Article

Comparative Analysis of Two Pear Pests, *Cacopsylla jukyungi* and *Cacopsylla burckhardtii* (Hemiptera: Psyllidae), Based on Complete Mitochondrial Genomes and Comparison to Confamilial Species

Ah Rang Kang 1,2, Min Jee Kim 3, Jeong Sun Park 2, Ho-Jin Seo 1, Jang-Hoon Song 1, Kyung-Ho Won 1, Eu Ddeum Choi 1 and Iksoo Kim 2,*

1. Introduction

After a substantial revision of east Palaearctic pear psyllid taxonomy, the genus *Cacopsylla Ossiannilsson*, 1970 [1] (Hemiptera: Psyllidae) was determined to comprise four species in Korea: *Cacopsylla burckhardtii* (Luo, Li, Ma & Cai, 2012) [2], *Cacopsylla jukyungi* (Kwon, 1983) [3], *Cacopsylla maculatili* (Li, 2011) [4], and *Cacopsylla sandolbaea* (Park & Lee, 1982) [5,6]. Among them, the polyvoltine *C. jukyungi* [3], which was previously misidentified as *Cacopsylla pyricola* (Foerster, 1848) [3,7], is the most damaging pest among commercial pears, particularly the southern type, *Pyrus pyrifolia* N., in Korea [8]. *C. jukyungi* overwinters under bark scale, after which it moves upward to tree branches, usually during February and March, and starts a new generation by ovipositing on branches. Ultimately, *C. jukyungi* will undergo 4–5 generations in a year [9,10]. The species displays...
seasonal dimorphism, converting from a light-colored form (summer form) to a dark overwintering form (winter form) in late summer and autumn in response to the shortening photoperiod [11]. The univoltine *C. burckhardti* [2], which was previously misidentified as *Cacopsylla pyrisuga* [7], is known to overwinter in the weeds around pear orchards. In South Korea, this psyllid enters commercial pear orchards in the spring, during which it damages pears, then disperses outside pear orchards [12]. A detailed, in-field lifecycle for *C. burckhardti* is not known. As is typical of psyllids, both nymphs and adults of *C. burckhardti* and *C. jukyungi* damage leaves and fruits by injecting a toxin while feeding that causes blackening and burning of the foliage (psylla shock) and by secreting honeydew that causes sooty mold and marks the fruit [13]. The other two pear psyllids occurring in Korea damage the northern-type pear (*P. ussuriensis* M.), which is a non-commercial pear, distributed infrequently in Korea [14].

Full and partial mitochondrial genome (mitogenome) sequences have been used for diverse purposes, such as evolutionary genomics, phylogenetic inference, and biogeographic inference [15–19]. Furthermore, mitogenomes have also been used to trace the origin of invasive species, evaluate donors for the genetic rescue of endangered species, and identify species, often using the mitochondrial COI gene segment [15,16,20–24].

Until now, the Psyllidae mitogenomes have been reported for 11 species. Four of them have been used for individual mitogenome reports, the main content of which includes genome annotation and nucleotide composition, along with phylogenetic inference, focusing on the phylogenetic position of the given species [25–28]. In this list, the *C. burckhardti* mitogenome, which has been reported this year, is also included [28]. On the other hand, the remaining seven species have been used to elucidate evolutionary relationships within the superfamily Psylloidea [29]. However, no study has been performed to illustrate the evolutionary characteristics of the family Psyllidae, particularly using a comparative approach, which will provide us with a further scrutinized understanding on mitogenome evolution. To control pear-damaging psyllids, the prompt application of proper control strategies upon detection of psyllids is important. This requires rapid identification of *Cacopsylla* species, but the co-occurrence of different species, life stages, and seasonal forms in pear orchards often complicates species identification in the field, especially when identification is performed by non-specialists. Thus, molecular marker-based identification is often required. Moreover, pest control using pesticides is often limited by the number and type of commercial pesticides available to control pear pests, and eco-friendly cultivation requires minimal application of pesticides. To overcome such difficulties, integrated pest-control strategies are required, involving an ecological understanding of pests, such as population genetic structure and gene flow [30–32]. For this, molecular markers, which provide variable regions, are essential for acquiring a meaningful understanding of population dynamics.

In this study, the complete mitogenomes of *C. jukyungi* and *C. burckhardti* were sequenced and the two genomes were characterized in terms of gene arrangement, codon composition, genome structure, and conserved sequence stretch. Available complete mitogenome sequences of other Psyllidae were also considered by a comparative approach. Thus, the newly sequenced mitogenomes of *C. jukyungi* and *C. burckhardti* are expected to enrich our understanding of mitogenome evolution in the family Psyllidae. Moreover, we compared the sequence divergence of individual genes to obtain a better understanding of individual gene-level evolution, which will be useful for the selection of candidate genes for subsequent population genetic analyses.

**2. Materials and Methods**

**2.1. Insects**

*C. jukyungi* and *C. burckhardti* were collected at Daap-myeon, Gwangyang city, Jeollanam-do Province, South Korea (35°04'00.50" N, 127°44'02.54" E) in April 2021. After live samples were transferred to the laboratory, samples were identified to species by examining their external morphology following Cho et al. [6], and also by sequencing the mitochondrial COI gene, corresponding to the DNA-barcoding region. Leftover specimen and DNA
were deposited at Chonnam National University with accession numbers CNU15412 for *C. jukyungi* and CNU15404 for *C. burckhardti*.

### 2.2. Genomic DNA Extraction and DNA Barcode Sequencing

Genomic DNA was extracted from adult hind legs using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), isopropyl alcohol, and 70% ethanol, following the manufacturer’s instructions. For confident species identification, a 658-bp DNA barcoding region was amplified using the primer set LCO1490 and HCO2198 [33]. Polymerase chain reaction (PCR) was conducted under the following conditions: initial denaturation for 4 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 51–52 °C, and 1 min at 72 °C, with a subsequent final 7 min extension at 72 °C. AccuPower PCR PreMix (Bioneer, Daejeon, Korea) was used for PCR. To confirm successful DNA amplification, electrophoresis was conducted using 0.5× TAE buffer on a 0.5% agarose gel. PCR products were purified using a PCR Purification Kit (Qiagen, Hilden, Germany). DNA sequencing was performed using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). All products were sequenced bi-directionally. A BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 4 March 2022) showed 100% identity to DNA-barcoding sequences of both *C. jukyungi* (GenBank no. JF327670) and *C. burckhardti* (GenBank no. MK039639) that were also sampled from Korea.

### 2.3. Next-Generation Sequencing

For library construction, 50 ng of genomic DNA was isolated and randomly sheared using the Covaris System (Woburn, MA, USA) to generate inserts of ~300 bp fragments. Library construction was performed using the TruSeq Nano DNA Kit (Illumina, San Diego, CA, USA) following the manufacturer’s instructions. The quality and size of DNA libraries were assessed using the Agilent 2100 BioAnalyzer (Agilent Technologies, Palo Alto, CA, USA). Libraries were quantified by qPCR using the CFX96 Real-Time System (BioRad, Hercules, CA, USA). After normalization, sequencing of the prepared library was conducted on the MGISEQ-2000 sequencing platform (MGI Tech Co. Ltd., Shenzhen, China). A quality analysis of the raw sequence data was performed using FastQC software [34]. Adapter sequence reduction and trimming of 5′- and 3′-ends were performed using Skewer ver. 0.2.2. [35]. Base-calling errors or insertions/deletions (indels) were corrected from the filtered set of reads using the alignment-based error correction tool Karect [36]. Consequently, ~3.99 gigabases (Gb) of nucleotides from 133 million *C. jukyungi* reads and ~4.38 Gb of nucleotides from 146 million *C. burckhardti* reads were obtained. The Phred quality score (Q) indicated that base call accuracy was 87.75% for *C. jukyungi* and 88.15% for *C. burckhardti* from the Q30 score.

