Taiwania 68(4): 398–406, **2023**
DOI: 10.6165/tai.2023.68.398

Genetic profile of the genus *Trichromothrips* (Thysanoptera: Thripidae) from China based on three molecular markers

Chun-Ying WU[1,2,3], Ya-Jin LI[1,2], Yun-Yu WANG[1,2,3], Hao-Ran GAO[1], Hong-Rui ZHANG[1,2,*]

1. *Plant Protection College, Yunnan Agricultural University, Kunming, Yunnan 650201, China.* ***2.*** *State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan Agricultural University, Kunming, Yunnan 650201, China.* ***3.*** *State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, China Academy of Sciences, Kunming, Yunnan 650201, China. *Corresponding author's email: hongruizh@126.com*

(Manuscript received 13 July 2023; Accepted 18 September 2023; Online published 25 September 2023)

ABSTRACT: Thrips are tiny insects that are difficult to identify morphologically, but molecular marker has become a well-established method of identification. To explore the phylogenetic relationships among species in the genus *Trichromothrips* of the large family Thripidae, 3 genes: a mitochondrial gene, COI (cytochrome c oxidase subunit I), and two nuclear genes, ITS2 (internal transcribed spacer 2) and 28S (28S ribosomal DNA), were used to construct single-gene and multi-locus phylogenetic trees of 24 samples in seven species. The single-gene BI (Bayesian inference) trees have different results, revealing the possible instability of constructing phylogenetic trees from single genes in this genus. Therefore, multi-locus phylogenetic trees were constructed using the BI and ML (Maximum likelihood) methods, and the results differ from all single-gene trees as well. Furthermore, this study proposed one potential new species (*T.* sp. "Jinping, Yunnan") and two newly recorded species (*T. alis* and *T. formosus*) in China. The results of this study improved our understanding of the evolutionary relationships among species within the genus *Trichromothrips*.

KEY WORDS: Genetic profile, molecular marker, new record, *Trichromothrips alis*, *Trichromothrips formosus*, Thrips.

INTRODUCTION

Insects in the family Thripidae are small, highly cryptic, and easily develop resistance to chemical pesticides. Thrips have gradually become one of the most important agricultural pests worldwide because of high reproductive rates, feeding activities, and transmission of viruses (Stuartv *et al.*, 2011). To study thrips biological characteristics and management, the correct identification of thrips species is essential.

Because of the small size of thrips, identifying species based on morphological characteristics alone is challenging. According to Mound *et al.* (2001), morphological data alone cannot establish reliable phylogenetic relationships, and molecular data are needed for verification. DNA barcoding and molecular marker are helpful to recognize species, and these have been used to identify thrips (Kumar *et al.,* 2014; Tyagi *et al.,* 2015*,* 2017a; Oktarianti *et al.*, 2021; Xie *et al*., 2022). The DNA data can quickly and accurately provide molecular-level information to verify morphological classification systems and establish phylogenetic relationships.

The genus *Trichromothrips* Priesner, 1930 belongs to the subfamily Thripinae, family Thripidae, and order Thysanoptera. It contains many species, with 38 species recorded globally and 16 species recorded in China (Li *et al.*, 2019; Bhatti *et al.*, 2000; Mound *et al.*, 2004). The genus is closely related to *Octothrips* (Moulton, 1940) and is part of the *Trichromothrips* genus group (Tyagi *et al.,* 2017a). *Trichromothrips* thrips are medium in size with body length about 1.0-1.6 mm, primarily leaf-feeding, fragile, and slender. The thrips usually have a uniform pale or contrasting two-color body and are often brightly colored when alive (Masumoto *et al.*, 2005, 2012; Tyagi *et al.,* 2017b). It is important to identify such morphologically complex species of which little is known. In this study, on the basis of previous morphological studies (Tyagi *et al.,* 2017a,b; Li *et al.*, 2019), phylogenetic relationships within the genus were explored.

