diversity

MDPI

Article

Genome Skimming of Thysanoptera (Arthropoda, Insecta) and Its Taxonomic and Systematic Applications

Emma Wahlberg

Department of Zoology, Swedish Museum of Natural History, P.O. Box 50007, 104 05 Stockholm, Sweden; emma.wahlberg@nrm.se

Abstract: High-throughput sequencing has transformed molecular systematics. This study presents a semi-automated pipeline for genome skimming in Thysanoptera, an insect order known for challenging species identification and cryptic relationships. By efficiently obtaining mitochondrial genomes and nuclear genes from multiple thrips specimens, the study evaluates the limitations of traditional barcoding and the data required for accurate species delimitation. The results highlight the importance of the sequencing data volume and this pipeline in reconstructing Thysanoptera phylogeny. This research also showcases the potential of advanced sequencing techniques for species delimitation and phylogenetics.

Keywords: Thysanoptera; high-throughput sequencing; species delimitation; phylogeny; mitochondrial genome; genome skimming

1. Introduction

Thrips (Thysanoptera Haliday, 1836) are generally very small insects, often not larger than 3 mm, and are found in various habitats and environments. Some are found in flowers or seed pods, others on bark or in soil and deadwood. Feeding is also variable, ranging from pollen and spores to fungi and predation. Compared to other insect orders, there are relatively fewer taxonomic and systematic studies of thrips; their small size and secluded lifestyles could be taxonomic obstacles as they often require laboursome collection methods and slide preparation. But some thrips taxa have been well studied because of their impact as pests on both commercial and private agriculture [1]. Additionally, some species have been noted as being potentially invasive [2].

DNA barcodes, most often utilizing ca 600 bp of the mitochondrial gene COI/COX1, have been utilized for a range of applications, including species delimitation, identification, and population studies [3,4]. In some cases, genetic identification can improve taxon discovery compared to traditional morphology-based identification, especially when carried out by non-experts [5]. Genetic species identification could also alleviate the problems of working with very small insects, such as thrips. Finally, the metabarcoding of environmental DNA can also provide a means of identifying species which are challenging to collect. The molecular identification of thrips has not been utilized extensively, but preliminary studies have been carried out of potential pest species [6]. Marquina et al. [7], however, have in silico inferred the barcoding gene COI as having a very low taxonomic resolution within Thysanoptera, with roughly half of the species detected.

Phylogenetic approaches to species delimitation using molecular data have been developed as a complement to traditional sequence reference-based species identification [8]. One large advantage of the former is that there is no need for a curated reference database to assign specimens to taxonomic units. Phylogenetic delimitation can be used as a first

Academic Editor: Luc Legal

Received: 4 March 2025
Revised: 19 March 2025
Accepted: 23 March 2025
Published: 24 March 2025

Citation: Wahlberg, E. Genome Skimming of Thysanoptera (Arthropoda, Insecta) and Its Taxonomic and Systematic Applications. *Diversity* **2025**, *17*, 226. https://doi.org/10.3390/d17040226

step in delineating species, including cryptic ones, from a specimen pool or to test previous species hypotheses. Phylogenetic species delimitation in thrips has not been widely explored, but a recent study has shown a conflict between molecular and morphologically defined species [9]. More generally, the amount of data, i.e., number of nucleotides, can, however, have a large effect on molecular species delimitation [10,11].

Systematic phylogenetic research into thrips has mainly focused on the deep relationships within the order as a whole [12,13]. The mitogenome has been examined in several studies, with Tyagi et al. [14] suggesting that extensive sampling is needed to investigate the genomic evolution in thrips. An investigation of mitochondrial genes could help to find suitable regions for species-level molecular identification. The use of mitochondrial genomes has increased due to the improved sequencing technology and lower costs. Mitochondrial genomes, in whole or part, have been shown to increase the resolution and support in phylogenies compared to a few individual Sanger-sequenced genes [15]. There are generally 37 genes in the insect mitochondrial genome, with a total length of 14–19 kilobases. Even though the number of genes is conserved, their arrangement within the genome varies. Within Thysanoptera, this is to a relatively high degree with genome synapomorphies identified for different groups [14]. This provides additional evidence for the higher-level relationships. Due to the rapid evolution of mitochondrial genes and the ease of sequencing even degraded specimens, it has shown to be a valuable source of data in species delimitations, population and biogeographical studies, and phylogenetic analyses.

Genome skimming is a method to acquire gene fragments through low-coverage shotgun sequencing of whole genomes [16], and it can be utilized to generate molecular data from fragmented genomes or small amounts of DNA. The sequence can be assembled into whole mitochondrial genomes using iterative assembly without the need for a complete reference genome [17], providing a fast pipeline for acquiring large amounts of data even from degraded material. The method has proven successful for the reconstruction molecular phylogenies for several different organism groups (e.g., [16,18,19]). High-throughput sequencing (HTS) can generate enormous amounts of data that can be time consuming and costly to process, and genome skimming can streamline this process by searching for selected regions. This is especially useful for acquiring individual nuclear genes from unassembled genome sequencing data. In this study, genome skimming is explored for specimens of thrips and the application for species delimitation and phylogenetic reconstruction of this insect order. A non-destructive lysis protocol is used together with efficient automated robot extraction. Furthermore, the amount of data required to successfully delimit species is examined, from short fragments to multiple loci.

2. Material and Methods

2.1. Sampling, DNA Extraction, and COI Amplification

The collection of material was carried out in Sweden during the period 2021–2022, with a focus on species in the family Phlaeothripidae Uzel, 1895. Additional species were included from the families Thripidae Stephens, 1829 and Aeolothripidae Uzel, 1895 to extend the sampling. Specimens were killed and stored in the field in 80% ethanol. If possible, several specimens from each species were included to facilitate the species delimitation process [20]. Extraction was carried out using a non-destructive process that preserves a voucher specimen for morphological studies and museum deposition [21]. Species identification was carried out using morphological methods and keys [22–26]. The DNA extraction was carried out using Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (Omega Bio-Tek, Norcross, GA, USA) with a Flex 96 extraction robot following the manufacturer's tissue protocol, with the modification of an additional 15 µL of proteinase K added into each sample after overnight lysis and DNA eluted in 100 µL of elution buffer

to yield higher concentrations of DNA. For the sequencing of the DNA-barcoding region, the primers LCO1490 and HCO2198 [3] were used. The PCR reaction was carried out with Ready-To-Go PCR beads (Amersham Biosciences, Amersham, UK), with 21 µL of H_2O, 1 µL of primer, and 2 µL of DNA-extract. The PCR reaction was set up with 95 °C (5 m), 40 cycles of 95 °C (30 s), 50 °C (30 s), 72 °C (50 s), followed by 72 °C (8 m).

2.2. Library Preparation and Sequencing

All extractions were fragmented on a Covaris sonicator at SciLife Lab (Solna, Sweden) to a target fragment length of 500 bp. Libraries for each sample were constructed following a modified version of the Meyer and Kircher [27] protocol for Illumina sequencing, using magnetic AMPure beads for cleaning steps [28]. Adapters used were IS1_adapter_P5.F, IS2_adapter_P7.F, and IS3_ adapter_P5+P7.R. Libraries were amplified with dual index primers. In order to assess how many PCR cycles are needed for each library, PCR reactions with 5 µL of DNA library were run for 12 cycles and visualized on a gel. Samples with weak bands needed either more input DNA (8 µL) or more PCR cycles. In total, 6 reactions per DNA library were performed. The fragment distribution of each library was measured using BioAnalyser, ensuring a 500 bp target fragment size. Concentrations were measured using Qubit and pooled accordingly. Sequencing was carried out on one Illumina NovaSeq6000 S4 lane, 2 × 150 bp reads, at SciLife Lab (Solna, Sweden). The Sanger sequencing of barcode regions was performed by Macrogen Inc. (Amsterdam, The Netherlands).

