and PCG123 datasets, only the BI trees are shown

417   in Fig. 5.

418   Each of the four subfamilies of Sphingidae was recovered as monophyletic (although

419   strictly the monophyly of Langiinae could not be tested as it comprises only a single

420   genus and species) and with very high support values (PP = 1, BS = 100). However, our

421   preferred topology based on the PCG12 data set, which excludes third codon positions

422   and rRNAs, places Langinae as sister to all remaining Sphingidae. This accords with the

423   results of Kawahara et al. (2009), which was based on a small number of nuclear genes,

424   rather than the more extensive phylogenomic analysis of Kawahara & Barber (2015),

425   which placed Langiinae as sister to Smerinthinae + Sphinginae and Macroglossinae as

426   sister to these three. This suggests that exclusion of third codon positions may perhaps be

427   introducing artefact rather than removing it, and further, even more comprehensive data

428   and analyses will be required to resolve this ambiguity.

429   Based on our included samples, subfamily Macroglossinae comprises two tribes,

430   Hemarini and Macroglossini. Subtribe Choerocampina (*Theretra japonica*, *T.*

431   *oldenlandiae*) is nested within subtribe Macroglossina, rendering the latter paraphyletic.

432   Like Langiinae, the monophyly of tribe Hemarina could not be tested as only a single

433   species, *C. hylas*, was included in our data set. Five taxa of subfamily Sphinginae were

434   included in our sampling, with the two subspecies of *Notonagemia analis, N. a. scribae*

435   and *N. a. analis*, grouping together as sister to *Psilogramma* in a monophyletic

436   "*Psilogramma* genus-group" (Kitching & Rougerie et al., 2018). Within subfamily

437   Smerinthinae, four groups were recovered. Three were well supported (PP = 1, BS = 100)

438   and correspond to the tribes Leucophlebiini, Sichiini and Ambulycini. The fourth group,

439   which is very poorly supported (PP = 0, BS = 59), comprises *Parum* (placed in

440   "Smerinthinae incertae sedis" by Kitching & Rougerie et al., 2018) and *Barbourion*

441   (placed by Kitching & Rougerie et al., 2018 in Ambulycini). A similar placement of

442   *Barbourion* outside Ambulycini was found by Timmermans et al. (2019), although they

443   were unsure whether or not this was an artefact of their limited sampling. Although our

444   sampling was only slightly more comprehensive, we nevertheless suggest that

445   *Barbourion* should be removed from Ambulycini, and placed in "Smerinthinae incertae

446   sedis", pending further studies with increased taxa sampling density of Smerinthinae.

447   Unsurprisingly, given that most of the data derives from their study, the pattern of

448   relationships among the remaining Ambulycini is identical with that found by

449   Timmermans et al. (2019), with the exception of the addition of *Ambulyx ochracea* to a

450   monophyletic genus *Ambulyx*.

4. Conclusion

452   In this study, we documented ten further complete mitogenomes of Sphingidae

453   (including the mitogenome of *T. oldenlandiae* previously reported by us (Wang et al.,

454   2020)), together with 24 other Sphingidae mitogenomes downloaded from GenBank, and

455   used different methods and datasets to identify and compare differences among them and

456   then analyze the phylogenetic relationships of the family. Our results support the four

subfamily classification of Sphingidae but our preferred PGC12 tree disagrees with the previously reported pattern of relationships, in that Langiinae, not Macroglossinae, is the first subfamily to diverge. Although the phylogenetic analysis presented here provide a hypothesis for the relationships within Sphingidae. However, further investigations are still necessary to fully elucidate and document the evolution of Sphingidae. Our taxon sampling is still sparse and so the relationships found here must still be considered tentative, Further research is required with denser sampling and additional molecular and morphological characters.

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602

603 **Tables**

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606 *oldenlandiae* sample reported by Wang et al. (2020).

607 Table 3. Nucleotide composition of separate regions on mitogenomes of Sphinginae.

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614 Table 10. Mitogenomic organization of Macroglossinae.

615

616 **Figures**

617
618 **Fig. 1** Circular maps of the mitochondrial genome *L. zenzeroides*. Protein-coding and ribosomal genes are

619 indicated using standard abbreviations. The J-strand is shown on the outer circle and the N-strand on the

620 inner circle.

621 **Fig. 2** Relative synonymous codon usage (RSCU) of the mitochondrial genomes of (a) Sphinginae, (b)

622 Langiinae, (c) Smerinthinae and (d) Macroglossinae.

623 **Fig. 3** Evolutionary rates of mitochondrial genomes in four subfamilies. The numbers of nonsynonymous

624 substitutions per nonsynonymous site (Ka), the number of substitutions per synonymous site (Ks), and the

625 ratio of Ka/Ks for every mitochondrial genome is given, using *Manduca sexta* as the reference sequence.

626 **Fig. 4** Predicted secondary cloverleaf structure for the tRNAs of *Langia zenzeroides.*

627 **Fig. 5** Phylogenetic tree produced by maximum likelihood and Bayesian inference analyses based on the

628 PCG12(a) and PCG123(b) dataset. Bootstrap (BS) and posterior probability (PP) values were shown on the

629 nodes. BS values lower than 50 were not shown.

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644 **Conflicts of Interest**: All authors declare no conflicting interests.

Abbreviation list

BI: Bayesian inference
mitogenome: mitochondrial genome
ML: maximum-likelihood
mtDNA: mitochondrial deoxyribonucleic acid
PCGs: protein-coding genes
RSCU: relative synonymous codon usage
WGS: whole genome shotgun

Table 1

Table 1. List of species investigated and their related information.