Three gene sequences were selected as molecular markers to help identify and differentiate between these species. The mitochondrial COI (cytochrome c oxidase subunit I) gene, which is usually used as a DNA barcode, is the most widely used molecular marker. Nuclear genes ITS2 (internal transcribed spacer 2) and 28S (28S ribosomal DNA) have also been commonly used in previous studies on thrips (Inoue *et al.,* 2007; Hoddle *et al.,* 2008; Glover *et al.,* 2010; Buckman *et al.,* 2013; Kumar *et al.,* 2017; Marullo *et al.,* 2020). The ITS2 gene is located between 5.8S rDNA and 28S rDNA (Bianciardi *et al.,* 2012). It is a non-coding region that is under less selective pressure and has relatively high significant relative variation. Therefore, ITS2 can provide a heritable trait needed for detailed phylogenetic analysis and is often used as a marker of genetic profile. Because of the high sequence variability, ITS2 is usually applied in lower taxonomic categories, especially for species recognition in plant, insect, and aquatic life (Kumar *et al.,* 2017; Glover *et al.,* 2010; Oktarianti *et al.,* 2021). As the

Table 1. *Trichromothrips* sample information used in this study.

Species	Voucher ID	Locality	Date	Host plant
T. alis	TH215300	Yanzi Cave, Jianshui, Yunnan	Apr. 2, 2019	*Poaceae barnhart*
T. elegans	TH103700	Chengdu botanical garden, Sichuan	Oct. 2, 2016	*Pteridiaceae*
T. elegans	TH103800	Chengdu botanical garden, Sichuan	Oct. 2, 2016	*Pteridiaceae*
T. elegans	TH216701	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. elegans	TH216702	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. elegans	TH216703	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. formosus	TH216401	XTBG, Menglun Yunnan	Nov. 18, 2018	*Bischofia polycarpa*
T. formosus	TH216402	XTBG, Menglun Yunnan	Nov. 18, 2018	*Bischofia polycarpa*
T. fragilis	TH210601	Wuding, Yunnan	Nov. 2, 2020	*Eleusine indica*
T. fragilis	TH210602	Wuding, Yunnan	Nov. 2, 2020	*Eleusine indica*
T. fragilis	TH210603	Wuding, Yunnan	Nov. 2, 2020	*Eleusine indica*
T. fragilis	TH216801	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. fragilis	TH216802	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. fragilis	TH216803	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. fragilis	TH216804	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. fragilis	TH216805	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. fragilis	TH216806	Maobiling, Xijiang, Guizhou	Aug. 5, 2017	*Stachyurus chinensis*
T. guizhouensis	TH215801	Maolan reserve, Guizhou	Sep. 5, 2019	*Artabotrys hexapetalus*
T. guizhouensis	TH215802	Maolan reserve, Guizhou	Sep. 5, 2019	*Artabotrys hexapetalus*
T. guizhouensis	TH215803	Maolan reserve, Guizhou	Sep. 5, 2019	*Artabotrys hexapetalus*
T. guizhouensis	TH215804	Maolan reserve, Guizhou	Sep. 5, 2019	*Artabotrys hexapetalus*
T. sp. "Jinping, Yunnan"	TH215100	Jinping, Yunnan	Apr. 1, 2019	*Isodon amethystoides*
T. trifasciatus	TH211801	Diaoluoshan reserve, Hainan	Jan. 8, 2021	*Ligustrum lucidum*
T. trifasciatus	TH211802	Diaoluoshan reserve, Hainan	Jan. 8, 2021	*Ligustrum lucidum*

28S rDNA gene is part of the nuclear DNA (nDNA) that encodes 28S rRNA, it is generally more conserved than the ITS2 region. Mitochondrial DNA (mtDNA) follows unique maternal inheritance characteristics, while nDNA inherits genetic information from both parents. In general, phylogenetic studies that consider both mtDNA and nDNA information can provide more comprehensive information on the evolution of species within a genus (Inoue *et al.,* 2007; Buckman *et al.,* 2013; Xie *et al.,* 2022). Therefore, in this study, COI, ITS2, and 28S genes were selected to explore the phylogenetic relationships among species in the genus *Trichromothrips*.

MATERALS AND METHODS

Sampling and morphological identification

Specimens were collected from southwestern China during 2016-2021 (**Table 1**) and stored in 75% ethanol at -20°C until DNA extraction. One to six specimens were collected of each species. After DNA extraction, which caused very little damage to specimens, specimens were mounted in Canada balsam on glass slides for morphological identification. Specimens were identified with standard morphological keys (Li *et al.*, 2019; Bhatti *et al.*, 2000). All specimens were deposited at Yunnan Agricultural University, Plant Protection College, Entomology Department, Kunming, Yunnan, China.

