Molecular Studies of Relationships and Identifications Among Insects of the Subfamily Panchaetothripinae (Thysanoptera, Thripidae)

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Abstract

The Panchaetothripinae comprises 42 genera and 146 species of leaf-feeding thrips, some of which are horticultural pests. We examined representatives of the 18 genera that include most of these pests. For species delimitation, we used DNA barcoding to produce 171 sequences for 40 morphospecies. Most species were found to be monophyletic, although cryptic diversity was evident in 8 presumptive species. A multilocus molecular phylogenetic assessment was based on one mitochondrial (COI) and three nuclear loci (EF-1α, ITS2, and 28S) from 132 specimens (18 genera and 33 species), representing all genera and ~82% of species in China. Maximum likelihood (ML) and Bayesian inference (BI) confirmed monophyly of each genus with strong support. Monophyly of tribes Panchaetothripini and Monilothripini were refuted, but the well supported tribe Tryphactothripini was confirmed. Rhipiphorothrips was recovered as a sister to the remainder of the genera of Panchaetothripinae combined. Both analyses revealed two major clades. Clade A comprised the majority of the genera, including tribe Tryphactothripini. Clade B included only four genera of which two, Helionothrips and Caliothrips, are particularly species rich. The relationships of some genera remain unresolved.

Key words: DNA barcoding, multilocus phylogeny, Panchaetothripinae, China

The Greenhouse Thrips, Heliothrips haemorrhoidalis (Bouche), is one of 146 species in the Panchaetothripinae, one of four subfamilies in the Thysanoptera family Thripidae. Currently, 42 genera are recognized in this subfamily, of which 23 are monobasic (ThripsWiki 2022). Members of the group are found worldwide, but some genera are restricted in their distribution with five known only in the Neotropics, eight only in the Australasian region, and nine in the Old World tropics particularly in Africa. The study reported here is based on the 18 genera known from China (Li et al. 2018, 2021; Xie et al. 2019), and these include all but two of the taxa commonly considered to be pests (Mound et al. 2022). In China, 46 species representing 17 genera are recorded and the reported diversity of Panchaetothripinae in this country is increasing, with one genus and nine species described in the last 5 years (Mirab-balou et al. 2016; Wang et al. 2017; Li et al. 2018, 2021).

These species all feed on mature leaves as larvae and adults, and most of them seem to have little host specificity. The greenhouse thrips, H. haemorrhoidalis, damages plants in many families (Scott-Brown and Simmonds 2006, Denmark and Fasulo 2010), and the red-banded thrips, Selenothrips rubrocinctus (Giard), infests various fruit, ornamental and shade trees (Wilson 1975, Denmark and Wolfenbarger 2010). In addition, Brachyurothrips anomalus (Bagnall), Hercinothrips bicinctus (Bagnall), Rhipiphorothrips cruentatus (Hood), Caliothrips fasciatus (Pergande), Parthenothrips dracaenae (Heeger), Retithrips syriacus (Mayet) and Panchaetothrips indicus (Bagnall) are recorded as pests on leaves of peppers, bananas, grapes, roses, castor oil, beans, palms, coffee, cotton, black vine and turmeric (Mound et al. 2001, Mound and Postle 2004, Lima et al. 2020). However, precise identification of species can be challenging, particularly in the larger genera such as Astrothrips, Helionothrips, and Rhipiphorothrips.
To avoid misidentifications based on morphology, and to recognize cryptic diversity amongst pest species, an accurate and effective molecular approach is required. The fragment of mitochondrial cytochrome c oxidase subunit I (COI) gene can be an effective marker for thrips species discrimination (Timm et al. 2008, Glover et al. 2010), new species discovery (Mound et al. 2010, 2017) and the resolution of cryptic species complexes (Rebijith et al. 2014, Iftikhar et al. 2016, Tyagi et al. 2017). However, DNA studies on Thripidae have been based mostly on Thripinae and not on Panchaetothripinae. Of this subfamily, the largest number of species examined before the present study was the 10 species included in barcoding studies on Indian thrips (Tyagi et al. 2017), and there has been very little phylogenetic analysis of relationships within the group.

Based on morphology, Wilson (1975) recognized three Panchaetothripinae tribes: Monilothripini with 2 monobasic genera based on two shared morphological character states (long prontal setae and lack of strong prontal sculpture); Tryphactothripini with 8 genera sharing these states: (abdominal segment II constricted at base and bearing laterally patches of strong ridges, wart-like tubercles or stoutly recurved microtrichia, and abdominal segment X tending to be asymmetrical); Panchaetothripini, comprising the remaining genera, undefined by any single apomorphy. However, Monilothripini and Panchaetothripini were not recovered as monophyletic in the latest morphological study (Mound et al. 2001), and the tribal delimitation and generic relationships remained inconclusive. Although that study made important contributions to our understanding of generic relationships within Panchaetothripinae, the backbone of the phylogenetic tree was poorly supported. In a subsequent re-classification, Bhatti (2006) recognized 7 families, Caliothripidae, Heliothripidae, Panchaetothripidae, Parthenothripidae, Rhipiphorothripidae, Retithripidae, and Tryphactothripidae for species currently placed in Panchaetothripinae. Although based on morphological characters, there was no interpretation of phylogenetic relationships, and this classification requires extensive re-consideration (Mound and Morris 2007).