### 2.4. Assembly and Gap Filling

The two mitogenomes were constructed using MITObim ver. 1.9 [37] by de novo assembly of the Illumina reads with *Cacopsylla citrisuga* [25]. The assembled mitogenomes were remapped with the whole genome sequence reads using Bowtie2 [38] before conducting manual curation. Mismatch calling and correction of the assembled sequences were conducted using GATK [39]. Finally, primary annotation of protein-coding genes (PCGs), tRNAs, rRNAs, and the A+T-rich region of each mitogenome was carried out using the MITOS Web Server [40] (http://mitos.bioinf.uni-leipzig.de/index.py, accessed on 10 April 2022).

Gap filling was usually not necessary, but the *C. jukyungi* A+T-rich region was unexpectedly long (1282 bp) and needed confirmation. Thus, a long fragment (LF) encompassing *lrRNA* and *COI*, in which the A+T-rich region is included, was amplified using a primer designed from the *C. jukyungi* mitogenome. Amplification of the LF was conducted using LA Taq (Takara Biomedical, Tokyo, Japan) under the following conditions: 94 °C for 4 min, 30 cycles at 98 °C for 10 s and 48 °C for 15 min, and a final extension step at 72 °C for 10 min. The PCR product was purified with a PCR purification kit (Bioneer, Daejeon, Korea).
and sequenced using barcode-tagged sequencing technology (Celemics, Inc., Seoul, Korea) and the Illumina MiSeq platform (Illumina, San Diego, CA, USA).

2.5. Gene Annotation

The identification, boundary delimitation, and secondary structure folding of tRNAs were performed using the MITOS Web Server by following the protocols presented by Cameron [41]. Twenty-one tRNA genes were well-identified, but trnS1, which has a truncated dihydrouridine (DHU) arm, was detected using a hand-drawn secondary structure in conjunction with an alignment of the predicted trnS1 regions of C. citrisuga [25]. Start and stop codons of PCGs were further confirmed by alignment against mitochondrial PCGs of Psyllidae species. The nucleotide sequences of the PCGs were translated based on the invertebrate mitochondrial DNA (mtDNA) genetic code. Sequence data were deposited into the GenBank database under accession numbers ON553958 for C. jukyungi and ON411626 for C. burckhardti.

2.6. Comparative Genome Analyses

For the comparative analysis, 11 Psyllidae mitogenomes were downloaded from the GenBank database. These sequences, along with the two mitogenome sequences obtained in the present study, were compared for several genomic characteristics. The A/T content of each gene and whole genome was calculated using DNASTAR (Madison, WI, USA). Codon usage was determined by MEGA 6 [42], and the gene overlap and intergenic spacer sequences were hand-counted. The genetic distance for each gene was calculated at within-Psyllidae and within-Cacopsylla levels using unrooted pairwise distances estimated with PAUP ver. 4.01b10 [43]. Genetic distances were then plotted with minimum, maximum, and median values presented with boxplots using JMP software ver. 15.0.0 (SAS Institute, Cary, NC, USA).

3. Results

3.1. General Mitochondrial Genome Features

Mitogenome sizes were 15,438 bp in C. jukyungi and 14,799 bp in C. burckhardti (Table 1). These sizes are within the range previously reported for complete Psyllidae mitogenomes, which range from 14,790 bp (Arytainilla spartiphila) to 16,047 bp (Russelliana solanica) (Table 2). The two mitogenomes contained 37 genes (13 PCGs, 22 tRNA genes, and two rRNA genes), which is typical in animals, and one major non-coding A+T-rich region [44]. In both species, thirteen PCGs had the typical ATN start codon, whereas the stop codons were incomplete in COII, ND5, and ND4 in C. jukyungi and COII, COIII, ND5, and ND4 in C. burckhardti; in these cases, stop codons were present only as a single thymine (Table 1). The gene arrangement was identical to that typically observed in other insects [41].

A total of 22 tRNA genes (one for each amino acid and two for leucine and serine) were identified for each mitogenome sequenced in this study (Figure S1 for C. jukyungi and Figure S2 for C. burckhardti). All tRNAs except trnS1, which lacked the DHU loop, folded into the expected cloverleaf secondary structures in both species. This incomplete trnS1 structure has been detected frequently in the mitogenomes of other animals, including insects [45]. The postulated tRNA cloverleaf structure of C. jukyungi and C. burckhardti harbors an invariant 7 bp in the aminoacyl stem, 5 bp in the anticodon stem, and 7 bp
in the anticodon loop, whereas the DHU and TΨC arms, particularly within the loops, were variable in length (3–5 bp in stems, but 0 bp in trnS1 DHU stems; 3–9 bp in loops; Figures S1 and S2). A total of 38 and 40 unmatched base pairs were detected in *C. jukyungi* and *C. burckhardti* tRNAs, respectively. Of these unmatched base pairs, 18 and 19 were G–U base-pairs, while 20 and 21 were non-Watson–Crick in *C. jukyungi* and *C. burckhardti*, respectively. The biased A/T content shown in the whole genome and genes reflects a biased usage of A/T-containing codons (Table 3). Among 64 codons, TTA (leucine), ATT (isoleucine), TTT (phenylalanine), and ATA (methionine), which are composed only of A and T nucleotides, were most frequently used, accounting for 31.43% of codons in *C. jukyungi* and 32.0% in *C. burckhardti*. In other species of Psyllidae, this bias is also obvious, with the frequency of these A/T-containing codons ranging from 28.91% in *Cacopsylla coccinea* to 36.59% in a *Heteropsylla* sp. (Table 3).