DNA sequencing and sequence procession

Thrips DNA was extracted following Buckman *et al.* (2013). TIANamp Genomic DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) was used to obtain genomic DNA solutions. Sequences of COI, ITS2, and 28S were amplified using TaKaRa Ex Taq hot start version DNA polymerase (Takara Bio Inc., Dalian, China). Three fragments of the mitochondrial genes COI and the nuclear genes ITS2 and 28S were amplifed and sequenced using the following primers: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG -3'; Folmer *et al.,* 2003) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA -3'; Folmer *et al.,* 2003), P1 (5'-ATCACTCGGCTCGTGGATCG-3'; Moritz *et al.,* 2002) and 52R (5'-GTTAGTTTCTTTTCCTCCCCT -3'; Moritz *et al.,* 2002), and 28SA (5'- TCGGARGGAACCAGCTACTA -3'; Inoue *et al.,* 2007) and 28SS (5'-GACCCGTCTTGAAMCAMGGA-3'; Chen *et al.,* 2003). Oligonucleotide primers were purchased from Tsingke Biotechnology Co., Ltd. (Beijing, China). PCR amplifications were carried out in a 25 μl reaction volume using the following protocol: an initial denaturing step at 95°C for 4 min, followed by 35 cycles of denaturing at 94°C for 40 s, annealing at 55°C for 1 min, and extending at 72°C for 1 min. The final extension was performed at 72°C for 10 min. The PCR products were sequenced using an ABl3730x1DNA automated sequencer (Applied Biosystems, UK).

Sequences were assembled, edited, and MUSCLE-aligned using Geneious Prime® 2022.2.1 (Biomatters Ltd Auckland, New Zealand.), FasParser (Sun, 2017), and,

Table 2. GenBank accession numbers of ***Tricromopthrips*** used in this study.

Species	Voucher ID	Genbank accession numbers		
		COI	ITS2	28S
Trichromothrips alis	TH215300	OP712631	OP724343	OP714422
Trichromothrips elegans	TH103700	OP712634	OP724350	OP714423
Trichromothrips elegans	TH103800	OP712635	OP724351	OP714424
Trichromothrips elegans	TH216701	OP712643	OP724335	NA
Trichromothrips elegans	TH216702	NA	OP724336	OP714425
Trichromothrips elegans	TH216703	OP712644	OP724337	OP714426
Trichromothrips formosus	TH216401	OP712632	OP724344	OP714436
Trichromothrips formosus	TH216402	OP712633	OP724345	OP714437
Trichromothrips fragilis	TH210601	OP703301	OP724348	OP714438
Trichromothrips fragilis	TH210602	OP703302	OP724349	OP714439
Trichromothrips fragilis	TH210603	OP703303	NA	OP714440
Trichromothrips fragilis	TH216801	OP703304	OP724352	OP714431
Trichromothrips fragilis	TH216802	OP703305	OP724353	OP714432
Trichromothrips fragilis	TH216803	OP703306	OP724354	OP714433
Trichromothrips fragilis	TH216804	OP703307	OP724355	OP714434
Trichromothrips fragilis	TH216805	OP703308	OP724356	OP714435
Trichromothrips fragilis	TH216806	OP703309	OP724357	NA
Trichromothrips guizhouensis	TH215801	OP712638	OP724339	OP714427
Trichromothrips guizhouensis	TH215802	OP712639	OP724340	OP714428
Trichromothrips guizhouensis	TH215803	OP712640	OP724341	OP714429
Trichromothrips guizhouensis	TH215804	OP712641	OP724342	OP714430
Trichromothrips sp. "Jinping, Yunnan"	TH215100	OP712642	OP724338	OP724334
Trichromothrips trifasciatus	TH211801	OP712636	OP724346	OP714441
Trichromothrips trifasciatus	TH211802	OP712637	OP724347	OP714442
Thrip knoxi (outgroup)	TH200201	OQ933018	OQ999872	OR005037

MEGA X (Kumar *et al.,* 2018). All sequences were blasted in GenBank to ensure they were the target sequences and then were submitted to obtain GenBank accession numbers (**Table 2**). Finally, COI gene sequences of 661 bp, 28S gene sequences of 357 bp, and the ITS2 genes ranging from 489 to 645 bp, were used for further analysis. When sequences were aligned, there were many gaps which also have phylogenetic information. Therefore, the aligned ITS2 sequences were trimmed to a total of 782 characters.