2.3. Bioinformatics and Alignments

The complete bioinformatic pipeline is illustrated in Figure 1, and the workflow was carried out on the Galaxy web platform at usegalaxy.eu [29] and is publicly available at https://usegalaxy.eu/u/emmawahl/w/mitochondrial-assembly-of-thysanoptera (accessed on 1 February 2025). Mitochondrial genome was retrieved and assembled using NOVOPlasty 4.3.1 [30], from untrimmed reads as recommended by the software instructions. The Sanger-sequenced barcode was used as an assembly seed for each species. The read length was set to 150 bp, the insert size was set to 350, and the overlap was set to 25. The mitochondrial genome was annotated using MITOS2 2.0.6 [31], with genetic code 5, RefSeq63 Metazoa reference data, final overlap at 50, fragment overlap at 20, and fragment quality factor 10. The other settings were left at default. Two nuclear genes were selected for gene fishing, 18S and 28S. These genes have been used in previous phylogenetic studies, and reference data are readily available in NCBI GenBank [32]. The reads were filtered and trimmed using Trimmomatic 0.38.1 [33], with standard settings. The reads were thereafter mapped to a reference sequence using Bowtie2 2.5.0 [34]. The reference sequences were retrieved from NCBI GenBank from as closely related species as possible. The resulting sequences were converted, the variant was called and normalized using mpileup, call, and norm from bcftools 1.15.1 [35], and finally a consensus was created with a consensus from the same package. The bcftools used default settings. The mapping depth was calculated using the Samtools depth 1.15.1 [35]. The bait sequences for NOVOPlasty and reference sequences for nuclear genes are shown in Table 1. Alignments of all genes were carried out using MAFFT 7.017 with an auto algorithm, scoring matrix of 2000 PAM/k = 2, gap open penalty of 1.53, and with the automatic determination of sequences' direction setting on. Alignments were refined with BMGE 1.12 [36], with the sequence coding set to auto, the name of PAM matrix of 250, a sliding window size of 3, maximum entropy threshold of 0.5, gap rate cut-off of 0.5, and minimum block size of 5. The Sanger-generated barcode sequences were inspected and assembled in Geneious 8 [37] and uploaded to the Barcode of Life Data System (BOLD) [38]. BOLD ID:s are presented in Table 1.

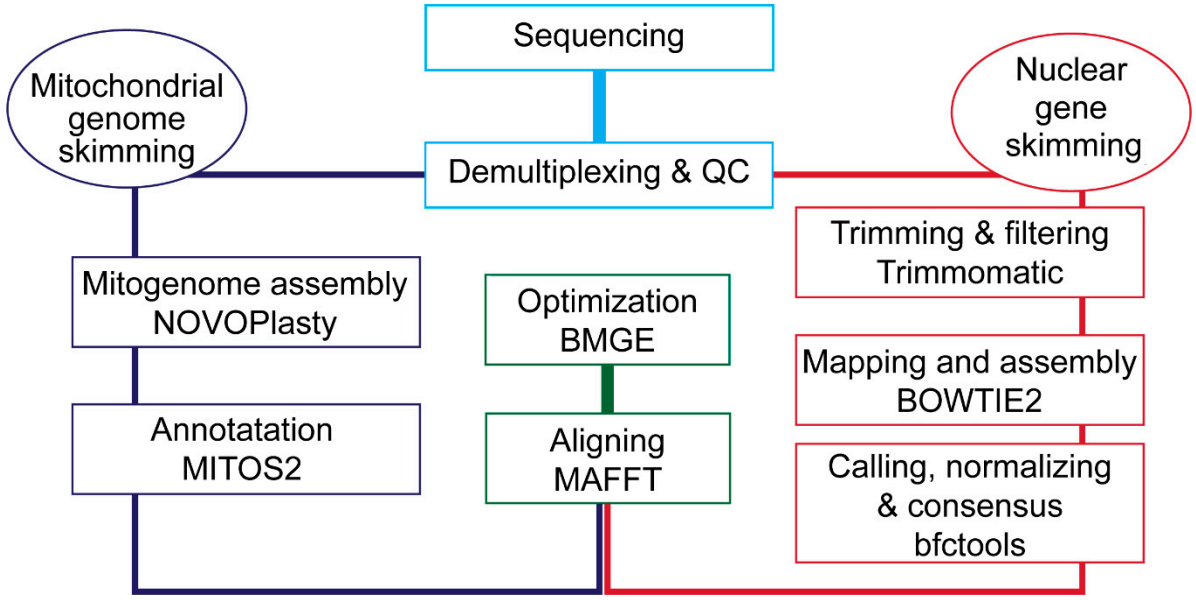

Figure 1. Bioinformatic pipeline for genome skimming, up until downstream analyses.

Table 1. Results from genome skimming, with number of sequencing reads in millions and quality. Families are abbreviated as Ae. = Aeolothripidae, Ph. = Phlaeothripidae, and Th. = Thripidae. Seed sequences are BOLD ID:s for sequences used as bait in NOVOPlasty, and 28S and 18S references are reference sequences from NCBI GenBank used in Bowtie2 mapping. Inc. under MITOS2 represents that mitogenome assemblies were not all genes that were recoverable. A minus sign represents failed recovery, assembly, or mapping.

Code	Species	Mreads	>=Q30(%)	Family	Sex	Seed Sequence	28S Reference	18S Reference	NOVOPlasty	MITOS2	28S	18S
AW7T	*Rhipidothrips brunneus*	53.81	90.90	Ae.	Female	SETHY150-22	KC513027	KC512922	OK	Inc.	OK	OK
AW6T	*Rhipidothrips brunneus*	44.62	91.70	Ae.	Female	SETHY149-22	KC513027	KC512922	OK	Inc.	OK	OK
AT9T	*Acanthothrips nodicornis*	63.01	90.79	Ph.	Female	SETHY125-22	KC5131161	KC513016	OK	Inc.	OK	OK
AU1T	*Acanthothrips nodicornis*	96.05	91.61	Ph.	Male	SETHY126-22	KC5131161	KC513016	-	-	OK	OK
AT8T	*Acanthothrips nodicornis*	61.06	90.87	Ph.	Female	SETHY124-22	KC5131161	KC513016	OK	Inc.	OK	OK
AQ1T	*Bolothrips dentipes*	377.04	88.94	Ph.	Male	BIOUG13498-F12	KC513078	KC512977	OK	Inc.	OK	OK
AW3T	*Cephalothrips monilicornis*	56.83	91.57	Ph.	Female	SETHY146-22	KC513067	KC512966	OK	Inc.	OK	OK
AW4T	*Cephalothrips monilicornis*	50.83	91.65	Ph.	Female	SETHY147-22	KC513067	KC512966	OK	Inc.	OK	OK
AW5T	*Cephalothrips monilicornis*	51.00	91.98	Ph.	Female	SETHY148-22	KC513073	KC512973	OK	Inc.	OK	OK
AY3T	*Cephalothrips monilicornis*	18.29	91.52	Ph.	Female	SETHY161-22	KC513067	KC512966	OK	Inc.	OK	OK
AV1T	*Cephalothrips monilicornis*	52.28	92.08	Ph.	Female	SETHY135-22	KC513067	KC512966	-	-	OK	OK
AS7T	*Cephalothrips monilicornis*	103.85	89.33	Ph.	Female	SETHY114-22	KC513073	KC512973	OK	Inc.	OK	Ok
AY4T	*Haplothrips hukkineni*	14.25	92.53	Ph.	Female	SETHY162-22	KC513067	KC512966	OK	Inc.	Ok	OK
AV5T	*Haplothrips leucanthemi*	14.85	93.22	Ph.	Female	SETHY139-22	KC513067	KC512966	OK	OK	Ok	OK
AR5T	*Haplothrips leucanthemi*	71.80	90.41	Ph.	Male	SETHY107-22	KC513067	KC512966	-	-	OK	OK
AR6T	*Haplothrips leucanthemi*	108.21	90.21	Ph.	Male	SETHY108-22	KC513067	KC512966	-	-	OK	OK
AR7T	*Haplothrips leucanthemi*	187.55	90.37	Ph.	Female	SETHY109-22	KC513067	KC512966	-	-	OK	OK
AT7T	*Haplothrips leucanthemi*	108.15	89.09	Ph.	Female	SETHY123-22	KC513067	KC512966	-	-	OK	OK
AV4T	*Haplothrips leucanthemi*	59.96	92.55	Ph.	Female	SETHY138-22	KC513067	KC512966	OK	OK	OK	OK