Given the limitations of morphological classification, mitochondrial and nuclear DNA sequence data could provide more robust hypotheses of phylogenetic relationships and contribute to the development of more stable classifications. Molecular phylogeny has been studied previously for only nine of the 42 genera in Panchaetothripinae tribes: Monilothripini with 2 monobasic genera, undefined by any single apomorphy. However, Monilothripini and Panchaetothripini were not recovered as monophyletic in the latest morphological study (Mound et al. 2001), and the tribal delimitation and generic relationships remained inconclusive. Although that study made important contributions to our understanding of generic relationships within Panchaetothripinae, the backbone of the phylogenetic tree was poorly supported. In a subsequent re-classification, Bhatti (2006) recognized 7 families, Caliothripidae, Heliothripidae, Panchaetothripidae, Parthenothripidae, Rhipiphorothripidae, Retithripidae, and Tryphactothripidae for species currently placed in Panchaetothripinae. Although based on morphological characters, there was no interpretation of phylogenetic relationships, and this classification requires extensive re-consideration (Mound and Morris 2007).

Given the limitations of morphological classification, mitochondrial and nuclear DNA sequence data could provide more robust hypotheses of phylogenetic relationships and contribute to the development of more stable classifications. Molecular phylogeny has been studied previously for only nine of the 42 genera in Panchaetothripinae (Mound et al. 2007, Buckman et al. 2013, Tyagi et al. 2017). However, Mound et al. (2007) and Tyagi et al. (2017) examined only a single nuclear locus (18S rDNA) or a single mitochondrial locus (COI), and Buckman et al. (2013) combined nuclear and mitochondrial markers (18S r DNA, 25S rDNA, Histione 3, Tubulinalpha 1 and COI). These studies confirmed the monophyly of Panchaetothripinae, but the limited taxonomic coverage provided no profound information on superspecific relationships within the subfamily. Although the COI gene has become a reliable marker to discriminate thrips species (Glover et al. 2010, Tyagi et al. 2017), some drawbacks such as heteroplasy, introgression, lack of neutrality, and deviations from the molecular clock indicated that the use of this mitochondrial marker alone is unsuited for inferring phylogenetic relationships (Galtier et al. 2009, Balloux 2010). The use of multiple genes, ideally both mitochondrial and nuclear, is preferable for generating robust phylogeny estimates. Therefore, in the study reported here we employed the mitochondrial COI gene combined with three nuclear genes, the partial elongation factor-1α (EF-1α), the internal transcribed spacer 2 (ITS2), and the third domain of the ribosomal 28S (28S rDNA). These loci have been used in previous DNA barcoding or phylogenetic analysis of thrips (Toda and Komazaki 2002, Inoue and Sakurai 2007, Glover et al. 2010, Tyagi et al. 2017).

Our study involved a broad sampling of all genera and most species of Panchaetothripinae in China, with most of them being examined in a molecular context for the first time. By using a comprehensive approach, we aim to: (1) generate a reference DNA barcodes database for the Chinese Panchaetothripinae fauna, (2) test the effectiveness of DNA barcoding in discriminating these morphologically challenging taxa, and ultimately to detect hidden species diversity, (3) provide a strong backbone tree and define a genus-level phylogeny to permit more focused phylogenetic research and taxonomic revision of specific clades in the future.

### Materials and Methods

**Sampling and Morphological Identification**

Specimens were collected from August 2011 to October 2018, mainly in China with 3 individuals from Indonesia (Supp Table 1 [online only]). Thrips were beaten from plant foliage onto a white plastic plate, and then preserved in 95% ethanol and stored at −20°C. After nondestructive DNA extraction, voucher specimens were slide-mounted into Canada balsam and identified based on morphological characters to species level according to the monograph of Wilson (1975), a series of publications (Bhatti 1967, Kudô 1992, Nonaka and Okajima 1992, Mound et al. 2012, Mirabalbalou et al. 2016, Li et al. 2018). Specimens used in this study were labeled and deposited at Yunnan Agricultural University, China.