Phylogenetic analysis

The three aligned gene (COI 661 bp, ITS2 782 bp, and 28S 357 bp) sequences were concatenated for each specimen by PhyloSuite (Zhang *et al.*, 2020). The entire sequence length is 1800 characters. Different genes often perform different biological functions and may therefore be subject to different selection pressures, leading to different rates of evolution. So we partition by gene and choose the best evolutionary model for each gene. PartitionFinder2 (Lanfear *et al.*, 2017), the greedy algorithm (Lanfear *et al.*, 2012), and the AICc (Akaike Information Criterion, corrected) criterion were used to select the best evolutionary model.

The maximum likelihood (ML) phylogenies were derived using IQ-TREE (Nguyen *et al.*, 2015) under an edge-linked partition model using 5,000 ultrafast bootstraps (Minh *et al.*, 2013) and an approximate likelihood-ratio test using the Shimodaira-Hasegawa-like theory (Guindon *et al.*, 2010).

Bayesian inference (BI) phylogenies were inferred using MrBayes 3.2.7 (Ronquist *et al.*, 2012) under the partition best fit model (two parallel runs, 2,000,000 generations), with the initial 25% of sampled data discarded as burn-in. The convergence of the Bayesian Inference tree was tested using the average standard deviation of split frequencies below 0.01.

Three single-gene (COI, ITS2, and 28S) trees were also constructed separately using PartitionFinder2 (Lanfear *et al*., 2017) and MrBayes 3.2.7 (Ronquist *et al*., 2012) for reference. These trees were included in the supplementary section of the article. *Thrip knoxi* of the genus *Thrip* (subfamily Thripinae, family Thripidae) was used as the outgroup in both single-gene and multi-locus trees.

RESULTS

Morphological analyses

24 samples of 7 species in the genus *Trichromothrips* were identified (**Fig. 1**). The species included: *T. alis*, *T. elegans*, *T. fragilis*, *T. formosus*, *T. guizhouensis*, *T. trifasciatus*, and *T*. sp. "Jinping, Yunnan".

Multi-locus ML and BI tree

Molecular experiments were performed on 1-6 individuals of each species. The thrips of each species