**Table 1. Summary of *Cacopsylla jukyungi* and *C. burckhardti* mitochondrial genomes.**

| Gene   | * D | ** AC | Cacopsylla jukyungi | Cacopsylla burckhardti |
|--------|-----|-------|---------------------|------------------------|
|        |     |       | Nucleotide Position (Size) | Anticodon Position | Start/Stop Codon | Nucleotide Position (Size) | Anticodon Position | Start/Stop Codon |
| trnI   | F   | GAT   | 1–66 (66)           | 33–35               | 1–66 (66)       | 33–35               |
| trnQ   | R   | TTA   | 71–136 (66)         | 106–108             | 71–136 (66)     | 106–108             |
| trnM   | F   | CAT   | 142–207 (66)        | 174–176             | 142–206 (65)    | 174–176             |
| ND2    | F   | 208–1179 (972) | ATA/TA             | 207–1178 (972)     | ATA/TA            |
| trnW   | F   | TCA   | 1202–1264 (63)      | 1232–1234           | 1177–1239 (63)  | 1208–1210           |
| trnC   | R   | GCA   | 1266–1362 (61)      | 1295–1297           | 1242–1302 (61)  | 1271–1273           |
| trnY   | R   | GTA   | 1328–1389 (62)      | 1358–1360           | 1303–1364 (62)  | 1333–1335           |
| COI    | F   | 1390–2922 (1533) | ATG/TA             | 1366–2898 (1533)   | ATG/TA            |
| trnL2  | F   | TAA   | 2922–2989 (68)      | 2953–2955           | 2988–2961 (64)  | 2928–2930           |
| COII   | F   | TTA   | 3729–3794 (66)      | 3763–3765           | 3694–3754 (61)  | 3724–3726           |
| ATP8   | F   | 3795–3947 (153) | ATC/TA             | 3755–3907 (153)    | ATC/TA            |
| ATP6   | F   | 3941–4615 (675) | ATG/TA             | 3901–4575 (675)    | ATG/TA            |
| COII   | F   | 4615–5394 (780) | ATG/TAG            | 4575–5352 (778)    | ATG/T             |
| trnG   | F   | TCC   | 5400–5457 (58)      | 5430–5432           | 5353–5414 (58)  | 5387–5388           |
| ND3    | F   | 5458–5808 (351) | ATG/TA             | 5415–5765 (351)    | ATG/TA            |
| trnA   | F   | TGC   | 5811–5870 (60)      | 5839–5841           | 5767–5827 (61)  | 5795–5797           |
| trnR   | F   | TCG   | 5872–5932 (61)      | 5903–5905           | 5831–5891 (61)  | 5862–5864           |
| trnN   | F   | GTT   | 5952–5997 (68)      | 5962–5964           | 5891–5955 (65)  | 5921–5923           |
| trnS1  | F   | GCT   | 5999–6052 (54)      | 6017–6019           | 5956–6009 (54)  | 5974–5976           |
| trnE   | F   | TTC   | 6053–6114 (62)      | 6085–6087           | 6010–6069 (60)  | 6040–6042           |
| trnF   | R   | GAA   | 6103–6165 (65)      | 6133–6135           | 6058–6120 (63)  | 6088–6090           |
| ND5    | R   | 6166–7778 (1621) | ATT/T              | 6121–7771 (1621)   | ATT/T             |
| trnH   | R   | GTG   | 7786–7824 (57)      | 7812–7814           | 7739–7800 (62)  | 7767–7769           |
| ND4    | R   | 7845–9087 (1243) | ATG/T              | 7801–9049 (1249)   | ATG/T             |
| ND4L   | R   | 9081–9362 (282) | ATG/TA             | 9043–9324 (282)    | ATG/TA            |
| trnT   | R   | TGT   | 9370–9428 (59)      | 9400–9402           | 9332–9392 (61)  | 9364–9366           |
| trnP   | R   | TGG   | 9429–9490 (62)      | 9458–9460           | 9393–9454 (62)  | 9422–9424           |
| ND6    | F   | 9493–9978 (486) | ATG/TAG            | 9457–9942 (486)    | ATG/TAG           |
| CytB   | F   | 9972–11,117 (1146) | ATG/TA         | 9936–11,078 (1143) | ATG/TA            |
| trnS2  | F   | TGA   | 11116–11,177 (62)   | 11,146–11,148       | 11,079–11,142 (64) | 11,109–11,111 |
| ND1    | R   | 11,205–12,119 (915) | ATG/TA         | 11,170–12,084 (915) | ATG/TA            |
| trnL1  | R   | TAG   | 12,120–12,182 (63)  | 12,151–12,153       | 12,085–12,147 (63) | 12,116–12,118 |
| lrRNA  | R   | 12,183–13,339 (1157) | ATT/TA         | 12,148–13,297 (1150) | ATT/TA            |
| trnV   | R   | TAC   | 13,340–13,402 (63)  | 13,371–13,373       | 13,298–13,359 (62) | 13,328–13,330 |
| srRNA  | R   | 13,403–14,156 (754) | ATG/TA         | 13,361–14,137 (777) | ATG/TA            |
| A+T-rich region | R | 14,157–15,438 (1282) | ATG/TA         | 14,138–14,799 (662) | ATG/TA            |

tRNAs are denoted as one-letter symbols in accordance with the IUPAC-IUB single-letter amino acid codes, except those encoding leucine and serine, which are labeled L1 for the CTN codon family, L2 for the TTR codon family, S1 for the AGN codon family, and S2 for the TCN codon family. * D, direction (F and R, forward and reverse transcriptional directions, respectively) and ** AC, anticodon.
Table 2. Characteristics of 13 mitochondrial genomes of Psyllidae species.

| Taxon | Size (bp) | A/T Content (%) | PCG | sRNA | tRNA | A+T-Rich Region |
|-------|-----------|-----------------|-----|------|------|-----------------|
|       |           |                 | No. Codons | AT (%) | Size (bp) | AT (%) | Size (bp) | AT (%) | Size (bp) | AT (%) |
| Psyllidae |           |                 |      |      |      |                  |
| Acizinae |           |                 |      |      |      |                  |
| Acizzia uncatooides | 14,957 | 72.75 | 3596 | 70.68 | 747 | 77.24 | 1145 | 75.9 | 1404 | 75.78 | 789 | 85.17 |
| Freysuila caesalpiniae | 15,327 | 75.62 | 3598 | 74.77 | 746 | 77.21 | 1146 | 77.31 | 1387 | 76.35 | 947 | 83.95 |
| Russelliana solanica | 16,047 | 72.23 | 3597 | 70.93 | 747 | 75.64 | 1147 | 76.20 | 1372 | 75.73 | 1430 | 79.16 |
| Ciriacreminae |           |                 |      |      |      |                  |
| Heteropsylla sp. | 15,284 | 73.18 | 3612 | 72.18 | 776 | 77.24 | 1150 | 76.17 | 1377 | 75.49 | 1652 | 82.04 |
| Acizzinae |           |                 |      |      |      |                  |
| Acizzia uncatoides | 14,790 | 75.73 | 3595 | 74.58 | 744 | 77.55 | 1146 | 77.84 | 1373 | 76.33 | 696 | 86.78 |
| Aphalaroidinae |           |                 |      |      |      |                  |
| Freysuila caesalpiniae | 15,327 | 75.62 | 3598 | 72.10 | 754 | 77.06 | 1157 | 76.40 | 1380 | 75.29 | 1282 | 82.20 |
| Russelliana solanica | 16,047 | 72.23 | 3597 | 72.20 | 777 | 77.48 | 1150 | 76.09 | 1376 | 75.38 | 662 | 82.93 |
| Psyllinae |           |                 |      |      |      |                  |
| Arytainilla spartiophila | 14,798 | 73.72 | 3601 | 70.44 | 773 | 78.01 | 1154 | 76.43 | 1383 | 74.69 | 695 | 79.28 |
| Cacopsylla jukyungi | 15,438 | 73.18 | 3602 | 72.34 | 755 | 77.35 | 1155 | 77.75 | 1385 | 76.75 | 660 | 83.00 |
| Cacopsylla burckhardti | 14,799 | 73.69 | 3597 | 72.20 | 777 | 77.48 | 1150 | 76.09 | 1376 | 75.38 | 662 | 82.93 |
| Cacopsylla burckhardti | 14,798 | 73.69 | 3612 | 72.18 | 776 | 77.45 | 1150 | 76.17 | 1377 | 75.33 | 662 | 83.00 |
| Cacopsylla citrisuga | 14,906 | 72.65 | 3598 | 72.10 | 754 | 77.06 | 1157 | 76.40 | 1380 | 75.29 | 1282 | 82.20 |
| Cacopsylla coccinea | 14,832 | 72.04 | 3597 | 72.20 | 777 | 77.48 | 1150 | 76.09 | 1376 | 75.38 | 662 | 82.93 |
| Cacopsylla pyri | 14,886 | 73.85 | 3597 | 72.20 | 777 | 77.48 | 1150 | 76.09 | 1376 | 75.38 | 662 | 82.93 |
| Cyamophila willieti | 14,971 | 73.66 | 3598 | 72.04 | 711 | 76.93 | 1145 | 77.73 | 1367 | 75.49 | 891 | 82.72 |
| Average | 15,142 | 73.88 | 3599 | 72.36 | 754 | 77.42 | 1152 | 76.82 | 1382 | 75.92 | 819 | 83.38 |