Table 1. *Cont.*

Code	Species	Mreads	>=Q30(%)	Family	Sex	Seed Sequence	28S Reference	18S Reference	NOVOPlasty	MITOS2	28S	18S
AY1T	*Haplothrips statices*	7.38	91.92	Ph.	Female	SETHY159-22	KC513067	KC512966	OK	Inc.	Ok	OK
AY2T	*Haplothrips statices*	10.71	92.22	Ph.	Female	SETHY160-22	KC513067	KC512966	OK	Inc.	OK	OK
AT1T	*Haplothrips statices*	107.64	89.43	Ph.	Female	SETHY117-22	KC513067	KC512966	-	-	OK	OK
AT2T	*Haplothrips statices*	120.22	89.71	Ph.	Female	SETHY118-22	KC513067	KC512966	OK	Inc.	OK	OK
AU5T	*Haplothrips subtilissimus*	20.25	92.35	Ph.	Female	SETHY130-22	KC513067	KC512966	OK	Inc.	OK	OK
AU7T	*Haplothrips subtilissimus*	36.14	92.56	Ph.	Female	SETHY132-22	KC513067	KC512966	OK	Inc.	OK	OK
AW2T	*Haplothrips subtilissimus*	56.51	92.16	Ph.	Female	SETHY145-22	KC513067	KC512966	OK	Inc.	OK	OK
AX2T	*Haplothrips subtilissimus*	45.36	91.92	Ph.	Female	SETHY153-22	KC513067	KC512966	OK	Inc.	Ok	OK
AX3T	*Haplothrips subtilissimus*	53.14	91.28	Ph.	Female	SETHY154-22	KC513067	KC512966	OK	Inc.	OK	OK
AX4T	*Haplothrips subtilissimus*	46.23	91.67	Ph.	Male	SETHY155-22	KC513067	KC512966	Ok	Inc.	OK	OK
AU6T	*Haplothrips subtilissimus*	18.35	92.70	Ph.	Female	SETHY131-22	KC513067	KC512966	OK	Inc.	OK	-
AW8T	*Haplothrips subtilissimus*	59.33	91.47	Ph.	Female	SETHY151-22	KC513067	KC512966	-	-	OK	Ok
AU3T	*Haplothrips subtilissimus*	50.24	92.55	Ph.	Male	SETHY128-22	KC513067	KC512966	Ok	Inc.	OK	OK
AP8T	*Haplothrips utae*	184.89	88.84	Ph.	Male	SETHY094-22	KC513067	KC512966	OK	OK	OK	OK
AY5T	*Haplothrips utae*	27.51	91.54	Ph.	Female	SETHY163-22	KC513067	KC512966	-	-	OK	OK
AP6T	*Haplothrips utae*	139.02	88.30	Ph.	Female	SETHY092-22	KC513067	KC512966	OK	OK	OK	OK
AU2T	*Hoplothrips corticis*	79.79	92.79	Ph.	Female	SETHY127-22	KC513073	KC512973	-	-	OK	OK
AW9T	*Hoplothrips longisetis*	60.81	92.50	Ph.	Male	SETHY152-22	KC513073	KC512973	OK	Inc.	OK	OK
AU4T	*Hoplothrips longisetis*	44.53	93.27	Ph.	Female	SETHY129-22	KC513073	KC512973	-	-	Ok	OK
AV8T	*Hoplothrips pedicularius*	1008.12	92.67	Ph.	Female	SETHY142-22	KC513073	KC512973	-	-	OK	OK
AT5T	*Hoplothrips semicaecus*	135.53	90.10	Ph.	Male	SETHY121-22	KC513073	KC512973	-	-	OK	OK
AT4T	*Hoplothrips semicaecus*	117.02	89.60	Ph.	Female	SETHY120-22	KC513073	KC512973	-	-	OK	OK
AS4T	*Xylaplothrips fuliginosus*	223.99	89.77	Ph.	Female	SETHY111-22	KC513067	KC512966	-	-	OK	OK
AS5T	*Xylaplothrips fuliginosus*	114.24	89.34	Ph.	Female	SETHY112-22	KC513067	KC512966	-	-	OK	OK
AU9T	*Xylaplothrips fuliginosus*	43.20	92.02	Ph.	Male	SETHY134-22	KC513067	KC512966	-	-	OK	OK
AV2T	*Xylaplothrips fuliginosus*	54.67	92.63	Ph.	Female	SETHY136-22	KC513067	KC512966	OK	OK	OK	OK
AS3T	*Chirothrips manicatus*	70.73	92.45	Th.	Female	SETHY110-22	KC513044	KC512943	-	-	OK	OK
AQ8T	*Thrips major*	51.89	92.89	Th.	Female	SETHY101-22	KC513123	KC513023	-	-	OK	OK
AU8T	*Thrips minutissimus*	4.26	92.45	Th.	Female	SETHY133-22	KC513123	KC513023	-	-	OK	OK
AR3T	*Thrips trehernei*	47.42	92.34	Th.	Male	SETHY105-22	KC513123	KC513023	-	-	Ok	OK
AS2T	*Tmetothrips subapterus*	71.90	91.01	Th.	Female	SETHY101-22	KC513123	KC513023	OK	Inc.	OK	OK

2.4. Species Delimitation Tests

The loci selected for individual analysis were those with the highest specimen coverage, both mitochondrial and nuclear genes. The specimen and taxa used in each dataset are provided in Table 2. For the COX1 gene, the shorter 658 bp "Folmer"-region barcoding fragment was analyzed, as well as an even shorter fragment based on the primer pairs BF1 and BR2 from Elbrecht and Leese [39]. This shorter fragment, ca 300 bp depending on the organism group, represents the shorter sequence reads often used in metabarcoding studies. Additional complete sequences from mitochondrial genomes and nuclear 18S and 28S sequences were retrieved from NCBI GenBank to increase the sample size and complexity, as well as aiding in correct alignment. Only sequences from trusted sources

with determined species with complete annotations were selected. For mitochondrial genomes, these were from 9 specimens, and for 18S 29 sequences and 28S 23 sequences.

Table 2. Results from species delimitation analyses in bPTP. A + symbol indicates presence in dataset, and a - indicates missing data. The number in parentheses represents the species' partition number. Green cells indicate correct partitioning and red indicates failed (oversplitting or lumping). The two last columns contain results from all mitochondrial genes (Mit. conc.) concatenated and all genes including 28S concatenated (All. concat.).