No.	Family	Subfamily	Taxa	GenBank Accession No.	Location/Refence
1.	Geometridae	Ennominae	*Biston panterinaria*	KU325533	(Cheng et al., 2017)
2.			*Phthonandria atrilineata*	EU569764	(Yang et al., 2009)
3.	Sphingidae	Smerinthinae	*Adhemarius dariensis*	MG747645	(Li et al., 2018b)
4.			*Adhemarius dentoni*	MK804148	(Timmermans et al., 2019)
5.			*Ambulyx dohertyi*	MK804150	(Timmermans et al., 2019)
6.			*Ambulyx ochracea*	MT712132	This study, Huangshan
7.			*Ambulyx substrigilis*	MK804151	(Timmermans et al., 2019)
8.			*Amplypterus mansoni*	MK804152	(Timmermans et al., 2019)
9.			*Amplypterus panopus*	MK804153	(Timmermans et al., 2019)
10.			*Barbourion lemaii*	MK804154	(Timmermans et al., 2019)
11.			*Batocnema coquerelii*	MK804155	(Timmermans et al., 2019)
12.			*Clanis bilineata*	MK804156	(Timmermans et al., 2019)
13.			*Clanis undulosa gigantea*	MT712135	This study, Huangshan
15.			*Leucophlebia lineata*	MK804158	(Timmermans et al., 2019)
16.			*Marumba gaschkewitschii*	MT712137	This study, Lu'an
17.			*Marumba sperchius*	MT712138	This study, Lu'an
18.			*Orecta lycidas*	MK804159	(Timmermans et al., 2019)
19.			*Parum colligata*	MG888667	(Li et al., 2019)
20.			*Protambulyx astygonus*	NC_046723	(Timmermans et al., 2019)
21.			*Protambulyx eurycles*	MK804161	(Timmermans et al., 2019)
22.			*Protambulyx ockendeni*	NC_046725	(Timmermans et al., 2019)
23.			*Protambulyx strigilis*	MK804163	(Timmermans et al., 2019)
24.			*Trogolegnum pseudambulyx*	MK804164	(Timmermans et al., 2019)
		Macroglossinae	*Acosmerycoides harterti*	MT712136	This study, Huangshan
25.			*Ampelophaga rubiginosa*	KT153024	(Li et al., 2018a)
26.			*Ampelophaga rubiginosa*	MT712133	This study, Anqing
27.			*Cephonodes hylas*	MT712134	This study, Chizhou
28.			*Macroglossum stellatarum*	MG747645	(Li et al., 2018b)
29.			*Theretra japonica*	MG655620	(Li et al., 2018a)
30.			*Theretra oldenlandiae*	MN885801	(Wang et al., 2020)
31.		Langiinae	*Langia zenzeroides*	MT922035	This study, Chengde
32.		Sphinginae	*Manduca sexta*	EU286785	(Kim et al., 2016)
33.			*Notonagemia analis scribae*	KU934302	(Kim et al., 2016)
34.			*Psilogramma increta*	MF974243	(Li et al., 2018b)
35.			*Notonagemia analis analis*	MT712143	This study, Huangshan
36.			*Sphinx morio*	KC470083	(Kim et al., 2013)

Table 2

Table 2. Nucleotide composition of nine newly generated mitogenomes and that of the *Theretra*

oldenlandiae sample reported by Wang et al. (2020).

Taxa	Length (bp)	A%	C%	G%	T%	G+C%	A+T%
Acosmerycoides harterti	14995	41.46	11.22	7.50	39.83	18.71	81.29
Ambulyx ochracea	15346	42.05	11.34	7.57	39.03	18.92	81.08
Ampelophaga rubiginosa (Anhui)	15064	41.39	11.07	7.47	40.07	18.54	81.46
Cephonodes hylas	15410	41.04	11.70	7.58	39.68	19.28	80.72
Clanis undulosa gigantea	15416	40.71	13.11	7.64	38.54	20.75	79.25
Langia zenzeroides	15366	41.35	11.79	7.67	39.19	19.45	80.55
Marumba gaschkewitschii	15501	40.67	11.33	7.43	40.56	18.77	81.23
Marumba sperchius	15669	40.75	11.39	7.36	40.50	18.75	81.25
Notonagemia analis analis	15099	40.90	12.44	8.04	38.62	20.48	79.52
Theretra oldenlandiae	15312	40.60	12.32	7.68	39.41	20.00	80.00

Table 3

Table 3. Nucleotide composition of separate regions on mitogenomes of Sphinginae.

N. analis analis

Regions	Size (bp)	A	C	G	T	AT(%)	GC-skew	AT-skew
PCGs	11193	40.4	13.2	8.8	37.6	78.0	-19.8%	3.5%
1st and 2nd codon position	9784	35.7	9.9	12.5	41.9	22.4	11.6%	-27.7%
A+T-rich region	112	40.2	1.8	3.6	54.5	94.6	33.3%	-15.1%
tRNAs	1446	41.4	11.0	8.3	39.3	80.7	-14.0%	2.7%
rRNAs	2186	42.8	10.8	4.7	41.7	84.5	-39.2%	1.4%
Full genome	15099	40.9	12.4	8.0	38.6	79.5	-21.5%	2.9%

table 4

Table 4. Nucleotide composition of separate regions on mitogenomes of Langiinae

L. zenzeroides								
Regions	**Size (bp)**	**A**	**C**	**G**	**T**	**AT(%)**	**GC-skew**	**AT-skew**
PCGs	11196	40.9	12.5	8.4	38.3	79.2	-19.8%	3.2%
1st and 2nd codon position	9644	34.5	9.3	11.2	45.0	79.5	9.3%	-13.2%
A+T-rich region	334	44.9	4.8	2.7	47.6	92.5	-28.0%	-2.9%
tRNAs	1449	40.9	10.7	8.5	40.0	80.8	-11.5%	1.1%
rRNAs	2202	43.6	10.4	4.9	41.1	84.7	-36.1%	2.9%
Full genome	15366	41.35	11.79	7.67	39.19	80.6	-21.2%	2.7%

Table 5

Table 5. Nucleotide composition of separate regions on mitogenomes among Smerinthinae.