Table 3. Frequency of the four most common codons in Psyllidae.

| Species | Codon (No/%) | TTA (L) | ATT (I) | TTT (F) | ATA (M) | Total |
|---------|--------------|---------|---------|---------|---------|-------|
| Acizinae |              |         |         |         |         |       |
| Acizzia uncatooides | 258/7.17 | 293/8.11 | 276/7.64 | 224/6.20 | 1051/29.10 |
| Freysuila caesalpiniae | 328/9.12 | 384/10.67 | 280/7.78 | 256/7.12 | 1248/34.69 |
| Russelliana solanica | 287/7.98 | 312/8.67 | 244/6.78 | 223/6.20 | 1066/29.64 |
| Ciriacreminae |              |         |         |         |         |       |
| Heteropsylla sp. | 392/10.90 | 368/10.23 | 316/8.79 | 240/6.67 | 1316/36.59 |
| Psyllinae |              |         |         |         |         |       |
| Arytainilla spartiophila | 316/8.79 | 354/9.85 | 337/9.37 | 204/5.67 | 1211/33.69 |
| Cacopsylla jukyungi (this study) | 316/8.78 | 329/9.14 | 292/8.12 | 194/5.39 | 1131/31.43 |
| Cacopsylla burckhardti (this study) | 315/8.76 | 325/9.04 | 299/8.31 | 212/5.89 | 1151/32.00 |
| Cacopsylla burckhardti | 315/8.72 | 324/8.97 | 300/8.31 | 213/5.90 | 1152/31.89 |
| Cacopsylla citrisuga | 321/8.90 | 297/8.23 | 296/8.20 | 188/5.21 | 1120/30.54 |
| Cacopsylla coccinea | 271/7.53 | 287/7.97 | 281/7.80 | 202/5.61 | 1041/28.91 |
| Cacopsylla pyri | 311/8.65 | 326/9.06 | 295/8.20 | 208/5.78 | 1140/31.69 |
| Cyamophila willieti | 284/7.90 | 337/9.37 | 281/7.81 | 202/5.62 | 1104/30.69 |
| Psylla alni | 307/8.53 | 339/9.42 | 287/9.78 | 192/5.34 | 1125/31.27 |
| Average | 309/8.59 | 329/9.13 | 291/8.08 | 212/5.89 | 1143/31.70 |

3.2. Intergenic Spacer Sequences

The genes of C. jukyungi and C. burckhardti mitogenomes were interleaved with 84 and 53 bp intergenic spacer sequences (ISSs), which were spread over 13 and 10 regions, respectively, ranging in size from 1 to 27 bp in both species (Table 4). In contrast, genes (including the A+T-rich region) overlapped by 39 and 43 bp in 9 and 10 regions in C. jukyungi and C. burckhardti, respectively, with the size of these overlapping sequences
ranging from 1–12 bp in both species (Table 4). The majority of ISS are short (1–10 bp), with a few exceptions, and variable in their position and length. Other species of Psyllidae also present similar overlapping sequence and ISS patterns to those of the two species sequenced in this study, with some notable exceptions. *Cyamophila willieti* [26] has an exceptionally long 871 bp ISS at the *trnL–trnQ* junction, whereas other species have either no ISS, a 2–4-bp long ISS, or a 3-bp long overlap at this junction. Furthermore, *Freyolla caesalpiniae* and *R. solanicola* [30] also have longer ISS at the *trnS* and ND1 junction (273 and 469 bp, respectively; Table 4). An ISS at the *trnS* and ND1 junction is common in all species of Psyllidae except *F. caesalpiniae* and *R. solanicola*. The length of this ISS is highly conserved at 23–36 bp, with the length in the two species considered in this study both being 27 bp. Sequence alignment of this junction allowed us to detect a well-conserved stretch of pentanucleotides, CGGTA, at the *trnS* and ND1 junction of all species, regardless of ISS length (Figure 1).

**Table 4. Overlapping and intergenic spacer sequences in the mitochondrial genomes of Psyllidae species.**

| Region | A | B | C | D | E | F | G | H | I | J | K | L | M |
|--------|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *trnL–trnQ* | (I)4 | (I)4 | (I)4 | (I)4 | (I)4 | (I)4 | (I)4 | (I)871 | (I)4 | (I)3 | (I)3 | (I)3 | (I)3 |
| *trnQ–trnM* | (I)5 | (I)5 | (I)5 | (I)5 | (I)5 | (I)5 | (I)5 | (I)3 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 |
| *trnM–ND2* | (I)5 | (I)5 | (I)5 | (I)5 | (I)5 | (I)5 | (I)5 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 |
| *ND2–trnW* | (I)22 | (O)2 | (I)26 | (I)22 | (I)26 | (I)26 | (I)26 | (I)3 | (I)2 | (I)2 | (I)2 | (I)2 | (I)2 |
| *trnW–trnC* | (I)1 | (I)2 | (O)2 | (I)2 | (I)2 | (I)2 | (I)2 | (I)1 | (I)6 | (I)6 | (I)6 | (I)6 | (I)6 |
| *trnC–trnY* | (I)1 | (I)2 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 |
| *trnY–COI* | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *COI–trnL2* | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *COI–trnK* | (I)5 | (O)2 | (O)2 | (I)17 | (I)17 | (I)17 | (I)17 | (I)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 |
| *trnK–trnD* | (I)5 | (O)2 | (O)2 | (I)17 | (I)17 | (I)17 | (I)17 | (I)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 |
| *ATP6–ATP8* | (I)5 | (O)2 | (O)2 | (I)17 | (I)17 | (I)17 | (I)17 | (I)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 |
| *ND5–ND3* | (O)5 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)2 | (I)2 | (I)2 | (I)2 | (I)2 |
| *trnS–trnA* | (I)2 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 | (I)1 |
| *trnA–trnR* | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *trnR–trnN* | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *trnN–trnS1* | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 | (O)2 |
| *trnS1–trnE* | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 |
| *trnE–trnF* | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 |
| *trnF–ND5* | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 | (O)12 |
| *ND5–trnH* | (I)2 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 | (O)5 |
| *trnH–trnD* | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 | (O)3 |
| *ND4–ND1* | (I)2 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *ND1–trnL1* | (I)2 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *trnL1–trnN* | (I)2 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *trnN–trnV* | (I)2 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *trnV–srRNA* | (I)2 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *srRNA–A+T-rich region* | (I)2 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| *A+T-rich region–* | (I)2 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 | (O)1 |
| Total nucleotides | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 | (O)39 |