Code	Species	28S	ATP6	COB	COX1	COX1 658	COX1 313	COX2	NAD4	NAD5	Mit. Conc.	All. Concat.
MZ645927	*Acaciothrips ebneri*	-	+ (13)	+ (8)	+ (14)	+ (1)	+ (11)	+ (1)	+ (5)	+ (13)	+ (10)	+ (9)
AT8T	*Acanthothrips nodicornis*	+ (20)	+ (16)	+ (18)	+ (1)	+ (1)	+ (24)	+ (1)	+ (20)	+ (27)	+ (8)	+ (27)
AT9T	*Acanthothrips nodicornis*	+ (20)	+ (16)	+ (19)	+ (1)	+ (1)	+ (25)	+ (1)	+ (21)	+ (28)	+ (8)	+ (27)
AU1T	*Acanthothrips nodicornis*	+ (20)	-	-	-	-	-	-	-	-	-	+ (27)
MW233591	*Bactrothrips quadrituberculatus*	-	+ (19)	+ (4)	+ (12)	+ (1)	+ (2)	+ (1)	+ (7)	+ (26)	+ (4)	+ (3)
AQ1T	*Bolothrips dentipes*	+ (15)	+ (18)	+ (3)	+ (11)	+ (1)	+ (2)	+ (1)	+ (6)	+ (26)	+ (3)	+ (29)
KC513043	*Cartomothrips browni*	+ (10)	-	-	-	-	-	-	-	-	-	+ (10)
AS7T	*Cephalothrips monilicornis*	+ (7)	+ (25)	+ (6)	+ (7)	+ (1)	+ (12)	+ (1)	+ (18)	+ (24)	+ (6)	+ (41)
AV1T	*Cephalothrips monilicornis*	+ (6)	-	-	-	-	-	-	-	-	-	+ (42)
AW3T	*Cephalothrips monilicornis*	+ (6)	+ (15)	+ (6)	+ (7)	+ (1)	+ (27)	+ (1)	+ (14)	+ (14)	+ (6)	+ (42)
AW4T	*Cephalothrips monilicornis*	+ (6)	+ (25)	+ (6)	+ (7)	+ (1)	+ (14)	+ (1)	+ (19)	+ (25)	+ (6)	+ (44)
AW5T	*Cephalothrips monilicornis*	+ (7)	+ (26)	+ (6)	+ (7)	+ (1)	+ (26)	+ (1)	+ (18)	+ (18)	+ (6)	+ (41)
AY3T	*Cephalothrips monilicornis*	+ (6)	+ (14)	+ (6)	+ (7)	+ (1)	+ (28)	+ (1)	+ (15)	+ (15)	+ (6)	+ (43)
AS3T	*Chirothrips manicatus*	+ (1)	-	-	-	-	-	-	-	-	-	+ (4)
KC513047	*Cryptothrips amneiu*	+ (16)	-	-	-	-	-	-	-	-	-	+ (22)
KC513053	*Dyothrips pallescens*	+ (6)	-	-	-	-	-	-	-	-	-	+ (16)
KC513064	*Gynaikothrips ficorum*	+ (12)	-	-	-	-	-	-	-	-	-	+ (17)
MK940484	*Gynaikothrips uzeli*	-	+ (12)	+ (7)	+ (13)	+ (1)	+ (19)	+ (1)	+ (4)	+ (12)	+ (9)	+ (8)
KP198620	*Haplothrips aculeatus*	-	+ (5)	+ (13)	+ (4)	+ (1)	+ (4)	+ (1)	+ (13)	+ (5)	+ (14)	+ (15)
KC513065	*Haplothrips froggatti*	+ (6)	-	-	-	-	-	-	-	-	-	+ (25)
KC513066	*Haplothrips graminis*	+ (6)	-	-	-	-	-	-	-	-	-	+ (37)
AY4T	*Haplothrips hukkineni*	+ (6)	+ (2)	+ (11)	+ (10)	+ (1)	+ (9)	+ (1)	+ (1)	+ (22)	+ (12)	+ (13)
AR5T	*Haplothrips leucanthemi*	+ (6)	-	-	-	-	-	-	-	-	-	+ (21)
AR6T	*Haplothrips leucanthemi*	+ (6)	-	-	-	-	-	-	-	-	-	+ (21)
AR7T	*Haplothrips leucanthemi*	+ (6)	-	-	-	-	-	-	-	-	-	+ (21)
AT7T	*Haplothrips leucanthemi*	+ (6)	-	-	-	-	-	-	-	-	-	+ (21)
AV4T	*Haplothrips leucanthemi*	+ (6)	+ (22)	+ (15)	+ (16)	+ (1)	+ (15)	+ (1)	+ (3)	+ (4)	+ (17)	+ (21)
AV5T	*Haplothrips leucanthemi*	+ (6)	+ (23)	+ (15)	+ (17)	+ (1)	+ (16)	+ (1)	+ (3)	+ (4)	+ (18)	+ (21)
KC513067	*Haplothrips leucanthemi*	+ (6)	-	-	-	-	-	-	-	-	-	+ (21)
AT1T	*Haplothrips statices*	+ (6)	-	-	-	-	-	-	-	-	-	+ (21)
AT2T	*Haplothrips statices*	+ (6)	+ (26)	+ (14)	+ (18)	+ (1)	+ (22)	+ (1)	+ (3)	+ (20)	+ (16)	+ (21)
AY1T	*Haplothrips statices*	+ (6)	+ (27)	+ (14)	+ (15)	+ (1)	+ (23)	+ (1)	+ (3)	+ (19)	+ (16)	+ (21)
AY2T	*Haplothrips statices*	+ (6)	+ (26)	+ (14)	+ (19)	+ (1)	+ (22)	+ (1)	+ (3)	+ (21)	+ (16)	+ (21)
AU3T	*Haplothrips subtilissimus*	+ (6)	+ (20)	+ (12)	+ (3)	+ (1)	+ (17)	+ (1)	+ (12)	+ (16)	+ (13)	+ (24)
AU5T	*Haplothrips subtilissimus*	+ (6)	+ (6)	+ (12)	+ (3)	+ (1)	+ (5)	+ (1)	+ (12)	+ (7)	+ (13)	+ (24)
AU6T	*Haplothrips subtilissimus*	+ (6)	+ (21)	+ (12)	+ (3)	+ (1)	+ (7)	+ (1)	+ (12)	+ (8)	+ (13)	+ (24)
AU7T	*Haplothrips subtilissimus*	+ (6)	+ (7)	+ (12)	+ (3)	+ (1)	+ (18)	+ (1)	+ (12)	+ (10)	+ (13)	+ (24)
AW2T	*Haplothrips subtilissimus*	+ (6)	+ (9)	+ (12)	+ (3)	+ (1)	+ (8)	+ (1)	+ (12)	+ (17)	+ (13)	+ (24)
AW8T	*Haplothrips subtilissimus*	+ (6)	-	-	-	-	-	-	-	-	-	+ (24)
AX2T	*Haplothrips subtilissimus*	+ (6)	+ (8)	+ (12)	+ (3)	+ (1)	+ (6)	+ (1)	+ (12)	+ (11)	+ (13)	+ (24)
AX3T	*Haplothrips subtilissimus*	+ (6)	+ (20)	+ (12)	+ (3)	+ (1)	+ (13)	+ (1)	+ (12)	+ (6)	+ (13)	+ (24)
AX4T	*Haplothrips subtilissimus*	+ (6)	-	-	-	-	-	-	-	-	-	+ (24)
AP6T	*Haplothrips utae*	+ (6)	+ (10)	+ (16)	+ (20)	+ (1)	+ (10)	+ (1)	+ (16)	+ (23)	+ (15)	+ (46)
AP8T	*Haplothrips utae*	+ (6)	+ (11)	+ (17)	+ (21)	+ (1)	+ (10)	+ (1)	+ (17)	+ (23)	+ (15)	+ (46)
AY5T	*Haplothrips utae*	+ (6)	-	-	-	-	-	-	-	-	-	+ (45)
KC513068	*Haplothrips victoriensis*	+ (6)	-	-	-	-	-	-	-	-	-	+ (26)
KC513073	*Holothrips cracens*	+ (7)	-	-	-	-	-	-	-	-	-	+ (34)
KC513116	*Hoplandrothrips quadriconus*	+ (21)	-	-	-	-	-	-	-	-	-	+ (28)
AU4T	*Hoplothrips longisetis*	+ (7)	-	-	-	-	-	-	-	-	-	+ (35)
AW9T	*Hoplothrips longisetis*	+ (7)	+ (17)	+ (10)	+ (5)	+ (1)	+ (21)	+ (1)	+ (8)	+ (2)	+ (7)	+ (36)
AV8T	*Hoplothrips pedicularius*	+ (7)	-	-	-	-	-	-	-	-	-	+ (32)
AT4T	*Hoplothrips semicaecus*	+ (7)	-	-	-	-	-	-	-	-	-	+ (33)
AT5T	*Hoplothrips semicaecus*	+ (7)	-	-	-	-	-	-	-	-	-	+ (31)
KC513078	*Idolothrips spectrum*	+ (15)	-	-	-	-	-	-	-	-	-	+ (30)
KC513079	*Kladothrips antennatus*	+ (9)	-	-	-	-	-	-	-	-	-	+ (12)
KC513080	*Leeuwenia scolopiae*	+ (13)	-	-	-	-	-	-	-	-	-	+ (18)
KC513081	*Leptothrips mali*	+ (6)	-	-	-	-	-	-	-	-	-	+ (38)
KC513045	*Liothrips reuteri*	+ (3)	-	-	-	-	-	-	-	-	-	+ (1)
KC513084	*Macrothrips papuensis*	+ (17)	-	-	-	-	-	-	-	-	-	+ (23)
KC513095	*Phaulothrips kranzae*	+ (4)	-	-	-	-	-	-	-	-	-	+ (2)
MW793907	*Psephenothrips eriobotryae*	-	+ (4)	+ (5)	+ (6)	+ (1)	+ (20)	+ (1)	+ (9)	+ (9)	+ (5)	+ (7)
AW6T	*Rhipidothrips brunneus*	+ (2)	+ (1)	+ (2)	+ (9)	+ (4)	+ (1)	+ (3)	+ (11)	+ (1)	+ (2)	+ (5)
AW7T	*Rhipidothrips brunneus*	+ (2)	+ (1)	+ (2)	+ (9)	+ (2)	+ (1)	+ (3)	+ (11)	+ (1)	+ (2)	+ (5)
KC513102	*Sacothrips catheter*	+ (14)	-	-	-	-	-	-	-	-	-	+ (19
KC513106	*Scopaeothrips bicolor*	+ (6)	-	-	-	-	-	-	-	-	-	+ (14)
KC513111	*Strepterothrips tuberculatus*	+ (5)	-	-	-	-	-	-	-	-	-	+ (6)
KC513114	*Teuchothrips ater*	+ (8)	-	-	-	-	-	-	-	-	-	+ (39)
KC513115	*Teuchothrips disjunctus*	+ (8)	-	-	-	-	-	-	-	-	-	+ (40)
AQ8T	*Thrips major*	+ (18)	-	-	-	-	-	-	-	-	-	+ (20)
AU8T	*Thrips minutissimus*	+ (19)	-	-	-	-	-	-	-	-	-	+ (47)