A. ochracea/ C. undulosa gigantea/ M. gaschkewitschii/ M. sperchius

Regions	Size (bp)	A %	C%	G%	T%	AT%	GC-skew	AT-skew
PCGs	11193/11219/11214/11211	40.4/40.2/40.3/40.1	13.2/14.2/12.1/12.3	8.8/8.5/8.2/8.2	37.6/37.1/39.5/39.4	78.0/77.9/79.8/79.4	-20.0%/-25.1%/-19.2%/-20.0%	3.6%/4.0%/1.0%/0.9%
1st and 2nd codon position	9590/9570/9578/9586	34.9/35.2/35.9/36.1	9.3/10.0/9.3/9.5	12.4/13.0/12.4/12.2	43.5/41.8/42.4/42.2	78.4/77.0/78.3/78.3	14.3%/13.0%/14.3%/14.3%	-11.0%/-8.6%/-8.3%/-7.8%
A+T-rich region	112/332/394/366	40.2/43.7/44.7/44.3	1.8/4.8/4.6/4.1	3.6/2.4/3.3/3.0	54.5/49.1/47.5/48.6	94.6/92.8/92.1/92.9	33.3%/-33.3%/-16.5%/-15.5%	-15.1%/-5.8%/-3.0%/-4.6%
tRNAs	1443/1482/1478/1491	41.5/42.8/41.0/41.1	11.0/11.4/9.7/10.2	8.1/8.0/7.7/7.6	39.4/40.5/41.5/41.0	80.7/83.3/82.5/82.2	-15.2%/-17.5%/-11.5%/-14.6%	2.6%/2.8%/-0.6%/0.1%
rRNAs	2186/2122/2133/2134	42.8/40.7/41.2/41.9	10.8/11.0/10.9/10.5	4.7/4.7/5.0/4.9	41.7/40.8/42.9/42.7	84.5/81.4/84.1/84.6	-39.4%/-40.1%/-37.1%/-36.4%	1.3%/-0.1%/-2.0%/-0.9%
Full genome	15346/15416/15501/15669	42.1/40.7/40.7/40.7	11.3/13.1/11.3/11.4	7.6/7.6/7.4/7.4	39.0/38.5/40.6/40.5	81.1/79.2/81.2/81.2	-19.6%/-26.6%/-20.9%/-21.3%	3.8%/2.8%/0.1%/0.2%

Table 6

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Table 6. Nucleotide composition of separate regions on mitogenomes among Macroglossinae.

A. rubiginosa (Anhui) */ C. hylas / A. harterti / T. oldenlandiae*

Regions	Size (bp)	A %	C%	G%	T%	AT%	GC- skew	AT-skew
PCGs	11192/11190/11192/11193	41.1/40.7/ 41.3/40.2	11.4/12.6/ 11.7/13.3	8.1/8.4/8.1 /8.5	39.3/38.3/ 38.9/38.0	80.5/79.0/ 80.2/78.2	-16.9/-20/-18.2/-22	2.2/3/3/2.8
1st and 2nd codon position	9480/9602/9555/9725	36.0/35.6/ 35.9/35.5	9.2/9.4/9.3 /9.8	11.4/12.5/ 12.1/12.4	43.3/42.4/ 42.8/42.2	79.3/78.0/ 78.7/22.3	10.7/14.2/ 13.1/11.7	-9.2/-8.7/-8.8/-8.6
A+T-rich region	118/332/54/423	38.7/41.9/ 25.9/44.4	3.9/3.3/1.9 /3.5	1.7/1.8/5.6 /0.9	55.8/53.0/ 66.7/51.1	94.5/94.9/ 92.6/95.5	-39.3/-29.4 /49.3/-59.1	-18.1/-11.7 /-44.1/-7
tRNAs	1462/1468/1441/1466	41.2/40.7/ 41.9/40.9	10.5/10.3/ 10.3/10.8	7.9/8.1/8.0 /7.9	40.4/40.9/ 39.8/40.5	84.5/81.6/ 81.7/81.3	-14.1/-12/-12.6/-15.5	1/-0.2/2.6/ 0.5
rRNAs	2118/2164/2117/2125	42.8/42.6/ 42.5/42.0	10.4/10.3/ 10.3/10.5	4.8/4.8/4.8 /4.9	42.0/42.4/ 42.5/42.6	84.8/85.0/ 85.0/84.6	-36.8/-36.4 /-36.4/-36.4	0.9/0.2/0/-0.7
Full genome	15064/15410/14995/15312	41.4/41.0/ 41.5/40.6	11.1/11.7/ 11.2/12.3	7.5/7.6/7.5 /7.7	40.1/39.7/ 39.8/39.4	81.5/80.7/ 81.3/80.0	-19.4/-21.2 /-19.8/-23	1.6/1.6/2.1 /1.5

Table 7

Table 7. Mitogenomic organization of Sphinginae.

	Position		Size	Intergenic	Codon		Strand
	From	**To**	**(bp)**	**nucleotides**	**Start**	**Stop**	
				Notonagemia analis analis			
trnM	1	68	68				J
trnI	76	140	65	7			J
trnQ	209	141	69				J
nad2	261	1274	1014	51	ATT	T	N
trnW	1274	1340	67	-1			J
trnC	1397	1333	65	-8			N
trnY	1462	1398	65				J
cox1	1470	3000	1531	7	CGA	T	N
trnL2	3001	3066	66				J
cox2	3067	3748	682		ATG	TAA	N
trnK	3749	3818	70				J
trnD	3819	3884	66				J
atp8	3885	4046	162		ATT	TAA	N
atp6	4040	4717	678	-7	ATG	TAA	J
cox3	4726	5517	792	8	ATG	TAA	N
trnG	5524	5589	66	6			J
nad3	5590	5943	354		ATC	TAA	N
trnA	5949	6005	57	5			J
trnR	6022	6086	65	16			J
trnN	6096	6161	66	9			J
trnS1	6162	6227	66				J
trnE	6239	6304	66	11			J
trnF	6367	6303	65	-2			N
nad5	8102	6368	1735		ATT	T	N
trnH	8169	8103	67				J
nad4	9507	8173	1335	3	ATG	TAA	N
nad4l	9801	9511	291	3	ATG	TAA	N
trnT	9812	9877	66	1			J
trnP	9941	9877	65	-1			N
nad6	9943	10470	528	1	ATG	TAA	N
cytb	10490	11644	1155	19	ATG	TAA	N
trnS2	11648	11712	65	3			J
nad1	12670	11735	936	22	ATG	TAA	N
trnL1	12737	12671	67				J
rrnL	14150	12738	1413				N
trnV	14214	14151	64				J
rrnS	14987	14215	773				N
AT-rich	14988	15099	112				N

Table 8

Table 8. Mitogenomic organization of Langiinae.