O, overlapping sequences; I, intergenic spacer sequences. Empty columns indicate that neighboring genes are abutting to each other. A, *Cacopsylla fukuangi* (this study); B, *Cacopsylla burchardti* (this study); C, *Cacopsylla burchardti*; D, *Cacopsylla citrisuga*; E, *Cacopsylla cocciarea*; F, *Cacopsylla pyrgaesi*; G, *Arysteina spartiophilae*; H, *Psylla abii*; I, *Cyamophila willieti*; J, *Acizziia uncataedes*; K, *Freyolla caesalpiniae*; L, *Heteropsylla sp.*; M, *Russelliana solanicola*. 
3.3. The A+T-Rich Region

The A+T-rich region of *C. jukyungi* was 1282 bp in size, which is nearly two-fold larger than that of *C. burckhardti* (662 bp; Table 2). Likewise, the size of this region is highly variable among species in Psyllidae, ranging from 355 bp (*Heteropsylla* sp.) to 1430 bp (*R. solanicola*) (Table 2). The *C. jukyungi* sequenced in this study showed exceptional structure, having the second-longest A+T-rich region in the family. It had two identical 540-bp repeat sequences surrounded by 46, 52, and 104 bp of non-repeat sequences, which do not have any homology (Figure 2A).

The longest *R. solanicola* A+T-rich region is more notable, having both a tandem repeat region and a peculiar poly-running microsatellite DNA-like region (Figure 2B). The tandem repeat region consisted of nine complete copies (49 bp) and one partial copy (28 bp), which lacks the end part. Eight of the nine copies are identical, but the fifth copy has one substitution (C → T) compared to the common copy. This repeat region is surrounded by 184 and 59 bp of non-repeat sequences. This 460-bp long repeat region is one source of longer sequences in this species, but the species additionally has a microsatellite DNA-like region (Figure 2B). This microsatellite-like region is unusual in Psyllidae, in that each copy is blended with different types of nucleotide repeats, such as CA, TA, GT, and ATA, infrequently interrupted by non-repeat sequences. This region also spans ~323 bp, making it another source of longer A+T-rich regions in this species. In other species of Psyllidae, such a microsatellite-like region also is present, but the length is much shorter than that of *R. solanicola* and is not blended with different nucleotide repeats as much as those of *R. solanicola* (data not shown).

In a comparison of the A+T-rich region, one highly conserved sequence stretch (conserved element [CE]), which spans ~33 bp, was detectable in Psyllidae, including in the two species analyzed in this study (Figure 2C). Located closer to the 5’-end of srRNA (beginning of the A+T-rich region), the CE was composed of poly-T and poly-A sequences, each of which abuts non-poly-running sequences. This CE was present in all species of Psyllidae, and was highly conserved in sequence identity and position in the family. In the case of *C. jukyungi*, which has two identical 540-bp repeat sequences, this CE also was duplicated within the repeats (Figure 2A).
Figure 2. Schematic map of the two longest A+T-rich regions detected in Psyllidae species. (A) Cacopsylla jukyungi (this study). (B) Russeliana solanicola (Percy et al., 2018). Yellow boxes, copies with substitutions. (C) An alignment of a conserved element (CE) detected in the A+T-rich region of Psyllidae. The arrows indicate the transcriptional direction of the neighboring two genes. *, consensus sequences.

3.4. Individual Gene Divergence

The genes srRNA, lrRNA, and COI, listed in order of increasing variation, provided the lowest variation of the 15 mitochondrial genes (13 PCGs and 2 rRNA genes) in the family Psyllidae (average median: 15.13–17.26%; Figure 3A). On the other hand, ATP8 provided the most variation, followed by ND6 and ND2 (average median: 36.89–27.55%). In the genus Cacopsylla, srRNA, lrRNA, and COI once again provided the lowest variation (10.86–14.86%), whereas ATP8, ND6, ND3 (=ND5), listed in order of decreasing variation, provided the highest variation (31.19–21.82%). Between two geographic samples of C. burckhardtii, only seven genes provided variation, ranging from 1–4 bp, with the first- and the second-highest variation existing in ND5 (4 bp) and COI (3 bp), respectively (Figure 3B).
Table 2), and such large variation resulted from the presence of large repeat sequences TAGTA in the hemipteran superfamily Fulgoroidea, with a slight sequence alteration in sequence divergence (%) in Psyllidae. (and minimum divergence (%), respectively; the red horizontal line represents the median of sequence divergence (%), whereas provided the highest variation (31.19–21.82 %). Between two geographic samples of 3B).

In the genus Cacopsylla Burckhardt is the second-highest variation existing in C. burckhardti, provided the most variation, followed by C. jukyungi and C. plot distribution of within-genus (Cacopsylla) and within-family (Psyllidae) genetic divergence for 13 PCGs and two rRNAs in Psyllidae. Top, middle, and bottom of bars indicate maximum, average, and minimum divergence (%), respectively; the red horizontal line represents the median of sequence divergence (%) in Psyllidae. (B) Divergence between two Cacopsylla burckhardti.

4. Discussion

4.1. Potential Motif Sequences in Intergenic Spacer Sequences

In contrast to the variability in length and position of the ISSs, one located at the trnS2 and ND1 junction is conserved in majority of Psyllidae and, more importantly, all species of Psyllidae consistently have penta-nucleotides, CGGTA at the junction (Figure 1). Considering that the evolutionary pressure on nucleotide substitution is higher in the ISS than in genic regions, an identical sequence stretch in an ISS may not be maintained in all members of Psyllidae if no functional role is granted. Previously, the ISS at the trnS2 and ND1 junction in insects was reported to contain conserved motif sequences, specifically TTAG- to determine the precise location and length of the motif.

4.2. The A+T-Rich Region Structure and Conserved Element

The size variation of the A+T-rich region among Psyllidae was substantial (355–1430 bp; Table 2), and such large variation resulted from the presence of large repeat sequences in C. burckhardti and R. solanicola (Figure 2A,B). One of the common interpretations of the occurrence of such identical repeats in animal mitogenomes includes slipped-strand
mispairing, in concert with unequal crossing over during DNA replication, resulting in an expanded repeat [51–53].

Previous studies have analyzed the A+T-rich region of some hemipteran groups, although no species in Psyllidae were considered [17,54,55]. These studies also found that hemipteran species often have a long A+T-rich region (>1500 bp in most and >2000 bp in a few species) and have tandem repeat sequences of variable copy number and length. For example, three species of Flatidae in the hemipteran infraorder Fulgoromorpha had 1702–1836 bp-long A+T-rich regions. These A+T-rich regions are commonly structured into four smaller regions, composed of one repeat region, a large non-repeat region, another repeat region, and a short non-repeat region [17]. However, copy number and length in each repeat region differed among all species, although each species commonly had four dividable regions. Therefore, the structural composition of the A+T-rich region in the Fulgoromorpha, particularly Flatidae, may require further analysis before generalizations can be made. Similarly, generalizing about the A+T-rich region in Psyllidae also is difficult in that, for the two species that have the longest A+T-rich regions (R. solanicola and C. jukyungi), these regions’ structure differs. In addition, other species in Psyllidae do not have any notable repeat elements. Thus, an extended exploration of the A+T-rich region of Psyllidae will be necessary to generalize the structural composition of this region across the family.