Table 2. *Cont.*

Code	Species	28S	ATP6	COB	COX1	COX1 658	COX1 313	COX2	NAD4	NAD5	Mit. Conc.	All. Concat.
AR3T	*Thrips trehernei*	+ (19)	-	-	-	-	-	-	-	-	-	+ (49)
AS2T	*Tmetothrips subapterus*	+ (19)	+ (1)	+ (1)	+ (8)	+ (3)	+ (1)	+ (2)	+ (10)	+ (1)	+ (1)	+ (50)
KC513125	*Treherniella amplipennis*	+ (11)	-	-	-	-	-	-	-	-	-	+ (11)
AS4T	*Xylaplothrips fuliginosus*	+ (6)	-	-	-	-	-	-	-	-	-	+ (48)
AS5T	*Xylaplothrips fuliginosus*	+ (6)	-	-	-	-	-	-	-	-	-	+ (52)
AU9T	*Xylaplothrips fuliginosus*	+ (6)	-	-	-	-	-	-	-	-	-	+ (51)
AV2T	*Xylaplothrips fuliginosus*	+ (6)	+ (3)	+ (9)	+ (2)	+ (1)	+ (3)	+ (1)	+ (1)	+ (3)	+ (11)	+ (53)
	Species total:	20	27	19	21	4	28	3	21	28	18	53

IQ-TREE 1.6.12 [40] was used to construct maximum likelihood trees for each gene, utilizing ModelFinder [41] to infer the most accurate model of substitution with the selection criterion set to BIC. Each tree was assigned 5000 ultrafast bootstrap replicates and 1000 Shimodaira–Hasegawa-like (SH-aLRT) approximate likelihood ratio tests [42]. The ML-tree from IQ-TREE was used as an input tree for species delimitation in the Bayesian Poisson Tree Process model in bPTP [43], with 500,000 generations and a 30% burn in (decided after examining the required burn in for stationary convergence).

To examine the results from multiple loci species delimitation, the alignments from the selected single loci were used, one with all mitochondrial genes and one with the 28S nuclear gene added. Single gene alignments were concatenated and thereafter analyzed in IQ-TREE, using gene partitioning followed by bPTP.

2.5. Phylogeny from Supermatrix

To infer the higher-level phylogeny of the family Phlaeothripidae, all the available loci were assembled in a supermatrix alignment, including non-coding mitochondrial genes (Table 3), as well as both nuclear genes 18S and 28S. To find the best partitioning and model scheme for the maximum likelihood analysis, ModelFinder was first run separately, with the Bayesian (BIC) selection criterion, new model selection procedure, and partition merging. Protein coding genes were divided into codons while non-coding genes were kept complete. The best scheme was used to infer a maximum likelihood phylogeny IQ-TREE 1.6.12, with 5000 ultrafast bootstrap replicates and 1000 Shimodaira–Hasegawa-like (SH-aLRT) approximate likelihood ratio tests. The best partitioning scheme from ModelFinder was also for the Bayesian inference, but models were assigned using bModelTest [44] in BEAST 2.7.5 [45]. BEAST was run for 100 million generations with a sampling frequency of 5000 in 2 independent runs and a burn in of 25%. The effective sampling size (ESS at least 200) and convergence were checked in Tracer v1.7.2 [46]. The trees from 2 runs were combined in LogCombiner, and a maximum clade credibility tree was summarized using TreeAnnotator. The resulting trees were inspected and exported using FigTree 1.4.4 [47], and finalized in Adobe Illustrator 27.7 (Adobe Inc., San Jose, CA, USA).

Table 3. Statistics for all alignments of mitochondrial and nuclear genes.

Gene	Specimens	Length	GC%	Pairwise Identity	Length After BMGE
28S	72	3120	60.5%	80.8%	2296
ATP6	33	705	19.3%	75.7%	674
COB	33	1224	22.5%	79.2%	1140
COX1	33	1647	25.5%	82.2%	1500
COX1 (HCO+LCO)	33	658	26.8%	83.8%	658
COX1 (BF1+BR2)	33	313	27.2%	82.1%	313
COX2	33	771	20.8%	79.2%	630
NAD4	33	1429	17.9%	75.9%	1315
NAD5	33	1836	17.7%	75.5%	1644
Concatenated excl. 28S	33	7612	20.8%	33.9%	7059
Concatenated incl. 28S	77	10,732	37.0%	43.9%	9355

Table 3. *Cont.*

Gene	Specimens	Length	GC%	Pairwise Identity	Length After BMGE
18S	77	2113	52.8%	93.9%	1915
ATP8	32	257	15.4%	61.9%	86
COX3	31	792	23.5%	84.0%	777
NAD1	32	987	20.6%	78.7%	885
NAD2	30	1150	15.0%	73.9%	881
NAD3	31	417	16.9%	80.5%	330
NAD4L	32	299	15.4%	79.4%	255
NAD6	32	657	13.8%	70.1%	446
rrnL	29	1380	21.3%	77.5%	595
rrnS	25	833	17.1%	76.4%	645
trnA	31	79	19.7%	80.8%	54
trnC	28	76	14.2%	79.3%	58
trnD	30	77	16.8%	84.6%	53
trnE	30	72	8.9%	86.4%	66
trnF	29	71	11.4%	82.2%	68
trnG	30	83	10.3%	86.1%	64
trnH	29	87	12.1%	83.1%	60
trnI	28	76	22.5%	78.9%	57
trnK	28	78	15.7%	85.2%	59
trnL1	27	72	16.9%	75.2%	68
trnL2	28	69	16.8%	83.7%	67
trnM	30	72	26.4%	82.7%	62
trnN	30	78	18.0%	87.6%	64
trnP	24	78	16.9%	82.0%	55
trnQ	25	76	16.0%	78.7%	61
trnR	30	81	22.4%	77.2%	60
trnS1	29	69	19.1%	74.9%	46
trnS2	28	81	10.4%	85.4%	66
trnT	29	71	9.0%	84.4%	62
trnV	29	85	13.2%	82.2%	49
trnW	29	85	14.0%	82.6%	62
trnY	30	74	19.4%	79.4%	64
Supermatrix	82	40,622	35.3%	52.8%	17,339

3. Results

Of the 50 sequenced specimens, it was possible to assemble 29 mitochondrial genomes through NOVOPlasty, but only 5 were completely annotated by MITOS2 (Table 1). However, many genes were still recovered and useable for downstream applications. The thrips mitochondrial genome consists of 13 protein coding genes, two ribosomal RNA, and 22 transfer RNA genes, and has a length of ca 15,000 bp [48]. Transfer RNA and RNA genes yielded the lowest success, but the protein-coding genes, ATP8, COX3, NAD1, NAD2, NAD3, NAD4L, and NAD6, were also missing on some occasions. These could possibly be recovered from manual assembly and annotation, as the NOVOPlasty produced full-length genome sequences. Based on the specimen coverage, the genes ATP6, COB, COX1, COX2, NAD4, and NAD5 were selected from the species delimitation analysis. In addition, the shorter fragments of COX1, the barcode regions (HCO+LCO), and COX1 metabarcoding region (BF1+BR2), were analyzed using the same taxon set. The skimming for nuclear genes using Bowtie2 and reference sequences was more successful, only failing to assemble 18S (Table 1). The average sequence depth was 6444. The total number of specimens and aligned length are provided in Table 3.