**DNA Extraction, Amplification, and Sequencing**

Specimens were pierced individually in an abdominal intersegmental region with a minor sterilized pin according to Rugman-Jones (2006). Total genomic DNA was extracted using the TIANamp Genomic DNA kit (Tiangen Biotech Co., Ltd, Beijing, China) following manufacturer’s protocol, after which the remaining carcasses were retained as vouchers for morphological studies. Four loci (COI, EF-1α, ITS2, and 28S rDNA) were amplified by polymerase chain reaction (PCR) with primers listed in Table 1. All PCRs were performed on an Eppendorf Mastercycler nexus instrument.

| Gene | Name | Sequence (5′–3′) | Source |
|------|------|----------------|--------|
| COI  | LCO1490 | GGTCAACAAATCATAAAGATATTGG | (Folmer et al. 1994) |
|      | HCO2198 | TAAACTTCAGGGTGACCAAAAAATCA | (Folmer et al. 1994) |
| EF1-α| EF1  | GACAACTTTGCGCTTCAACGTAAGACG | (Palumbi 1996) |
|      | EF2  | ATGTGAGCAGTGTGGCAATCCAA | (Palumbi 1996) |
| ITS2 | P1   | ATACACTTGGCTCGTCTGAGATCG | (Moritz et al. 2002) |
|      | S2R  | GTGAGGCACGTTGCCAACATGCCAA | (Moritz et al. 2002) |
| 28S rDNA | 28SA | TCCGARGGAACAGCCTGACTA | (Inoue et al. 2007) |
|      | 28SS | GACCCCTCTTGAAMCGGGA | (Chen et al. 2003) |
(Eppendorf, Germany) under the following protocol: 5 min at 95°C followed by 35 cycles of 10 s at 94°C, 30 s at 47°C, and 1 min at 72°C and with a final extension of 10 min at 72°C. PCR products were visualized on GoldView-stained 1.5% agarose gel. Successful amplifications were bidirectionally sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems) at Sangon Biotech Co., Ltd. (Shanghai, China).

**Data Processing**

Bidirectional sequences were assembled and manually edited in Sequen Pro 7.1.0 (DNASTar, Madison, WI). All sequences generated in this paper were deposited in the GenBank under accession numbers: COI (MT275826–MT275965), EF-1α (MT292657–MT292785), ITS2 (MT316836–MT316964), and 28S (MT294440–MT294568) as indicated in Supp Table 1 (online only). Three species of Aelothripidae were used as outgroups (Genbank number: MT275966–MT275968, MT292786–MT292788, MT316965–MT316967, and MT294568–MT294571). To increase taxon sampling and test further species delimitation ability of COI barcodes in a larger geographical dimension, 31 additional COI sequences assigned to 13 species were downloaded from Genbank (Supp Table 2 [online only]). Finally, we compiled two datasets of Panchaetothripinae, one mainly for species delimitation procedure with 171 COI sequences containing newly generated and GenBank derived data, another for phylogenetic reconstruction with 129 sequences per each for COI, EF-1α, ITS2, and 28S, samples with more than one missing locus were discarded from the analyses. Sequences were aligned separately for each gene using MAFFT version 7.047 (Katoh and Standley 2013), the G-INS-i strategy employing for COI and EF-1α fragments, and the L-INS-i strategy for ITS2 and 28S fragments.Aligned sequences were manual adjustment and trimmed to the appropriate length using MEGA X (Kumar et al. 2018). The aligned gap regions might contain some potentially meaningful phylogenetic information (Redelings and Suchard 2009) therefore were retained for later analysis. Nucleotide substitution saturation within each gene was tested by the Xia test (Xia et al. 2003) implemented in DAMBE version 5.2.74 (Xia 2013), in addition, the transition/transversion versus the genetic distance (F84) was also estimated. The four genes aligned were concatenated into one merged matrix using SequenceMatrix -Windows-1.7.8 (Vaidya et al. 2011).

**COI Barcodes-Based Species Delimitation**

The dataset of 171 mitochondrial COI sequences of Panchaetothripinae was applied to calculate intra- and interspecific genetic divergence values based on the Kimura 2-parameter (K2P) distance model (Kimura 1980), using MEGA X.

To test the reciprocal monophyly of each species, a Bayesian inference (BI) tree of COI barcodes was created in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) under the GTR + I + G model selected by MrModeltest 2.2 (Nylander et al. 2004) according to Akaike information criterion (AIC). The parameters set were: ngen = 30, 000, 000, samplefreq = 1000, nchains = 4, and a burn-in of 25%. Bayesian inference tree mainly focused on whether individuals of each morphospecies clustered together or segregated into different clades, rather than on the evolutionary relationships between species.