Due to the high variability in length and composition of the A+T-rich region in Hemiptera, detection of CE, except for simple poly-running sequences, has not been feasible [17,54,55]. However, a comparison of this region allowed us to detect a CE within the region (Figure 2C). Such a CE in the insect A+T-rich region has been searched for because it contains a stretch of sequences that are responsible for functional roles such as signaling for replication and transcription initiation [56–58]. In the insect order Lepidoptera, an ATAGA motif that is located immediately upstream of the poly-T sequence, close to the 5′-end of srRNA in the A+T-rich region, has been suggested to be the precise position of the origin of replication. The poly-T sequence has been suggested to function as a structural signal for protein recognition in the initiation of replication for minor-strand mtDNA in Bombyx mori Linnaeus (Bombycidae: Lepidoptera) [58]. Indeed, both the motif and poly-T sequences have been found to be well-conserved in diverse taxonomic groups within Lepidoptera, although the length of poly-T sequence varies [24,47,59]. Currently, no functional study of the hemipteran mitogenome that includes the CE of the A+T-rich region is available. However, the findings that the CE was located at a relatively conserved position with high sequence homology in all species of Psyllidae may suggest that the CE has a functional signal, although current understanding is too limited to make such a conclusion.

4.3. Individual Gene Divergence

The species in the genus Cacopsylla have relatively higher sequence variation even in the genes with lower variation (10.86–14.86% in srRNA, trRNA, and COI). Thus, the result of current mitogenome comparison emphasizes that pest species identification in the genus can casually be possible with any mitochondrial gene, although two other Cacopsylla species occurring in Korea (C. maculatili and C. sandolbaea) are additionally required for their mitogenome sequences for further accurate estimation of sequence divergence. Given the limited availability in mitogenome sequences for conspecifics, further data are required, but available data indicate that COI and ND5, which were not highly variable relative to other genes at both the family and genus levels, were the most variable genes at the intra-species level. These results suggest that evolutionary pressure for nucleotide substitution among mitochondrial genes differs among taxonomic levels. Thus, for population-level studies, particularly those involving C. burckhardti, COI and ND5 could be suitable markers to uncover population structures if mtDNA is considered. On the other hand, ATP8 and ND6 can be considered for phylogenetic inference up to the family level if gene segments of mtDNA are considered.
In the case of Fulgoroidea, in which 2–8 mitogenomes are available per species, a higher genetic divergence was detected in ATP6, ND5, and COI in Laodelphax striatellus; ND5, lrRNA, and COIII in Lycorma delicatula; ND2, ND4L, and ATP6 in Nilaparvata lugens; and ND4, ND2, and srRNA in Sogatella furcifera [17]. This result differs from that of the C. burckhardti mitogenome, for which the three most variable genes were ND5, COI, and COIII, although these genes appeared at least once as highly variable genes in L. striatellus and L. delicatula. These results further reinforce the importance of understanding variability among mitochondrial genes of target species to draw meaningful results in the study of intraspecific variation. One of the utilities of mitogenome sequences is their role as molecular markers for intraspecific variation. These markers are involved in diagnostics (e.g., origins of inset-driven foods), history tracing (e.g., origins of invasive populations, genetically compatible populations for restoration), and population dynamics (e.g., genetic diversity and gene flow), in which one or more portions of the mitochondrial genic regions are used [17,18,22–24,60,61]. In such studies, COI is often used to trace population history and dynamics as well as conduct species identification using DNA barcoding [21,62]. Nevertheless, this gene alone infrequently provided low variability and low numbers of haplotypes within species, particularly in introduced species [24,63–67]. In such cases, understanding the variability among mitochondrial genes may provide the knowledge needed to select the proper genes for the study of population structure and diversity.

5. Conclusions

The addition of two mitogenome sequences to the Psyllidae family that contains notorious pear pests will enhance our understanding of the mitogenome characteristics of the family, particularly by comparative approach, which we employed firstly for the family in this study. The Psyllidae including the two mitogenomes sequenced in this study have several features shared with other members (e.g., codon usage, arrangement, presence of motif sequences at the trnS2 and ND1 junction, and presence of a conserved element in the A+T-rich region). However, the family Psyllidae, in detail, has divergent characteristics compared to non-Psyllidae members: conserved CGGTA at the trnS2 and ND1 junction, a conserved element in the A+T-rich region, and different groups of genes with higher or lower intra-specific variation. Furthermore, the C. jukyungi sequenced in this study is unique in that the A+T-rich region has two identical 540-bp repeat sequences surrounded by non-repeat sequences, whereas the majority of other species in Psyllidae do not have repeat sequences. Our data suggest that sequencing of C. jukyungi and C. burckhardti mitogenomes and comparing their genomic characteristics to the family Psyllidae offers a promising avenue for pursuing further mitogenome sequences of the family members and extended taxonomic groups for a better understanding of the evolution of mitogenome characteristics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092037/s1, Figure S1: Predicted secondary cloverleaf structures for the tRNA genes of Cacopsylla jukyungi; Figure S2: Predicted secondary cloverleaf structures for the tRNA genes of Cacopsylla burckhardti.

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References

1. Ossiannilsson, F. The Psylloidea (Homoptera) of Fennoscandia and Denmark. In Fauna Entomologica Scandinaevia; Brill: Leiden, The Netherlands, 1992; Volume 26, p. 346.

2. Luo, X.; Li, F.; Ma, Y.; Cai, W. A revision of Chinese pear psyllids (Hemiptera: Psylloidea) associated with Pyrus ussuriensis. Zootaxa 2012, 3489, 58–80. [CrossRef]

3. Kwon, Y.J. Psylloidea of Korea (Homoptera: Sternorrhyncha); Editorial Committee of Insecta Koreana: Seoul, Korea, 1983; p. 181.

4. Li, F. Psyllidomorpha of China (Insecta: Hemiptera). Science Press: Beijing, China, 2011; p. 1976.

5. Park, H.C.; Lee, C.E. A new species of Psylla from Korea (Homoptera: Psylloidea). Nat. Life 1982, 12, 19–21.

6. Cho, G.; Burckhardt, D.; Inoue, H.; Luo, X.; Lee, S. Systematics of the east Palaearctic pear psyllids (Hemiptera: Psylloidea) with particular focus on the Japanese and Korean fauna. Zootaxa 2017, 4362, 75–98. [CrossRef]

7. Foerster, A. Uebersicht der Gattungen und Arten in der Familie der Psylliden. Verhandlungen des Naturhistorischen Vereins der Niederlande, 1848, 5, 65–98.

8. KREI. Agricultural Outlook Korea, 2021; Volume 2, p. 736–742.

9. Kim, D.S.; Yang, C.Y.; Jeon, H.Y. An empirical model for the prediction of the onset of upward-movement of overwintered Cacopsylla pyricola (Homoptera: Psylloidea) in pear orchards. Korean J. Agric. For. Meteorol. 2007, 9, 228–233. [CrossRef]

10. Park, J.S.; Park, J.W.; Kang, A.R.; Lee, S.H.; Yang, K.-Y.; Kim, W.S.; Kim, I. Analysis of occurrence pattern of the pear psylla, Cacopsylla pyricola, in the pear exporting complex. J. Agri. Sci. Technol. 2014, 48, 1–8. [CrossRef]

11. An, J.H.; Yiem, M.S.; Kim, D.S. Effects of photoperiod and temperature on formation and fecundity of two seasonal forms of Psylla pyricola (Homoptera: Psylloidea). Korean J. Appl. Entomol. 1996, 35, 205–208.