For the species delimitation analysis, a total of 44 species were included (including imported), of which 17 were represented by mitochondrial genes (Table 2). Out of all the mitochondrial genes, COB performed the best, only oversplitting two species (*Acanthothrips nodicornis* and *Haplothrips utae*). The 658 bp long barcoding region of COX1 performed the

worst, only being able to correctly delimit one species (represented by only one specimen). The oversplitting of species is common across genes, except for the COX1 barcoding region and COX2 where extreme lumping was observed. Concatenating all the mitochondrial genes, however, yielded the best overall result, only splitting *Haplothrips leucanthemi*. The nuclear gene, 28S, lumped together a large number of species, mostly *Haplothrips* and related species. Combining 28S with the mitochondrial genes did improve the results slightly but was far from comparable to only mitochondrial genes.

A total of 49 species were included in the supermatrix phylogeny (Supplementary Table S1), represented by 82 specimens. The 18S and 28S sequences from *Liothrips reuteri* (KC513045 and KC51294) were found to be for an unidentified species of the subfamily Idolothripinae Bagnall, 1908 (as opposed to the correct Phlaeothripinae Uzel, 1895), likely representing contamination or misidentification, and was removed from the final supermatrix alignment. Phylogenetic analysis combining all mitochondrial genes with 18S and 28S showed Phlaeothripidae to be monophyletic in regard to other families (Figure 2). The subfamilies, Phlaeothripinae and Idolothripinae, were also supported in the ML analysis but not in the BI where *Carientothrips mjobergi* was found within Phlaeothripinae. Within Phaleothripinae, the tribe, Haplothripini, was the only well-supported subgroup. Within Idolothripinae, both tribes were paraphyletic. Maximum Likelihood (Figure 2A) and Bayesian inference (Figure 2B) showed similar higher-level topologies. The differences at lower taxonomic levels, however, did not affect the monophyly or paraphyly of tribe-level groups. Support values were in general high but varied across different nodes depending on the analysis.

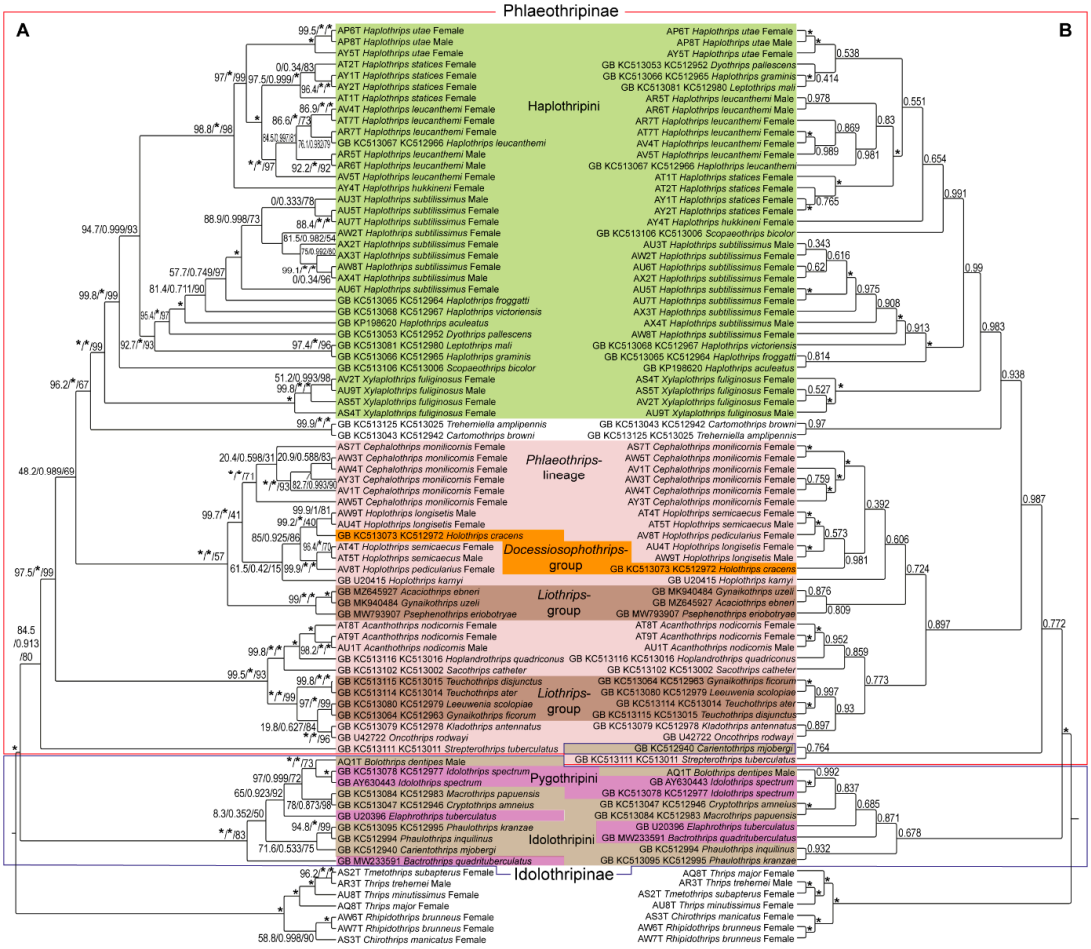

Figure 2. Phylogenetic trees of the thrips family, Phlaeothripidae, from Maximum Likelihood (**A**) and Bayesian inference (**B**). Support values are given in SH-aLRT/aBayes/ultrafast bootstrap for (**A**) and

posterior probabilities for (**B**). Full support is indicated by an asterisk, one asterisk alone in (**A**) indicated full support in all statistics. The coloured groups represent tribes and genus groups, and the coloured lines are bordering the subfamilies in Phlaeothripidae.

4. Discussion and Conclusions

The species delimitation analysis illuminates the limitations of using one or few markers for Thysanoptera. Prior investigations into molecular identification of thrips have found similar results [49]. These issues extend over several loci, as indicated by Ghosh et al. [49], suggesting broader challenges when distinguishing closely related species through genetic means.

Genetic sequence variation can reflect the population structure or geographic distribution rather than taxonomic identity alone [50]. Thus, the accurate delimitation of molecular taxonomic units necessitates a more comprehensive and integrated approach. Beyond the protocols of COX1 barcoding, the incorporation of complete mitochondrial genomes and several nuclear genes has emerged as imperative.

Cryptic species complexes pose significant challenges to taxonomists due to their morphological similarity despite their genetic divergence. Traditional taxonomy, reliant on phenotypic traits, often fails to detect these cryptic entities. Taxonomic projects are often limited in scope and funding, making whole-genome sequencing and assembly unfeasible. But genome skimming is cost-effective and computationally efficient, making it a viable tool for taxonomic studies, as it circumvents the often time-consuming parts of, e.g., assembly and annotation. Furthermore, it is a logical step from Sanger sequencing individual fragments to high-throughput sequencing when the costs are equalizing or even surpassing it.

Other modern methods such as metabarcoding in environmental studies also benefit from genome skimming. Accurate species identification is crucial for conservation planning. Cryptic species often have distinct ecological requirements and conservation statuses. Genome skimming helps in recognizing these species, ensuring that appropriate conservation strategies are implemented.

It is also very important to point out that for taxonomic studies that lead to taxonomic changes and new descriptions, it is necessary to perform the analyses using additional models in addition to, for example, bPTP. There are today a number of different approaches, and comparative studies are needed before drawing conclusions leading to revised taxonomies. The same is also valid for substation model selections.