Attempting to resolve ambiguous identifications, three methods were used to explore delimitation of molecular species by inferring molecular operational taxonomic units (MOTUs): Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012), the Generalized Mixed Yule Coalescent (GMYC) (Pons et al. 2006, Monaghan et al. 2009, Fujisawa and Barraclough 2013) and bayesian Poisson-Tree-Processes (bPTP) (Zhang et al. 2013, Kapli et al. 2017). ABGD analysis sorts sequences into hypothetical molecular species based on automatic identification of barcode gaps between inter- and intraspecific distances. We performed ABGD analysis on the webserver platform (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with parameters set as: Pmin = 0.001, Pmax = 0.1, Steps = 50, X (relative gap width) = 1.0, Nb bins (distance distribution) = 20 and Kimura (K80) Distance. GMYC and bPTP methods were designed for the analysis of single-locus data, and unique haplotype sequences of COI were extracted from DnaSP 5.10 (Librado and Rozas 2009) for both analyses first. To implement GMYC, an ultrametric tree was generated in BEAST v1.10.4 (Suchard et al. 2018) under an uncorrelated lognormal relaxed clock, GTR + I + G model and Yule process tree prior. The analysis was run for 50 million generations and a sampling frequency of 1,000. Convergence was monitored with Tracer v1.7 (Rambaut et al. 2018). The maximum clade credibility tree was computed using TreeAnnotator (available from the beast 1.10.4 package) and as an input for the single-threshold GMYC analysis. For bPTP analysis, the unrooted phylogenetic tree was drawn in raxmlGUI 1.5 (Silvestro and Michalak 2012) under GTR + G + I model and was run using 100,000 MCMC generations, with a thinning of 100 and burn-in of 0.1.

**Phylogenetic Analysis**

To explore phylogenetic relationships among Panchaetothripinae species, Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed based on a concatenated dataset of the four gene fragments. Three Aelothripidae species (Mymanthus garuda, Franklinthrips tami, and Aelothrips sp.) were used as outgroups. When analyzed phylogenetically, the dataset was partitioned by gene. The first ML phylogenetic tree was reconstructed with IQ-TREE 1.6.8 (Nguyen et al. 2015) under the best-fit substitution model GTR + F + I + G4 for COI, ITS2, and 28S, and SYM + R3 for EF-1α, respectively, according to the AIC criterion by ModelFinder (Kalyaanamoorthy et al. 2017). Support for the inferred ML tree was inferred by ultrafast bootstrap approximation (UFBS) with 10,000 replicates. Standard ML analysis was conducted using raxmlGUI 1.5 (Silvestro and Michalak 2012) under the GTR + G + I model, with a rapid bootstrap analysis of 1,000 bootstrap replicates to assess node support. BI analyses were performed in MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Before BI analysis, GTR + I + G model was selected as the optimal substitution model under the AIC criterion for each gene. Two replicates of Markov Chain Monte Carlo (MCMC) algorithm were run for at least 30,000,000 generations, sampling every 1,000 generations and with the first 25% of samples discarded as burn-in. Convergence was inferred when a standard deviation of split frequencies <0.01 was completed. Tracer v1.7 (Rambaut et al. 2018) was employed to assess convergence and to evaluate the effective sample sizes (EES) for each model parameter. The trees were visualized and edited in the program FigTree v1.4.4 (Rambaut 2018). Bayesian posterior probability (BP) values provide a measure of statistical support at each node.

**Results**

**Genetic Divergence of COI Barcodes**

A total of 140 individuals representing 33 morphological species in 18 genera of Panchaetothripinae were newly amplified and sequenced. No stop codons or indels were found, suggesting no pseudogenes were present in those sequences. Combined with the data download from GenBank, 171 COI sequences belonging to 40
species in 22 genera were used for further analysis: this comprised all genera and 82% of the Panchaethrothripinae species currently recorded in China. Sample size per species ranged from 1 to 12 with an average of 4.3, and 3 species out of 40 were singletons. The aligned sequences were trimmed to 645 bp, except for Heliothrips cephalicus 437 bp, Hercinothrips femoralis and Parthenothrips dracaenae 417 bp, and Neoheliothrips sylvarum 407 bp.

Frequency distribution histograms of K2P pairwise distances were shown in Fig. 1, an overlap between the intra- and interspecific distances of congeneric sequences was observed. The intraspecific divergences ranged from 0 to 13.71% with an average of 1.89%, whereas the interspecific divergences ranged from 1.85 to 18.70% with an average of 12.42%. High intraspecific genetic distances (>3%) were observed in seven species: Rhipiphorothrips pulchellus, Heliothrips rugatus, Astrothrips chisinaoensis, H. aino, H. mube, Caliothrips quadrifasciatus, and H. cephalicus. Low levels of interspecific genetic distances (<2%) were found between two species H. brunneipennis and H. shennongjiaensis. Between two morphologically similar species H. aino and H. mube, ambiguous interspecific distances from 3.85 to 5.69% were detected.