| | Position | | Size (bp) | Intergenic nucleotides | Codon | | Strand |
	From	To			Start	Stop	
Langia zenzeroides							
trnM	1	64	64				J
trnI	65	128	64				J
trnQ	194	126	69	-3			N
nad2	246	1259	1014	51	ATT	TAA	J
trnW	1272	1338	67	12			J
trnC	1393	1331	63	-8			N
trnY	1457	1394	64				N
cox1	1469	2999	1531	11	CGA	T	J
trnL2	3000	3065	66				J
cox2	3066	3747	682		ATG	T	J
trnK	3748	3817	70				J
trnD	3818	3882	65				J
atp8	3883	4050	168		ATT	TAA	J
atp6	4044	4721	678	-7	ATG	TAA	J
cox3	4726	5517	792	4	ATG	TAA	J
trnG	5520	5585	66	2			J
nad3	5586	5939	354		ATT	TAA	J
trnA	5948	6015	68	8			J
trnR	6020	6086	67	4			J
trnN	6087	6151	65				J
trnS1	6158	6223	66	6			J
trnE	6224	6288	65				J
trnF	6411	6345	67	56			N
nad5	8146	6412	1735		ATT	T	N
trnH	8212	8147	66				N
nad4	9549	8212	1338	-1	ATG	TAA	N
nad4l	9840	9550	291		ATG	TAA	N
trnT	9847	9912	66	6			J
trnP	9976	9912	65	-1			N
nad6	9978	10499	522	1	ATG	TAA	J
cytb	10507	11658	1152	7	ATG	TAA	J
trnS2	11657	11720	64	-2			J
nad1	12697	11759	939	38	ATG	TAA	N
trnL1	12765	12699	67	1			N
rrnL	14193	12766	1428				N
trnV	14258	14194	65				N
rrnS	15032	14259	774				N
AT-rich	15033	15366	334				N

Table 9

Table 9. Mitogenomic organization of Smerinthinae.

	Position		Size (bp)	Intergenic nucleotides	Codon		Strand
	From	To			Start	Stop	
A. ochracea/ C. undulosa gigantea/ M. gaschkewitschii/ M. sperchius							
trnM	1/1/1/1	68/68/69/68	68/68/69/68				J/J/J/J
trnI	77/69/78/70	141/132/141/133	65/64/64/64	8/-/8/1			J/J/J/J
trnQ	207/198/207/199	139/130/139/131	69/69/69/69	-3/-3/-3/-3			N/N/N/N
nad2	260/255/267/260	1273/1268/1280/1273	1014/1014/1014/1014	52/56/59/6	ATT/ATT/TAA/ATT	TAA/T/TAA/TAA	N/N/N/N
trnW	1278/1267/1279/1273	1345/1337/1349/1343	68/71/71/71	4/-2/-2/-1			J/J/J/J
trnC	1401/1393/1405/1399	1338/1330/1342/1336	64/64/64/64	-8/-8/-8/-8			N/N/N/N
trnY	1471/1462/1472/1466	1407/1396/1406/1400	65/67/67/67	5/2/-/-			J/J/J/J
cox1	1476/1477/1489/1512	3006/3007/3019/3042	1531/1531/1531/1531	4/14/16/45	CGA/CGA/CGA/CGA	T/T/T/T	N/N/N/N
trnL2	3007/3008/3020/3043	3073/3074/3088/3111	67/67/69/69				J/J/J/J
cox2	3074/3075/3089/3112	3755/3756/3770/3793	682/682/682/682		ATG/ATG/ATG/ATG	T/T/TAA/T	N/N/N/N
trnK	3756/3757/3771/3794	3827/3826/3841/3864	72/70/71/71				J/J/J/J
trnD	3838/3832/3854/3877	3903/3902/3921/3948	66/71/68/72	1/5/12/12			J/J/J/J
atp8	3904/3903/3922/3949	4065/4070/4089/4113	162/168/168/165		ATC/ATC/ATT/ATT	TAA/TAA/TAA/TAA	N/N/N/N
atp6	4059/4064/4083/4107	4736/4741/4760/4784	678/678/678/678	-7/-7/-7/-7	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	J/J/J/J