12. Kim, D.S.; Jeon, H.Y.; Yiem, M.S.; Cho, M.R.; Kim, S.B. Ecological studies on the pear psylla. In Annual Report of NHRI; No. 31235–51850–56–2; RDA: Suwon, Korea, 1995; pp. 736–742.

13. Burts, E.C.; van den Baan, H.E.; Croft, B.A. Pyrethroid resistance in pear psylla, Psylla pyricola Forster (Homoptera: Psylloidea), and synergism of pyrethroids with piperonyl butoxide. Can. Entomol. 1989, 121, 219–223. [CrossRef]

14. Lee, D.C.; Lee, H.J.; Jung, S.T.; Han, K.P.; Han, H.Y. Special Treatise on Fruit Crops and Horticulture; Hyangmunsa: Seoul, Korea, 1983.

15. Cameron, S.L. Insect ecotaxonomic genomics: Implications for evolution and phylogeny. Annu. Rev. Entomol. 2014, 59, 95–117. [CrossRef]

16. Kim, M.J.; Kim, I.; Cameron, S.L. How well do multispecies coalescent methods perform with mitochondrial genomic data? A case study of butterflies and moths (Insecta: Lepidoptera). Syst. Entomol. 2020, 45, 857–873. [CrossRef]

17. Kim, M.J.; Lee, K.H.; Park, J.S.; Jeong, J.S.; Jeong, N.R.; Lee, W.; Kim, I. Complete mitochondrial genomes of Metcalfa pruinosa and Salurnis marginella (Hemiptera: Flatidae): Genomic comparison and phylogenetic inference in Fulgoroidea. Curr. Issues Mol. Biol. 2021, 43, 1391–1418. [CrossRef] [PubMed]

18. Kim, M.J.; Park, J.S.; Kim, H.; Kim, S.-R.; Kim, S.-W.; Kim, K.-Y.; Kwak, W.; Kim, I. Phylogeographic relationships among Bombyx mandarina (Lepidoptera: Bombycidae) populations and their relationships to B. mori inferred from mitochondrial genomes. Biology 2022, 11, 68. [CrossRef] [PubMed]

19. Tiwari, J.K.; Mandadi, N.; Sridhar, J.; Mandal, V.; Ghosh, A.; Kardile, H.B.; Naga, K.C.; Shah, M.A.; Rawat, S.; Venkateswarlu, V.; et al. Draft genome sequencing of the foxglove aphid (Aulacorthum solani Kaltenbach), a vector of potato viruses, provides insights on virulence genes. J. Asia-Pac. Entomol. 2021, 24, 93–102. [CrossRef]

20. Kim, M.J.; Kim, S.-S.; Choi, S.-W.; Kim, I. Saturnia jonasii Butler, 1877 on Jejudo Island, a new saturnid moth of South Korea with DNA data and morphology (Lepidoptera: Saturniidae). Zootaxa 2015, 3946, 374–386. [CrossRef] [PubMed]

21. Kim, M.J.; Cho, Y.; Wang, A.R.; Kim, S.-S.; Choi, S.-W.; Kim, I. Population genetic characterization of the black-veined white, Aporia crataegi (Lepidoptera: Pieridae), using novel microsatellite markers and mitochondrial DNA gene sequences. Conserv. Genet. 2020, 21, 359–371. [CrossRef]

22. Lee, K.H.; Jeong, J.S.; Park, J.S.; Kim, M.J.; Jeong, N.R.; Jeong, S.Y.; Lee, G.S.; Lee, W.; Kim, I. Tracing the invasion and expansion characteristics of the flatid planthopper, Metcalfa pruinosa (Hemiptera: Flatidae), in Korea using mitochondrial DNA sequences. Insects 2021, 12, 4. [CrossRef]

23. Jeong, N.R.; Kim, M.J.; Kim, S.-S.; Choi, S.-W.; Kim, I. Morphological, ecological, and molecular divergence of Conogethes pinicolalis from C. punctiferalis (Lepidoptera: Crambidae). Insects 2021, 12, 455.
24. Jeong, N.R.; Kim, M.J.; Park, J.S.; Jeong, S.Y.; Kim, I. Complete mitochondrial genomes of Conogethes punctiferalis and C. pincolalis (Lepidoptera: Crambidae): Genomic comparison and phylogenetic inference in Pyrauloidea. *J. Asia Pac. Entomol.* 2021, 35, 1179–1186. [CrossRef]

25. Wang, Y.; Cen, Y.; He, Y.; Wu, Y.; Huang, S.; Lu, J. The first complete mitochondrial genome sequence of Cacopsylla citrisuga (Yang & Li), a new insect vector of Huanglongbing in Yunnan Province, China. *Mitochondrial DNA B Resour.* 2021, 6, 575–577.

26. Song, X.; He, Y.; Wang, X.; Gu, X. The complete mitochondrial genome of Cymophila willietti (Wu) (Hemiptera: Psyllidae). *Mitochondrial DNA B Resour.* 2019, 4, 3758–3759. [CrossRef]

27. Jo, E.; Cho, G. The complete mitochondrial genome of *Cacopsylla burchardi* (Hemiptera, Psyllioidea, Psyllidae). *Biodivers. Data J.* 2022, 10, e85094. [CrossRef]

28. Que, S.; Yu, L.; Xin, T.; Zou, Z.; Hu, L.; Xia, B. Complete mitochondrial genome of *Cacopsylla coccinea* (Hemiptera: Psyllidae). *Mitochondrial DNA A DNA Mapp. Seq. Anal.* 2015, 27, 3169–3170. [CrossRef]

29. Percy, D.M.; Crampton-Platt, A.; Sveinsson, S.; Lemmon, A.R.; Lemmon, E.M.; Ouvrard, D.; Burchhardt, D. Resolving the psyllid tree of life: Phylogenomic analyses of the superfamily Psylloidea (Hemiptera). *Syst. Entomol.* 2018, 43, 762–776. [CrossRef]

30. Shea, K.; Thrall, P.H.; Burdon, J.J. An integrated approach to management in epidemiology and pest control. *Ecol. Lett.* 2000, 3, 150–158. [CrossRef]

31. Kang, A.R.; Baek, J.Y.; Lee, S.H.; Cho, Y.S.; Kim, W.S.; Han, Y.S.; Kim, I. Geographic homogeneity and high gene flow of the pear psylla, *Cacopsylla pyricola* (Hemiptera: Psyllidae), detected by mitochondrial COI gene and nuclear ribosomal internal transcribed spacer 2. *Anim. Cells. Syst.* 2012, 16, 145–153. [CrossRef]

32. Park, J.W.; Park, J.S.; Kang, A.R.; Na, I.S.; Cha, G.H.; Oh, H.J.; Lee, S.H.; Yang, K.Y.; Kim, W.S.; Kim, I. Establishment of pest forecasting management system for the improvement of pass ratio of Korean exporting pears. *Int. J. Indust. Entomol.* 2012, 25, 163–169. [CrossRef]

33. Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Marine Biol. Biotechnol.* 1994, 3, 294–299.

34. Andrews, S. FastQC: A Quality Control Tool for High Throughput Sequence Data. 2010. Available online: http://www.bioinformatics.babraham.ac.uk/?/projects/fastqc/ (accessed on 6 June 2022).