Tree-Based Analysis

The Bayesian inference tree provided high resolution in species discrimination (Fig. 2). The results showed that all species represented by two or more individuals were recovered as monophyletic with moderate to high Bayesian posterior possibilities (BP > 0.95), except for three species in Heliothrips: H. shennongjiaensis, H. aino, and H. mube (BP < 0.7) this value is no support. Seven H. shennongjiaensis individuals together with a singleton H. brunneipennis were grouped in a robust monophyletic clade (BP = 1), with low interspecies divergence of 1.91%. Correspondingly, the intraspecies divergences for H. aino and H. mube were 0.33–5.11% (mean 2.38%) and 0.00–5.69% (mean 2.48%) respectively, and their interspecies divergence was 4.75%, showing an overlap between intra- and interspecific divergences.

Specimens of Rhipiphorothrips pulchellus, Heliothrips rugatus, and Astrothrips chisinaoensis were supported as monophyletic but had deep internal splits in their monophyletic clusters with BP > 0.9 support values. Rhipiphorothrips pulchellus with seven individuals formed three distinct clades. Three specimens from Guizhou and three specimens from Hainan were clustered respectively, with a high internal clade divergence of 14.88%, while the remaining one from Yunnan was distinct from Guizhou and Hainan individuals with mean genetic divergences of 15.32 and 14.18% respectively. Nine specimens of Heliothrips rugatus from Yunnan formed a clade sister to the other two Hainan specimens (10.72% internal clade divergence). Six specimens of Astrothrips chisinaoensis were divided into two clades with a divergence of 8.04%, one clade with specimens from Hainan and Hunan, and the other clade with specimens from Yunnan. Also puzzling was Caliothrips quadrifasciatus that formed two internal clades with BP support values 1 and 0.7 respectively; one clade with 2 specimens from Mengla, Yunnan, and Mengzi, Yunnan, the other with 3 specimens from Lushui, Yunnan, and showing inter-clade divergence of 3.71%.

Molecular Species Delimitation

Molecular operational taxonomic units (MOTUs) are defined as diagnosable molecular lineages that could be delineated by DNA sequences (Vogler and DeSalle 1994, Vogler and Monaghan 2007). The clustering of the MOTUs obtained by the present analysis was shown in Fig. 2. The consensus delimitation scheme based on the three methods yielded 45 MOTUs from 40 morphological Panchaethrothripinae species, but also revealed several conflicts. For ABGD analysis, distinct barcode gaps within the 0.0202–0.0324 distance range were identified, and primary partitions using this priori genetic distance thresholds delineated 43 MOTUs. The bPTP analysis identified 48 MOTUs under the highest Bayesian supported solution and 43 MOTUs under the maximum likelihood solution respectively. We chose the latter as it is close to the results in the other methods. Overall, ABGD and bPTP methods gave the same results, with 32 MOTUs corresponding to morphological species. However, Rhipiphorothrips pulchellus was detected as three MOTUs, and three morphological species (Astrothrips chisinaoensis, Caliothrips quadrifasciatus, and Heliothrips rugatus) were congruently split into two MOTUs, indicating that putative cryptic species may exist. Furthermore, H. aino and H. mube are morphologically similar, these two species clustered as a single MOTU. Surprisingly, two well identified morphological species, H. shennongjiaensis and H. brunneipennis were also recovered as a single MOTU. For the GMYC method, the likelihoods of null model and GMYC model were 320.7642 and 360.3001 respectively, GMYC model was preferred over the null model (likelihood ratio = 79.07179), with single threshold method resulting in 44 MOTUs (confidence interval: 40–51). This result was similar to the number and compositions of MOTUs obtained from ABGD and bPTP, but in different estimates for 4 morphospecies. GMYC was the only method that detected two MOTUs of H. cephalicus, recovered a single MOTU of Caliothrips quadrifasciatus, and successfully discriminated between H. aino and H. mube.

Phylogenetic Reconstruction

The final concatenated dataset included 132 sequences representing 33 species (3 as outgroups), and the rest included all genera of Panchaethrothripinae and about 69.0% of the species morphologically recognized in China. The final concatenated sequences of the four aligned genes yielded 2,935 bp, including 645 bp of COI, 195 bp of EF-1a, 1,642 bp of ITS2, and 453 bp of 28S characters. The phylogenetic signal analysis using a transition/transversion ratio vs. divergence graph and the Xia’s test (P < 0.0001) did not show evidence for substitution saturation, which indicated that the four genes possess adequate signals to infer phylogenetic relationships.