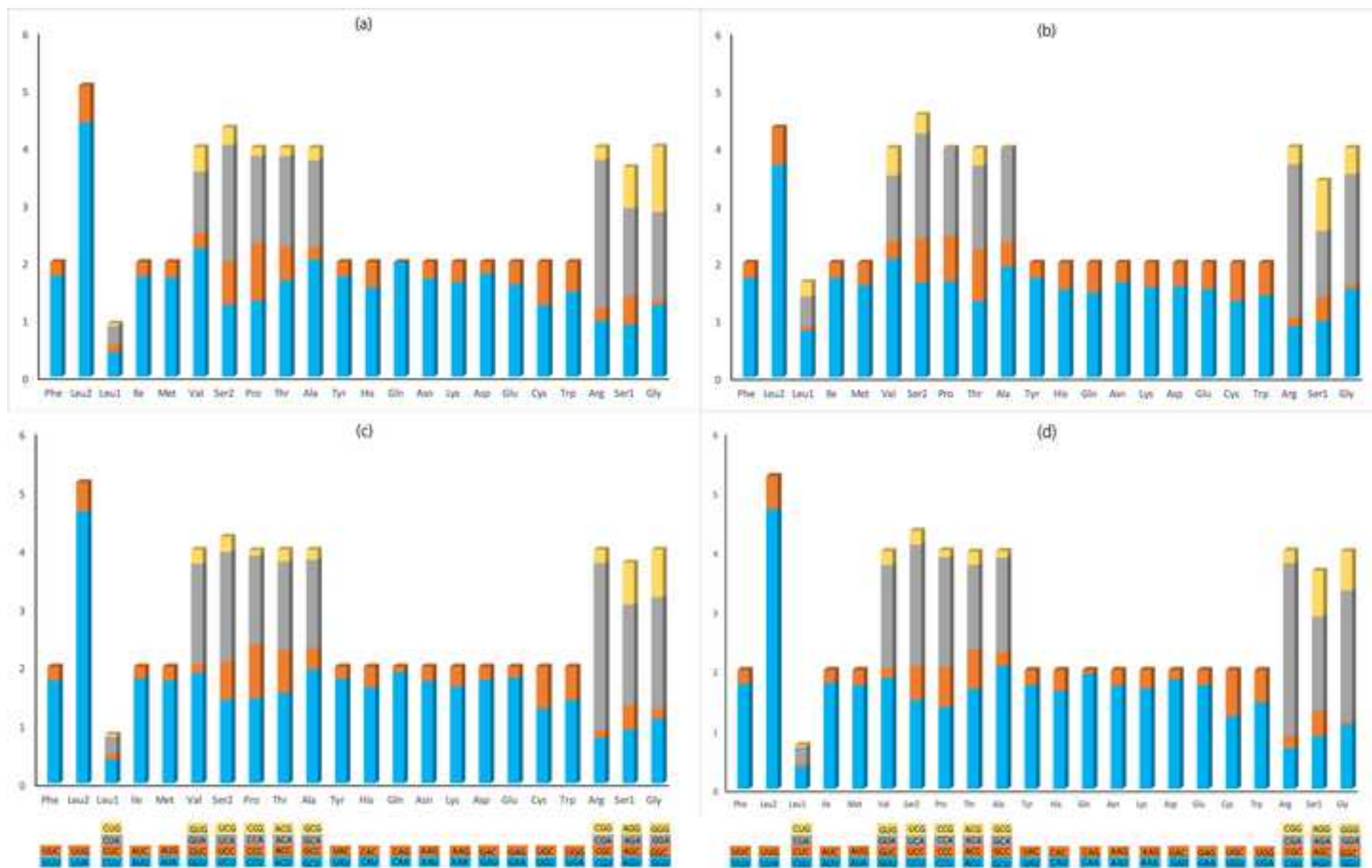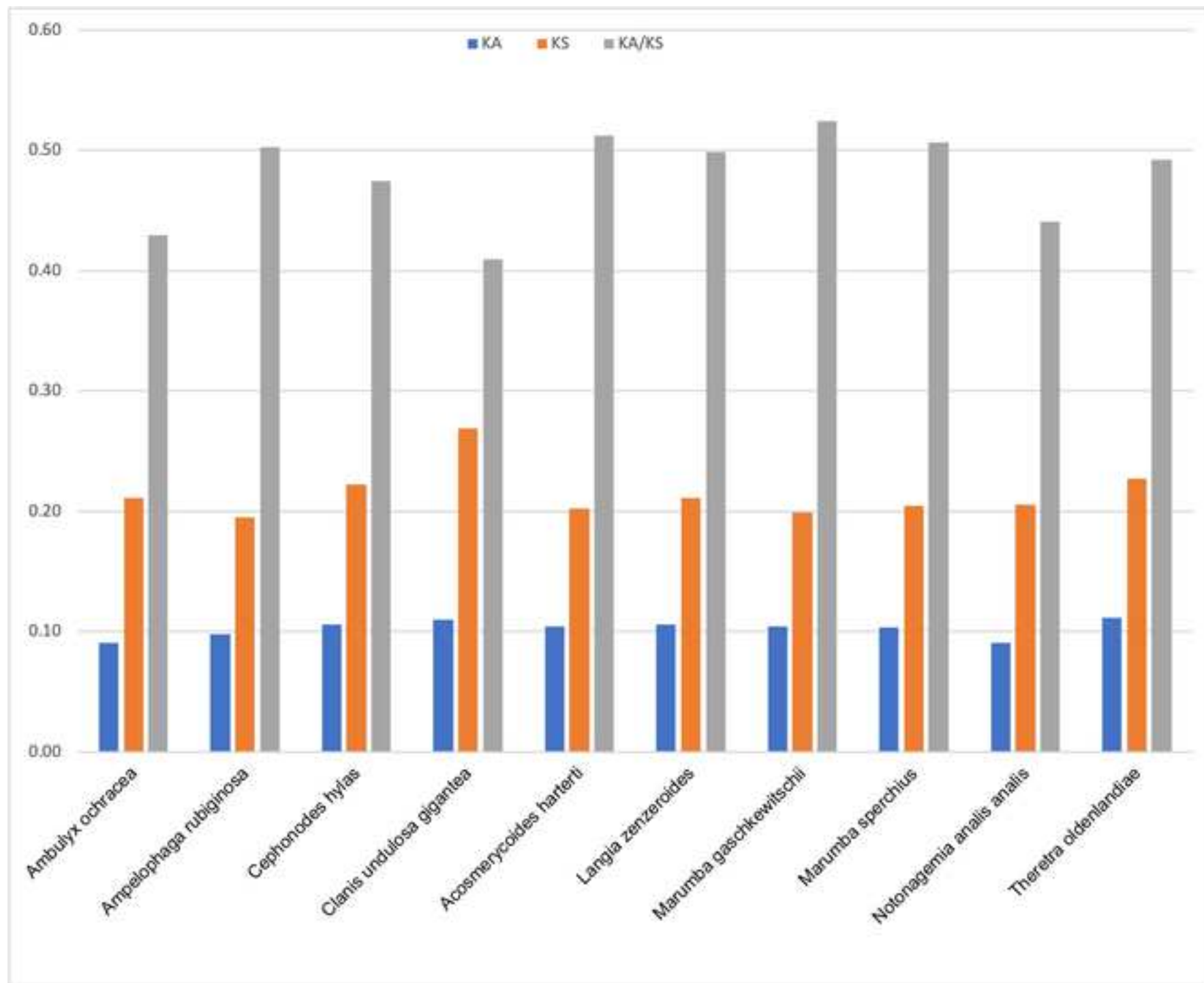| Gene							
cox3	4740/4741/4765/4788	5531/5529/5556/5579	792/789/792/792	3/-1/4/3	ATG/ATG/ATG/ATG	TAG/TAA/TAA/TAG	N/N/N/N
trnG	5534/5536/5629/5701	5600/5601/5694/5767	67/66/66/67	2/6/72/121			J/J/J/J
nad3	5601/5602/5695/5768	5954/5955/6048/6121	354/354/354/354		ATT/ATT/ATT/ATC	TAG/TAA/TAA/TAG	N/N/N/N
trnA	5953/5958/6052/6120	6019/6024/6116/6189	67/67/65/70	-2/2/3/-2			J/J/J/J
trnR	6020/6027/6117/6190	6085/6090/6179/6225	66/64/63/36	-/2/-/-			J/J/J/J
trnN	6090/6091/6180/6256	6156/6158/6245/6320	67/68/66/65	4/-/-/3			J/J/J/J
trnS1	6157/6161/6253/6359	6222/6229/6320/6426	66/69/68/68	-/2/7/38			J/J/J/J
trnE	6224/6284/6381/6463	6290/6351/6449/6532	67/68/69/70	1/54/6/36			J/J/J/J
trnF	6389/6485/6521/6694	6323/6418/6454/6627	67/68/68/68	32/66/4/94			J/J/J/J
nad5	8127/8220/8256/8432	6390/6486/6522/6695	1738/1735/1735/1738		ATT/ATT/ATT/ATT	T/T/T/T	N/N/N/N
trnH	8193/8291/8324/8499	8128/8221/8257/8433	66/71/68/67				J/J/J/J
nad4	9534/9628/9680/9842	8197/8291/8337/8502	1338/1338/1344/1341	3/1/12/2	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	N/N/N/N
nad4l	9829/9918/9977/10160	9539/9628/9687/9870	291/291/291/291	4/-1/6/27	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	N/J/N/N
trnT	9834/9924/9987/10165	9900/9989/10052/10230	67/66/66/66	4/5/9/4			J/J/J/J
trnP	9965/10053/10116/10297	9900/9989/10052/10230	66/65/65/68	-1/-1/-1/-1			N/N/N/N
nad6	9972/10065/10125/10324	10502/10598/10658/10857	531/534/534/534	6/11/8/26	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	N/N/N/N
cytb	10502/10603/10658/10857	11656/11760/11812/12011	1155/1158/1155/1155	-1/4/-1/-1	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	J/N/J/J
trnS2	11721/11811/11816/12014	11785/11876/11882/12081	65/66/67/68	64/5/3/2			J/J/J/J
nad1	12741/12829/12839/13035	11806/11893/11904/12100	936/937/936/936	2/16/21/18	ATG/ATG/ATG/ATG	TAG/T/TAA/TAA	N/N/N/N