In this study, the phylogenetic reconstruction of the Phlaeothripidae family was also examined, and the results align with previous investigations, e.g., Buckman et al. [13]. Some of the nuclear data reanalyzed here are from Buckman et al. [13], and while this dataset is predominantly mitochondrial data, the core structure of the resulting phylogeny is very similar.

Buckman et al. [13] recovered a paraphyletic Phlaeothripinae, but monophyletic Idolothripinae. Here, one taxon of Idolothripinae is found within Phaleothripinae in the Bayesian inference. The paraphyly of tribes and genus groups, as outlined by Dang et al. [51] and Mound and Palmer [12], has been indicated previously [13,52], but is here further strengthened. The Pygothripini and Idolothripini in Idolothripindae are found to be paraphyletic as well. However, there are also differences in the results presented here, for example, the *Liothrips*-group is paraphyletic, while it was monophyletic in Buckman et al. [13]. The *Phlaeothrips* lineage was not specifically examined previously, but is here found to be non-monophyletic. The monophyly of Haplothripini is the only tribe withstanding phylogenetic testing.

Many species of thrips have historically been difficult to identify via morphological methods. Molecular methods can alleviate these problems, but only with adequate data. Larger amounts of data than provided by barcodes and single gene markers are necessary, at least for some closely related and cryptic species groups. For evolutionary studies, nuclear genes are also of importance. With the possibility to sequence a large number of specimens in one high-throughput sequencing event and thereafter process the data in a semi-automated pipeline is here demonstrated to provide new possibilities for not only acquiring species level data but also reconstructing relationships with high support. Further additions of taxa, e.g., broader taxon sampling and geographical range, and gene data can be collected in a standardized workflow towards a complete and stable Thysanoptera phylogeny, with complete specimens saved for morphological studies and future research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17040226/s1. Supplementary Table S1. Sample data.

Funding: This work was supported by The Swedish Taxonomy Initiative at the SLU Swedish Species Information Centre (SLU Artdatabanken, Uppsala, Sweden) (grant numbers SLU.dha.2020.4.3-228, SLU.dha.2022.4.3-206 and SLU.dha.2023.4.3-247).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The sequence read data are deposited in the NCBI Sequence Read Archive with accession number PRJNA1008054. Assemblies, alignments, and trees are deposited at Zenodo: https://zenodo.org/doi/10.5281/zenodo.11196144 (accessed on 1 February 2025).

Acknowledgments: Rasa Bukontaite (Swedish Museum of Natural History) provided invaluable support during the development of the laboratory protocols. Carl-Axel Gertsson, Sverre Kobro, and Manfred R. Ulitzka have been very helpful and provided reference specimens for morphological species identifications.

Conflicts of Interest: The author declares no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Mound, L.A.; Teulon, D.A. Thysanoptera as Phytophagous Opportunists. *Thrips Biol. Manag.* **1995**, *3*, 3–19.
2. Reynaud, P. Thrips (Thysanoptera). *BioRisk* **2010**, *4*, 767–791. [CrossRef]
3. Folmer, O.; Hoeh, W.R.; Black, M.B.; Vrijenhoek, R.C. DNA Primers for Amplification of Mitochondrial Cytochrome c Oxidase Subunit I from Diverse Metazoan Invertebrates. *Mol. Mar. Biol. Biotechnol.* **1994**, *3*, 294–299. [PubMed]
4. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; deWaard, J.R. Biological Identifications through DNA Barcodes. *Proc. R. Soc. Lond. Ser. B* **2003**, *270*, 313–321. [CrossRef]
5. Sweeney, B.W.; Battle, J.M.; Jackson, J.K.; Dapkey, T. Can DNA Barcodes of Stream Macroinvertebrates Improve Descriptions of Community Structure and Water Quality? *J. N. Am. Benthol. Soc.* **2011**, *30*, 195–216. [CrossRef]
6. Jaramillo, J.; Chapman, E.G.; Vega, F.E.; Harwood, J.D. Molecular Diagnosis of a Previously Unreported Predator–Prey Association in Coffee: *Karnyothrips flavipes* Jones (Thysanoptera: Phlaeothripidae) Predation on the Coffee Berry Borer. *Naturwissenschaften* **2010**, *97*, 291–298. [CrossRef]
7. Marquina, D.; Andersson, A.F.; Ronquist, F. New Mitochondrial Primers for Metabarcoding of Insects, Designed and Evaluated Using In Silico Methods. *Mol. Ecol. Resour.* **2019**, *19*, 90–104. [CrossRef]
8. Pons, J.; Barraclough, T.G.; Gomez-Zurita, J.; Cardoso, A.; Duran, D.P.; Hazell, S.; Kamoun, S.; Sumlin, W.D.; Vogler, A.P. Sequence-Based Species Delimitation for the DNA Taxonomy of Undescribed Insects. *Syst. Biol.* **2006**, *55*, 595–609. [CrossRef]
9. Xie, Y.L.; Mound, L.A.; Lima, É.F.B.; He, S.Q.; Zhang, H.R.; Li, Y.J. Molecular Studies of Relationships and Identifications among Insects of the Subfamily Panchaetothripinae (Thysanoptera, Thripidae). *J. Insect Sci.* **2022**, *22*, 6. [CrossRef]
10. Huang, J.-P. What Have Been and What Can Be Delimited as Species Using Molecular Data under the Multi-Species Coalescent Model? A Case Study Using Hercules Beetles (*Dynastes*; Dynastidae). *Insect Syst. Divers.* **2018**, *2*, 3. [CrossRef]
11. Leaché, A.D.; Zhu, T.; Rannala, B.; Yang, Z. The Spectre of Too Many Species. *Syst. Biol.* **2019**, *68*, 168–181. [CrossRef] [PubMed]