The results of the ML analysis from IQ-TREE and raxmlGUI produced identical topologies (Fig. 3, Supp Fig. 1 [online only]). The BI tree had similar topologies to ML trees, except for the placement of Monilothrips and the differences in support values of several nodes (Fig. 4). Within the subfamily, each genus was recovered.
Fig 2. Bayesian tree based on the COI dataset and the delimited groups obtained by four species delimitation approaches: Morphology, ABGD, GMYC and bPTP. Bayesian posterior probabilities (BP) > 0.8 are indicated at the nodes. Clades that contain potential cryptic species or species complexes are highlighted in blue and orange, respectively. Insect panels provide for four morphospecies: (a) Rhipiphorothrips pulchellus; (b) Astrothrips chisinlaomensis; (c) Caliothrips quadrifasciatus; (d) Heliononthrips rugatus.
Fig 3. Phylogenetic tree from maximum likelihood analysis (IQ-TREE) based on the concatenated dataset of four genes. Ultrafast bootstrap support values (UFBS) > 80 are indicated at the nodes. Clades that contain potential cryptic species or species complexes are not collapse, and with specimens showing voucher ID and collection site on the right.
Fig 4. Phylogenetic tree from Bayesian analysis based on the concatenated dataset of four genes. Bayesian posterior probabilities (BP) > 0.8 are indicated at the nodes. Clades that contain potential cryptic species or species complexes are not collapse, and with specimens showing voucher ID and collection site on the right.
as monophyletic with strong support (UFBS = 100, BP = 1). The genus *Rhipiphorothrips* is the first branch of Panchaetothripinae in both ML and BI analyses, and was recovered as a sister taxon to the rest of the genera with strong support (UFBS = 100, BP = 1). The genus *Phialothrips* with two species formed a sub-basal clade. With strong support in BI (PP = 0.99) while moderate support in ML (UFBS = 90), the remaining Panchaetothripinae genera are subdivided into two sister genus-groups, but inter-relationships remained uncertain. The position of *Moniolothrips* was not satisfactorily resolved here, flipping from one group in ML topologies to another group in BI topologies (Figs. 3 and 4). Two major clades (A and B) were obtained in both ML and BI analyses that are worth more attention (Figs. 3 and 4). Clade A included ten genera in all, tribe Tryphaeothripini with six genera (((Astrothrips + Copadothrips) + Anisoptothrips) + Elixothrips) + (Opinothrips + Noathrips) plus *Zamiothrips* and *Selenothrips* were grouped into a relatively credible cluster with moderate ML but strong BI support (UFBS = 90, BP = 0.99), yet were placed as sister to *Retithrips + Heliothrips* with poor support. Clade B comprised a total of four genera (((Heliothrips + Aoratothrips) + Calothrips) + Avallacothrips) with full support (UFBS = 100, BP = 1). ML analysis placed *Panchaetothrips* and *Moniolothrips* as sister groups to Clade B, whereas BI analysis placed only *Panchaetothrips* as a sister, but both without support (UFBS = 21, BP = 0.42).

Although the monophyly of *Heliothrips* received strong support, the inter-species relationships remained unresolved (Figs. 3 and 4). Similar to the COI Bayesian inference results, *H. mube* and *H. aino* combined formed a strongly supported clade (UFBS = 100, BP = 1), also *H. shennongjaensis* individuals with *H. brunneipennis* nested within it were recovered as one cluster (UFBS = 100, BP = 1). and no sufficient morphological differences could be observed. These species might have experienced strong environmental selection on behavioral or physiological characteristics that resulted in cryptic speciation, but have not yet evolved diagnosable morphological traits (Schönrogge et al. 2002). Overall, the relationship between intraspecific divergence and geographical distance was not strong. For example, sequences of *Heliothrips haemorrhoidalis* obtained from China, Spain, Australia, and United Kingdom lacked barcode divergence, but *Calothrips quadrisulcatus* collected from sites in China <700 km apart showed 2.30% divergence. However, our study demonstrated that once cryptic species were revealed, they usually showed allopatric distribution and a large internal cryptic species divergence. Previous studies of thrips have also hinted at the importance of geographic segregation to cryptic diversity (Tyagi et al. 2017).

In genus *Heliothrips*, the length of antennal sense cones is often regarded as a reliable feature for specific differentiation (Wilson 1975). In *H. brunneipennis* the forked sense cone on antennal segment IV extends to the middle of VIII and is easily distinguished from *H. shennongjaensis* in which this sense cone scarcely surpasses the middle of VI (Fig. 5). Unexpectedly, DNA barcoding showed *Heliothrips shennongjaensis* and *H. brunneipennis* with low interspecific divergence (1.91%) and clustered in a single MOTU (Fig. 2). When concatenated with three nuclear genes, we recovered the same results with *H. brunneipennis* nested within *H. shennongjaensis* in both ML and BI analyses (Figs. 3 and 4).