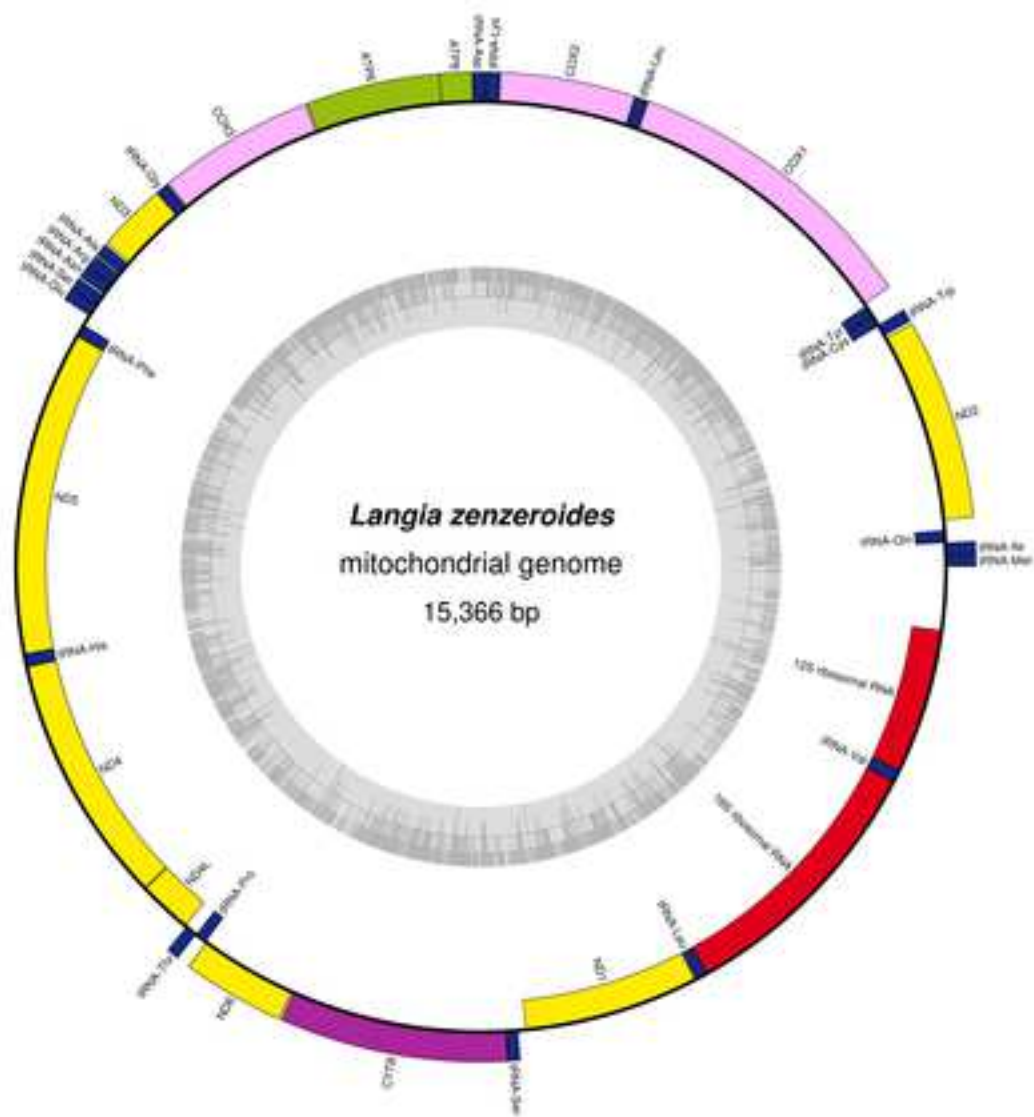|				
trnL1	12808/12897/12908/13103	12742/12830/12840/13037	67/68/69/67	-/-/-/1	J/J/J/J
rrnL	14174/14241/14259/14460	12809/12898/12909/13104	1366/1344/1351/1357		N/N/N/N
trnV	14239/14306/14325/14526	14175/14242/14260/14461	65/65/66/66		J/J/J/J
rrnS	15017/15084/15107/15303	14240/14307/14326/14527	778/778/782/777		N/N/N/N
AT-rich	15018/15085/15108/15304	15346/15416/15501/15669	329/332/394/366		N/N/N/N

Table 10

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Table 10. Mitogenomic organization of Macroglossinae.