35. Jiang, H.; Lei, R.; Ding, S.-W.; Zhu, S. Skewer: A fast and accurate adapter trimmer for next-generation sequencing paired-end reads. *BMC Bioinform.* 2014, 15, 182. [CrossRef]

36. Allam, A.; Kalnis, P.; Solovyev, V. Karect: Accurate correction of substitution, insertion and deletion errors for next-generation sequencing data. *Bioinformatics* 2015, 31, 3421–3428. [CrossRef]

37. Hahn, C.; Bachmann, L.; Chevreux, B. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. *Nucleic Acids Res.* 2013, 41, e129. [CrossRef]

38. Langmead, B.; Salzberg, S.L. Fast Gapped-Read Alignment with Bowtie 2. *Nat. Methods.* 2012, 9, 357–359. [CrossRef] [PubMed]

39. De Pristo, M.A.; Banks, E.; Poplin, R.; Gamirrella, K.V.; Maguire, J.R.; Hartl, C.; Philippakis, A.; Del Angel, G.; Rivas, M.A.; Hanna, M.; et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat. Genet.* 2011, 43, 491–498. [CrossRef] [PubMed]

40. Bernt, M.; Donath, A.; Donath, A.; Externbrink, F.; Florentz, C.; Fritzsch, G.; Pütz, J.; Middendorf, M.; Stadler, P.F. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mitochondrial DNA A DNA Mapp. Seq. Anal.* 2015, 31, 3421–3428. [CrossRef]

41. Cameron, S.L. How to sequence and annotate insect mitochondrial genomes for systematic and comparative genomics research. *Syst. Entomol.* 2014, 39, 400–411. [CrossRef]

42. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis ver. 6.0. *Mol. Biol. Evol.* 2013, 30, 2725–2729. [CrossRef]

43. Swofford, D.L. PAUP*: Phylogenetic Analysis Using Parsimony (and Other Method) Version 4.0 Beta; Sinauer Associates: Sunderland, MA, USA, 2002.

44. Boore, J.L. Animal mitochondrial genomes. *Nucleic Acids Res.* 1999, 27, 1767–1780. [CrossRef]

45. Wolstenholme, D.R. Animal mitochondrial DNA: Structure and evolution. *Int. Rev. Cytol.* 1992, 141, 173–216.

46. Kim, M.I.; Baek, J.Y.; Kim, M.J.; Jeong, H.C.; Kim, K.-G.; Bae, C.H.; Han, Y.S.; Jin, B.R.; Kim, I. Complete nucleotide sequence and organization of the mitogenome of the red-spotted aphid butterfly, *Parnassius bremeri* (Lepidoptera: Papilionidae) and comparison with other lepidopteran insects. *Mol. Cells* 2009, 29, 347–363. [CrossRef]

47. Kim, J.S.; Kim, M.J.; Jeong, J.S.; Kim, I. Complete mitochondrial genome of *Saturnia jonasii* (Lepidoptera: Saturniidae): Genomic comparisons and phylogenetic inference among Bombycoidea. *Genomics* 2018, 110, 174–282. [CrossRef]

48. Jeong, J.S.; Kim, M.J.; Kim, I. The mitochondrial genome of the dung beetle, *Copris tripartitus*, with mitogenomic comparisons within Scarabaeidae (Coleoptera). *Int. J. Biol. Macromol.* 2020, 144, 874–891. [CrossRef]

49. Taanman, J.W. The mitochondrial genome: Structure, transcription, translation and replication. *Biochim. Biophys. Acta–Bioenerg.* 1999, 1410, 103–123. [CrossRef]

50. Cameron, S.L.; Whiting, M.F. The complete mitochondrial genome of the tobacco hornworm, *Manduca sexta*, (Insecta: Lepidoptera: Sphingidae) and an examination of mitochondrial gene variability within butterflies and moths. *Gene* 2008, 408, 112–123. [CrossRef] [PubMed]

51. Moriz, C.; Brown, W.M. Tandem duplications of D-loop and ribosomal RNA sequences in lizard mitochondrial DNA. *Science* 1986, 233, 1425–1427. [CrossRef] [PubMed]
52. Moriz, C.; Brown, W.M. Tandem duplication in animal mitochondrial DNAs: Variation in incidence and gene content among lizards. *Proc. Natl. Acad. Sci. USA* 1987, 84, 7183–7187. [CrossRef] [PubMed]

53. Levinson, G.; Gutman, G.A. Slipped-strand mispairing: A major mechanism for DNA sequence evolution. *Mol. Biol. Evol.* 1987, 4, 203–221.

54. Wang, Y.; Chen, J.; Jiang, L.Y.; Qiao, G.X. Hemipteran mitochondrial genomes: Features, structures and implications for phylogeny. *Int. J. Mol. Sci.* 2015, 16, 12382–12404. [CrossRef] [PubMed]

55. Li, K.; Liang, A.-P. Hemiptera mitochondrial control region: New sights into the structural organization, phylogenetic utility, and roles of tandem repetitions of the noncoding segment. *Int. J. Mol. Sci.* 2018, 19, 1292. [CrossRef] [PubMed]

56. Fauron, C.M.; Wolstenholme, D.R. Intraspecific diversity of nucleotide sequences within the adenine+ thymine-rich region of mitochondrial DNA molecules of *Drosophila mauritiana*, *Drosophila melanogaster* and *Drosophila simulans*. *Nucleic Acids Res.* 1980, 8, 5391–5410. [CrossRef]

57. Clary, D.O.; Wolstenholme, D.R. *Drosophila* mitochondrial DNA: Conserved sequences in the A + T-rich region and supporting evidence for a secondary structure model of the small ribosomal RNA. *J. Mol. Evol.* 1987, 25, 116–125. [CrossRef]

62. Choi, D.S.; Park, J.S.; Kim, M.J.; Jeong, S.Y.; Jeong, J.S.; Park, J.; Kim, I. Geographic variation in the spotted-wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae), based on mitochondrial DNA sequences. *Mitochondrial DNA A DNA Mapp. Seq. Anal.* 2018, 29, 312–322. [CrossRef]

63. Wang, A.R.; Kim, M.J.; Cho, Y.B.; Wan, X.; Kim, I. Geographic genetic contour of a ground beetle, *Scarites aterrimus* (Coleoptera: Carabidae) on the basis of mitochondrial DNA Sequence. *Int. J. Indust. Entomol.* 2011, 22, 65–74. [CrossRef]

64. Lee, J.Y.; Wang, A.R.; Choi, Y.S.; Thapa, R.; Kwon, H.W.; Kim, I. Mitochondrial DNA variations in Korean *Apis cerana* (Hymenoptera: Apidae) and development of another potential marker. *Apidologie* 2016, 47, 123–134. [CrossRef]

65. Arca, M.; Mougel, F.; Guillemaud, T.; Dupas, S.; Rome, Q.; Ferrard, A.; Muller, F.; Fossoud, A.; Capdevielle-Dulac, C.; Torres-Leguizamon, M.; et al. Reconstructing the invasion and the demographic history of the yellow-legged hornet, *Vespa velutina*, in Europe. *Biol. Invas.* 2015, 17, 2357–2371. [CrossRef]

66. Takeuchi, T.; Takahashi, R.; Kiyoshi, T.; Nakamura, M.; Minoshima, Y.N.; Takahashi, J. The origin and genetic diversity of the invasive species *Mecalba pruinose* (Hemiptera: Flatidae) in the Republic of Korea. *J. Econ. Entomol.* 2016, 109, 1897–1906. [CrossRef]