In addition, *H. aino* and *H. mube* are morphologically similar differing by the yellow extreme base and apex of mid and hind tibiae of *H. mube*.
in *H. aino*, while in *H. mube* the tibiae are yellow only at extreme apex (Fig. 6). However, color of tibiae might vary to some extent, and it is far from clear that this is a valid or stable distinction. Kudô (1992) pointed that these species could be satisfactorily distinguished only in males, with pore plates on abdominal sternites VII–VIII in *H. aino*, but on VI–VIII in *H. mube* (Fig. 6). Nevertheless, Kudô (1992) also mentioned that some males of *H. mube* could have vestigial pore plate on VI (Fig. 6). Our years of collections indeed found some males in *H. mube* populations with absent VI pore plate. With ambiguous inter- and intra-specific distances, DNA barcoding could not resolve the identification of those two morphological species, as both species formed a single MOTU according to ABGD and bPTP approaches, only GMYC separated them into two MOTUs. The concatenated matrix analyses also grouped all samples together and the two species remained unresolved (Figs. 3 and 4). We infer that the two species are potential synonyms, but confirmation requires further morphological, genetic, ecological, and geographical information.

Phylogenetic Relationships Within Panchaetothripinae

This is the most detailed and up-to-date multilocus molecular phylogeny of the subfamily Panchaetothripinae. In agreement with previous molecular (Mound et al. 2007, Buckman et al. 2013) and morphological studies (Mound et al. 2001), our concatenated matrix resolved the monophyly of Panchaetothripinae with confidence, and also supported the reciprocal monophyly of all genera, although the internal relationships showed some contradictions with the previous morphology-based studies (Mound et al. 2001).

Bhatti (2006) proposed placing the genus *Rhipiphorothrips* in a superfamily Rhipiphorothripoidea, based on the mesopleuron angularly produced forward on each side and the mesothoracic spiracle greatly enlarged. However, this classification remains unaccepted by most thrips taxonomists (Mound and Morris 2007, Zhang et al. 2019). Our results recovered the Old World tropical genus *Rhipiphorothrips* at the base of Panchaetothripinae as a sister group to the other genera (Figs. 3 and 4). Species of this genus have a curious rugose sculpture on the head and body that is unique amongst Panchaetothripinae genera. However, features such as the fore wing with postermarginal cilia straight and the absence of anterior fringe cilia are shared with *Phibalothrips*, *Retithrips*, and *Australothrips*, and this might imply a relationship as indicated by Wilson (1975). Indeed, ML and BI analyses both showed *Phibalothrips* as a sub-base branch that is closest to *Rhipiphorothrips* (Figs. 3 and 4). The occurrence of basal polytomies in the multilocus phylogenetic tree probably reflect rapid diversification early in the evolutionary history of the subfamily (Barco et al. 2017). The broader taxonomic samples of COI data as a supplementary result showed *Rhipiphorothrips*, *Retithrips*, *Phibalothrips*, *Australothrips*, and *Heliothrips* formed a monophyletic clade, presumably reflecting their affinity.

The *Retithrips–Astrothrips* clade (Clade A) comprises more than half of the genera in our present study (Figs. 3 and 4). In congruence with a previous morphological study (Mound et al. 2001), *Heliothrips* was revealed as a sister group to *Retithrips*, although with weak support. *Heliothrips* and *Retithrips* originated in different regions, South America and Old World tropics respectively. However, physiologically, the members of these two genera are polyphagous and live on older leaves, not on newly emerged leaves,
and *H. haemorrhoidalis* and *R. syriacus* are particularly widespread as important pests on various agricultural and horticultural crops.

Our results refute Wilson’s three tribe division of subfamily Panchaetothripinae (Wilson 1975) because two of the three tribes, Panchaetothripini and Monilothripini were recovered as polyphyletic or paraphyletic, only tribe Tryphactothripini with six genera formed a convincing monophyletic clade. Two genera *Zaniothrips* and *Selenothrips* were related to the tribe Tryphactothripini, grouping into a moderately supported cluster. However, this relationship needs further testing, because no reasonable morphological apomorphy is shared between them. The previous morphology-based study also showed *Zaniothrips* and *Selenothrips* formed a monotypic clade, together with *Euhydratothrips*, *Xestothrips*, *Brachyurothrips*, *Chaeturothrips*, and *Panchaetothrips* (Mound et al. 2001).

Another major clade (Clade B) with strong statistical support contains four genera *Helionothrips*, *Caliothrips*, *Araliacothrips*, and *Aoratothrips* (Figs. 3 and 4), of which the first two are the most species-rich genera within Panchaetothripinae. The four genera shared common character states such as the antenna 8-segmented, segments III and IV each constricted into an apical neck and with developed long forked sense cone, head, and pronotum. The monobasic genus *Insularis* was placed at the basal branch of Clade B and was fully supported as a sister to the other three genera, *Helionothrips*, *Caliothrips*, and *Aoratothrips*. The original description of this genus has indicated this genus is the colorless body, elevated cylindrical ocellar hump and elongate mouth cone of *Aoratothrips* are distinct from *Helionothrips*. The worldwide distributed genus *Caliothrips* is weakly supported as a sister to *Aoratothrips* + *Helionothrips*. Mound and Infante (2017) discussed the patterns of fore wing color and tergal sculpture of the 23 *Caliothrips* species, but could not deduce clear relationships among these various species. Our multilocus analysis of a limited number of *Caliothrips* species indicated a well resolved (*C. quadrifasciatus* + (*C. tongi* + *C. indicus*)) as the sister group of *C. insularis*. The monobasic genus *Araliacothrips* was placed at the basal branch of Clade B and was fully supported as a sister to the other three genera, *Helionothrips*, *Caliothrips*, and *Aoratothrips*. The original description of this genus has indicated this genus resembles morphologically both *Helionothrips* and *Aoratothrips*. Currently, *Araliacothrips* is known only from Southwestern China.