	Position		Size (bp)	Intergenic nucleotides	Codon		Strand
	From	To			Start	Stop	
A. rubiginosa (Anhui) / *C. hylas* / *A. harterti* / *T. oldenlandiae*							
trnM	1/1/1/1	68/68/69/68	68/68/69/68				J/J/J/J
trnI	69/72/70/69	132/136/134/133	64/65/65/65	-/3/-/-			J/J/J/J
trnQ	198/202/200/199	130/134/132/131	69/69/69/69	-3/-3/-3/-3			N/N/N/N
nad2	255/259/254/254	1268/1272/1267/1267	1014/1014/1014/1014	56/56/53/54	ATT/ATT/ATT/ATC	TAA/TAA/T/TAA	N/J/J/J
trnW	1267/1275/1272/1266	1334/1342/1339/1333	68/68/68/68	-2/2/4/-2			J/J/J/J
trnC	1391/1398/1395/1389	1327/1335/1332/1326	65/64/64/64	-8/-8/-8/-8			N/N/N/N
trnY	1456/1464/1460/1456	1392/1399/1396/1390	65/66/65/67				J/J/J/J
cox1	1463/1467/1469/1464	2993/2997/2999/2994	1531/1531/1531/1531	6/2/8/7	CGA/CGA/CGA/CGA	T/T/T/TTG	N/J/J/J
trnL2	2994/2998/3000/2995	3061/3064/3066/3061	68/67/67/67				J/J/J/J
cox2	3062/3065/3067/3062	3743/3746/3748/3743	682/682/682/682		ATG/ATG/ATG/ATG	TAA/T/T/T	N/J/J/J
trnK	3744/3747/3749/3744	3814/3817/3819/3814	71/71/71/71				J/J/J/J
trnD	3816/3843/3821/3816	íí3883/3908/3887/3881	68/66/67/66	1/25/1/1			J/J/J/J
atp8	3884/3909/3888/3882	4045/4070/4049/4046	162/162/162/165		ATT/ATC/ATC/ATC	TAA/TAA/TAA/TAA	N/J/J/J
atp6	4039/4064/4043/4040	4716/4741/4720/4717	678/678/678/678	-7/-7/-7/-7	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	J/N/N/N
cox3	4717/4741/4720/4717	5508/5532/5511/5508	792/792/792/792	-/-1/-1/-1	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	N/J/J/J
trnG	5511/5535/5514/5511	5576/5601/5580/5577	66/67/67/67	2/2/2/2			J/J/J/J
nad3	5577/5602/5581/5578	5930/5955/5934/5931	354/354/354/354		ATT/ATT/ATT/ATT	TAG/TAA/TAG/TAA	N/J/J/J

trnA	5929/5963/5933/5931	5995/6030/5997/5997	67/68/65/67	-2/7/-2/-1			J/J/J/J
trnR	5997/6068/5998/5998	6060/6133/6061/6063	64/66/64/66	1/37/-/-			J/J/J/J
trnN	6061/6134/6062/6074	6126/6200/6127/6139	66/67/66/66	-/-/-/1			J/J/J/J
trnS1	6127/6201/6128/6140	6188/6266/6189/6205	62/66/62/66				J/J/J/J
trnE	6187/6268/6194/6210	6266/6333/6262/6276	80/66/69/67	-2/1/4/4			J/J/J/J
trnF	6330/6407/6326/6343	6265/6341/6261/6277	66/67/66/67	-2/7/-2/-			N/J/N/J
nad5	8092/8142/8099/8081	6356/6411/6360/6344	1737/1732/1740/1738	25/3/33/-	ATT/ATT/ATT/ATT	TAA/T/TAA/T	N/J/J/J
trnH	8156/8208/8163/8147	8093/8143/8100/8082	64/66/64/66				J/J/J/J
nad4	9491/9594/9558/9482	8157/8257/8227/8151	1335/1338/1332/1332	-/48/63/3	ATG/ATG/ATG/ATG	TAA/TAG/TAA/TAA	N/J/J/J
nad4l	9782/9885/9849/9774	9492/9595/9559/9484	291/291/291/291	-/-/-/1	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	N/J/J/J
trnT	9787/9892/9854/9789	9852/9956/9920/9854	66/65/67/66	4/6/4/14			J/J/J/J
trnP	9917/10022/9985/9919	9852/9957/9920/9854	66/66/66/66	-1/-/-1/-1			N/J/N/N
nad6	9924/10063/9992/9934	10454/10590/10522/10464	531/528/531/531	6/4/6/14	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	N/J/J/J
cytb	10461/10590/10522/10464	11609/11741/11670/11612	1149/1152/1149/1149	6/-1/-1/-1	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	N/N/N/N
trnS2	11609/11760/11669/11611	11673/11826/11733/11675	65/67/65/65	-1/18/-2/-2			J/J/J/J
nad1	12630/12780/12688/12631	11695/11845/11753/11696	936/936/936/936	21/18/19/2	ATG/ATG/ATG/ATG	TAA/TAA/TAA/TAA	N/J/J/J
trnL1	12697/12848/12758/12700	12631/12782/12690/12633	67/67/69/68	-/1/1/1			J/J/J/J
rrnL	14041/14235/14099/14051	12698/12849/12759/12701	1344/1387/1341/1351				N/J/J/J
trnV	14109/14301/14165/14115	14042/14236/14100/14052	68/66/66/64				J/J/J/J
rrnS	14883/15078/14941/14889	14110/14302/14166/14116	774/777/776/774				N/J/J/J
AT-rich	14884/15079/14942/14í890	15064/15410/14995/15312	181/332/54/423				N/J/J/J