12. Mound, L.A.; Palmer, J.M. The Generic and Tribal Classification of Spore-Feeding Thysanoptera (Phlaeothripidae: Idolothripinae). *Bull. Br. Mus. Nat. Hist. Entomol.* **1983**, *46*, 1–174.
13. Buckman, R.S.; Mound, L.A.; Whiting, M.F. Phylogeny of Thrips (Insecta: Thysanoptera) Based on Five Molecular Loci. *Syst. Entomol.* **2013**, *38*, 123–133. [CrossRef]
14. Tyagi, K.; Chakraborty, R.; Cameron, S.L.; Sweet, A.D.; Chandra, K.; Kumar, V. Rearrangement and Evolution of Mitochondrial Genomes in Thysanoptera (Insecta). *Sci. Rep.* **2020**, *10*, 695. [CrossRef]
15. Botero-Castro, F.; Tilak, M.K.; Justy, F.; Catzeflis, F.; Delsuc, F.; Douzery, E.J. Next-Generation Sequencing and Phylogenetic Signal of Complete Mitochondrial Genomes for Resolving the Evolutionary History of Leaf-Nosed Bats (Phyllostomidae). *Mol. Phylogenet. Evol.* **2013**, *69*, 728–739. [CrossRef]
16. Magnussen, T.; Johnsen, A.; Kjærandsen, J.; Struck, T.H.; Søli, G.E. Molecular Phylogeny of *Allodia* (Diptera: Mycetophilidae) Constructed Using Genome Skimming. *Syst. Entomol.* **2022**, *47*, 267–281. [CrossRef]
17. Trevisan, B.; Alcantara, D.M.; Machado, D.J.; Marques, F.P.; Lahr, D.J. Genome Skimming Is a Low-Cost and Robust Strategy to Assemble Complete Mitochondrial Genomes from Ethanol Preserved Specimens in Biodiversity Studies. *PeerJ* **2019**, *7*, e7543. [CrossRef]
18. Ren, Z.; Harris, A.J.; Dikow, R.B.; Ma, E.; Zhong, Y.; Wen, J. Another Look at the Phylogenetic Relationships and Intercontinental Biogeography of Eastern Asian–North American *Rhus* Gall Aphids (Hemiptera: Aphididae: Eriosomatinae): Evidence from Mitogenome Sequences via Genome Skimming. *Mol. Phylogenet. Evol.* **2017**, *117*, 102–110. [CrossRef]
19. Zhang, X.; Kang, Z.; Ding, S.; Wang, Y.; Borkent, C.; Saigusa, T.; Yang, D. Mitochondrial Genomes Provide Insights into the Phylogeny of Culicomorpha (Insecta: Diptera). *Int. J. Mol. Sci.* **2019**, *20*, 747. [CrossRef]
20. Zhang, C.; Zhang, D.-X.; Zhu, T.; Yang, Z. Evaluation of a Bayesian Coalescent Method of Species Delimitation. *Syst. Biol.* **2011**, *60*, 747–761. [CrossRef]
21. Wahlberg, E. A Modern Workflow for Non-Destructive DNA Extraction and Slide Preparation of Thrips (Insecta, Thysanoptera) for Taxonomic Studies and Collection Deposition. *Norw. J. Entomol.* **2023**, *70*, 1–5.
22. Mound, L.A.; Morison, G.D.; Pitkin, B.R.; Palmer, J.M. Part 11—Thysanoptera. In *Handbooks for the Identification of British Insects*; Royal Entomological Society: London, UK, 1976; Volume 1, p. 79.
23. Mound, L.A.; Collins, D.W.; Hastings, A. *Thysanoptera Britannica et Hibernica–Thrips of the British Isles*; Lucidcentral.org, Identic Pty Ltd.: Brisbane, QLD, Australia, 2018.
24. zur Strassen, R. *Die Terebranten Thysanopteren Europas und des Mittelmeer-Gebietes*; Die Tierwelt Deutschlands; Verlag Goecke & Evers: Keltern, Germany, 2003; Volume 74, pp. 1–271.
25. Wahlberg, E.; Gertsson, C.-A. Identification Key to and Checklist of the Swedish Phlaeothripidae (Thysanoptera). *ZooKeys* **2022**, *1096*, 161–187. [CrossRef]
26. Wahlberg, E. The Swedish Aeolothripidae and Melanthripidae (Thysanoptera) with a Redescription of *Rhipidothrips niveipennis* Reuter, 1899. *Taxonomy* **2024**, *4*, 163–183. [CrossRef]
27. Meyer, M.; Kircher, M. Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing. *Cold Spring Harb. Protoc.* **2010**, *2010*, pdb.prot5448. [CrossRef] [PubMed]
28. Irestedt, M.; Thörn, F.; Müller, I.A.; Jønsson, K.A.; Ericson PG, P.; Blom, M.P.K. A Guide to Avian Museomics: Insights Gained from Resequencing Hundreds of Avian Study Skins. *Mol. Ecol. Resour.* **2022**, *22*, 2672–2684. [CrossRef]
29. Afgan, E.; Nekrutenko, A.; Grüning, B.A.; Blankenberg, D.; Goecks, J.; Schatz, M.C.; Briggs, P.J. The Galaxy Platform for Accessible, Reproducible, and Collaborative Biomedical Analyses: 2022 Update. *Nucleic Acids Res.* **2022**, *50*, W345–W351. [CrossRef]
30. Dierckxsens, N.; Mardulyn, P.; Smits, G. NOVOPlasty: De Novo Assembly of Organelle Genomes from Whole Genome Data. *Nucleic Acids Res.* **2016**, *44*, e18. [CrossRef]
31. Donath, A.; Jühling, F.; Al-Arab, M.; Bernhart, S.H.; Reinhardt, F.; Stadler, P.F.; Middendorf, M.; Bernt, M. Improved Annotation of Protein-Coding Gene Boundaries in Metazoan Mitochondrial Genomes. *Nucleic Acids Res.* **2019**, *47*, 10543–10552. [CrossRef]
32. Wheeler, D.L.; Barrett, T.; Benson, D.A.; Bryant, S.H.; Canese, K.; Chetvernin, V.; Yaschenko, E. Database Resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* **2007**, *35* (Suppl. S1), D5–D12. [CrossRef]
33. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A Flexible Trimmer for Illumina Sequence Data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef]
34. Langmead, B.; Salzberg, S.L. Fast Gapped-Read Alignment with Bowtie 2. *Nat. Methods* **2012**, *9*, 357–359. [CrossRef] [PubMed]
35. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R. The Sequence Alignment/Map Format and SAMtools. *Bioinformatics* **2009**, *25*, 2078–2079. [CrossRef] [PubMed]
36. Criscuolo, A.; Gribaldo, S. BMGE (Block Mapping and Gathering with Entropy): A New Software for Selection of Phylogenetic Informative Regions from Multiple Sequence Alignments. *BMC Evol. Biol.* **2010**, *10*, 210. [CrossRef]
37. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data. *Bioinformatics* **2012**, *28*, 1647–1649. [CrossRef]

38. Ratnasingham, S.; Hebert, P.D.N. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Mol. Ecol. Notes* **2007**, *7*, 355–364. [CrossRef]
39. Elbrecht, V.; Leese, F. Validation and Development of COI Metabarcoding Primers for Freshwater Macroinvertebrate Bioassessment. *Front. Environ. Sci.* **2017**, *5*, 11. [CrossRef]
40. Trifinopoulos, J.; Nguyen, L.T.; von Haeseler, A.; Minh, B.Q. W-IQ-TREE: A Fast Online Phylogenetic Tool for Maximum Likelihood Analysis. *Nucleic Acids Res.* **2016**, *44*, W232–W235. [CrossRef]
41. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.; von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nat. Methods* **2017**, *14*, 587–589. [CrossRef]
42. Guindon, S.; Dufayard, J.F.; Lefort, V.; Anisimova, M.; Hordijk, W.; Gascuel, O. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Syst. Biol.* **2010**, *59*, 307–321. [CrossRef]
43. Zhang, J.; Kapli, P.; Pavlidis, P.; Stamatakis, A. A General Species Delimitation Method with Applications to Phylogenetic Placements. *Bioinformatics* **2013**, *29*, 2869–2876. [CrossRef]
44. Bouckaert, R.; Drummond, A. bModelTest: Bayesian Phylogenetic Site Model Averaging and Model Comparison. *BMC Evol. Biol.* **2017**, *17*, 42. [CrossRef]
45. Bouckaert, R.; Vaughan, T.G.; Barido-Sottani, J.; Duchêne, S.; Fourment, M. BEAST 2.5: An Advanced Software Platform for Bayesian Evolutionary Analysis. *PLoS Comput. Biol.* **2019**, *15*, e1006650. [CrossRef]
46. Rambaut, A.; Drummond, A.J.; Xie, D.; Baele, G.; Suchard, M.A. Posterior Summarisation in Bayesian Phylogenetics Using Tracer 1.7. *Syst. Biol.* **2018**, *67*, 901–904. [CrossRef] [PubMed]
47. Rambaut, A. *FigTree*, version 1.4.4; Institute of Evolutionary Biology, University of Edinburgh: Edinburgh, UK, 2018. Available online: https://github.com/rambaut/figtree (accessed on 1 January 2024).
48. Pakrashi, A.; Kumar, V.; Stanford-Beale, D.A.; Cameron, S.L.; Tyagi, K. Gene Arrangement, Phylogeny and Divergence Time Estimation of Mitogenomes in Thrips. *Mol. Biol. Rep.* **2022**, *49*, 6269–6283. [CrossRef]
49. Ghosh, A.; Jangra, S.; Dietzgen, R.G.; Yeh, W.B. Frontiers Approaches to the Diagnosis of Thrips (*Thysanoptera*): How Effective Are the Molecular and Electronic Detection Platforms? *Insects* **2021**, *12*, 920. [CrossRef]
50. Sukumaran, J.; Knowles, L.L. Multispecies Coalescent Delimits Structure, Not Species. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 1607–1612. [CrossRef]
51. Dang, L.H.; Mound, L.A.; Qiao, G.X. Conspectus of the *Phlaeothripinae* Genera from China and Southeast Asia (*Thysanoptera*, *Phlaeothripidae*). *Zootaxa* **2014**, *3807*, 1–82. [CrossRef]
52. Mound, L.A.; Minaei, K. Australian Thrips of the *Haplothrips* Lineage (Insecta: *Thysanoptera*). *J. Nat. Hist.* **2007**, *41*, 2919–2978. [CrossRef]