The Old World tropical genus *Panchaetothrips* is allied to Clade B with weak support (Figs. 3 and 4). The tube-like abdominal segment X of *Panchaetothrips* species is unusual in Panchaetothripinae, as is the conspicuous array of stout spines on the terminal abdominal segments. However, the phylogenetic significance of these character states remains unclear. Our ML results suggest that *Monilothrips* is the sister genus of *Panchaetothrips*, but that relationship has low support and is unresolved in the BI analysis. Wilson (1975) placed *Monilothrips* and *Zaniothrips* in tribe Monilothripini because both of these genera have very long pronotal setae and lack strong pronotal sculpture. In contrast, both a morphology-based study (Mound et al. 2001) and this molecular analysis reject this classification. The exact position of the genus *Monilothrips* within Panchaetothripinae remains uncertain.

**Conclusion**

DNA barcoding results from this study are valuable in the identification of species of Panchaetothripinae. Comprehensive molecular analyses provided herein indicate that thrips biodiversity is underestimated due to the presence of cryptic species. Our results also point to some ambiguous taxonomic decisions that need further investigation, such as *H. aino* and *H. mube*, and also *H. shennongiaensis* and *H. brunneipennis*. Furthermore, using multilocus methods we demonstrate reciprocal monophyly of all genera, and to provide a novel perspective of generic phylogenetic relationships within Panchaetothripinae. Although our study is only a partial picture of the phylogenetic relationships within this subfamily, it provides useful information on the thrips fauna of China as well as a basis for broader studies in other parts of the world. We hope this study will trigger further research using broader taxon sampling to establish a larger barcode dataset for species identification, as well as broader phylogenetic studies within Panchaetothripinae including morphological, genetic, ecological and biogeographic information.

**Conflict of Interest**

The authors declare that there is no conflict of interest with regard to this work.

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**Author Contributions**

H.R.Z. and Y.J.L. conceived and designed the study; Y.J.L., Y.L.X., E.F.B.L., performed the experiments and sub-tropics, with one aberrant species, *H. fanebris*, from South America (Wang et al. 2017). Interestingly, our phylogenetic analysis found *Aoratothrips* was closest to *Helionothrips*, although the colorless body, elevated cylindrical ocellar hump and elongate mouth cone of *Aoratothrips* are distinct from *Helionothrips*. The worldwide distributed genus *Caliothrips* is weakly supported as a sister to *Aoratothrips* + *Helionothrips*. Mound and Infante (2017) discussed the patterns of fore wing color and tergal sculpture of the 23 *Caliothrips* species, but could not deduce clear relationships among these various species. Our multilocus analysis of a limited number of *Caliothrips* species indicated a well resolved (*C. quadrifasciatus* + (*C. tongi* + *C. indicus*)) as the sister group of *C. insularis*. The monobasic genus *Araliacothrips* was placed at the basal branch of Clade B and was fully supported as a sister to the other three genera, *Helionothrips*, *Caliothrips*, and *Aoratothrips*. The original description of this genus has indicated this genus resembles morphologically both *Helionothrips* and *Aoratothrips*. Currently, *Araliacothrips* is known only from Southwestern China.

The Old World tropical genus *Panchaetothrips* is allied to Clade B with weak support (Figs. 3 and 4). The tube-like abdominal segment X of *Panchaetothrips* species is unusual in Panchaetothripinae, as is the conspicuous array of stout spines on the terminal abdominal segments. However, the phylogenetic significance of these character states remains unclear. Our ML results suggest that *Monilothrips* is the sister genus of *Panchaetothrips*, but that relationship has low support and is unresolved in the BI analysis. Wilson (1975) placed *Monilothrips* and *Zaniothrips* in tribe Monilothripini because both of these genera have very long pronotal setae and lack strong pronotal sculpture. In contrast, both a morphology-based study (Mound et al. 2001) and this molecular analysis reject this classification. The exact position of the genus *Monilothrips* within Panchaetothripinae remains uncertain.

**Supplementary Data**

Supplementary data are available at *Journal of Insect Science* online.

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