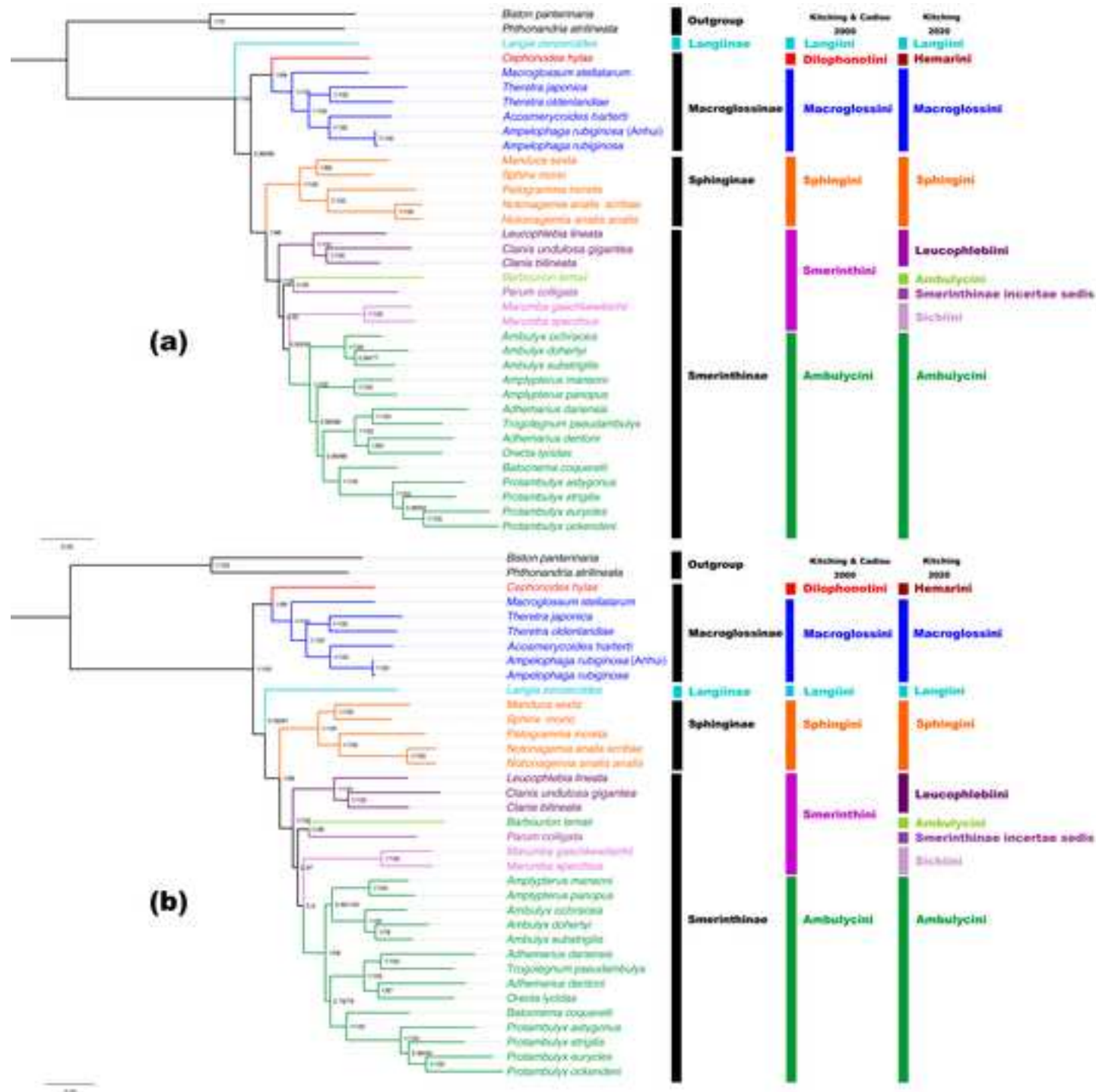Fig 2   Click here to access/download;Figure(s);Fig.2.tif ±

Fig 3

Fig 1

Fig 4

Fig 5   Click here to access/download;Figure(s);Fig.5.tif ±
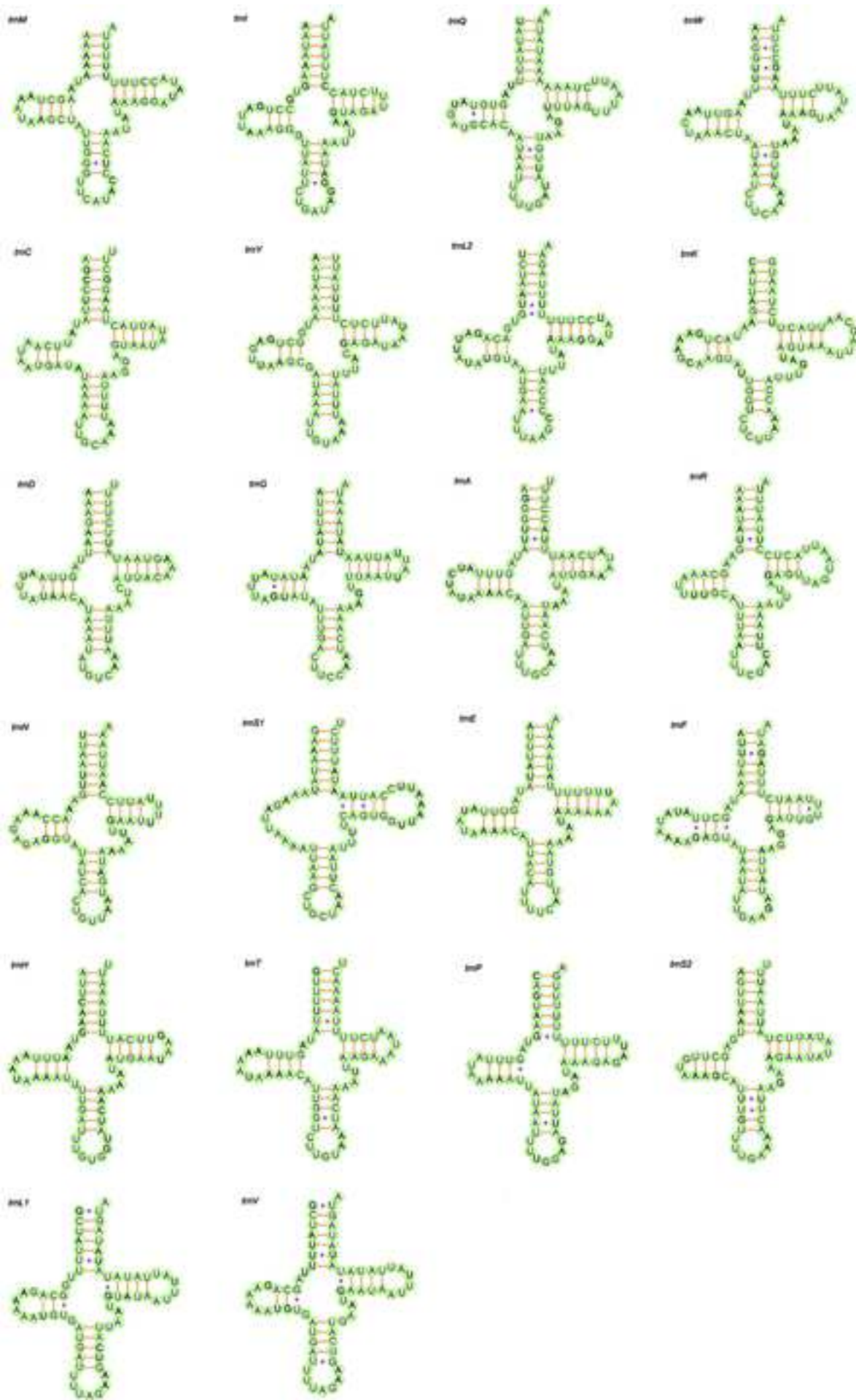
Declaration of Interest Statement

All authors declare no conflicting interests.

First mitogenome of subfamily Langiinae (Lepidoptera: Sphingidae) with its phylogenetic implications

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