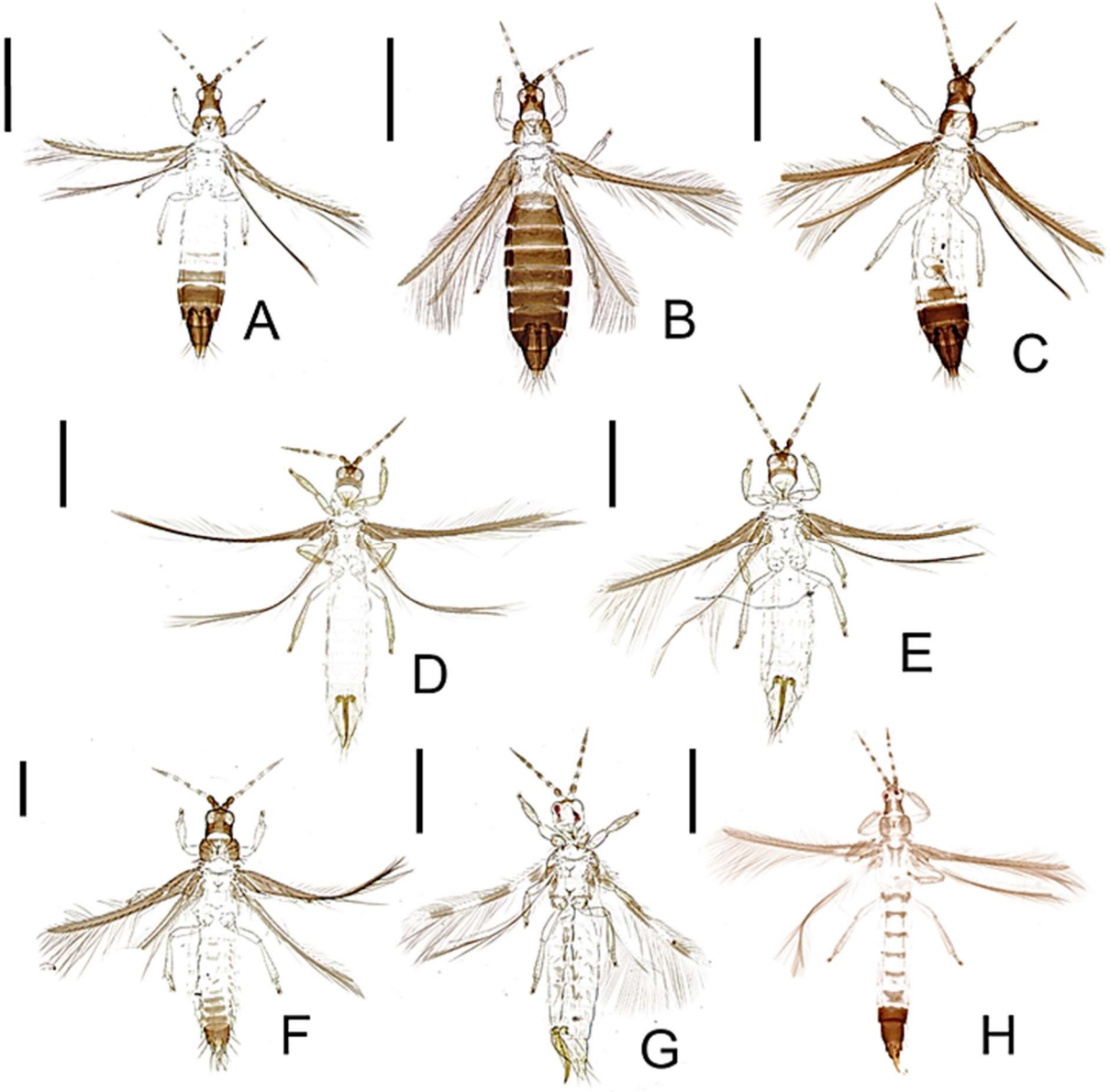

Fig. 1. ***Trichromothrips*** genus species. **A.** ***Trichromothrips alis***, female; **B.** ***T. elegans***, female; **C.** ***T. formosus***, female; **D.** ***T. fragilis*** "Yunnan, Wuding", TH210601, female; **E.** ***T. fragilis*** "Guizhou, Xijiang", TH216701, female; **F.** ***T. guizhouensis***, female; **G.** ***T. trifasciatus***, female; **H.** *T.* sp. "Jinping, Yunnan". Scale: 300 μm.

were morphologically identical, and molecularly clustered into the same clades. *T.* sp. "Jinping, Yunnan" TH215100 had not been described or collected before. It was only one specimen, tehrefore we defined it as *T.* sp. "Jinping, Yunnan" here. The multi-gene concatenated sequences are partitioned by gene, and the best-fit models for each of the three genes (COI, ITS2, and 28S) are GTR+I+G, SYM+G, and TRN+G respectively. These models are then used to construct the phylogenetic trees. The single-gene BI trees were provided in the Supplementary section. Different single-gene trees showed different results, indicating the possible instability of single-gene construction of phylogenetic trees of this genus.

Since the ML and BI methods produce topologically consistent multi-locus phylogenetic trees, we matched the ML tree to the BI tree to make it more concise (**Fig. 2**). On the whole, the same species were well clustered together, and the *Trichromothrips* species were divided into two clades. The first clade includes *T. farmosus*, *T. alis*, *T. elegans*, *T.* sp. "Jinping, Yunnan", and *T. guizhouensis*, of which *T. farmosus* and *T. alis* are new records in China, and *T.* sp. "Jinping, Yunnan" is a potential new species. *T.* sp. "Jinping, Yunnan", and *T. guizhouensis* form a sister group. The second clade includes *T. trifasciatus* and *T. fragilis*. *T. trifasciatus*

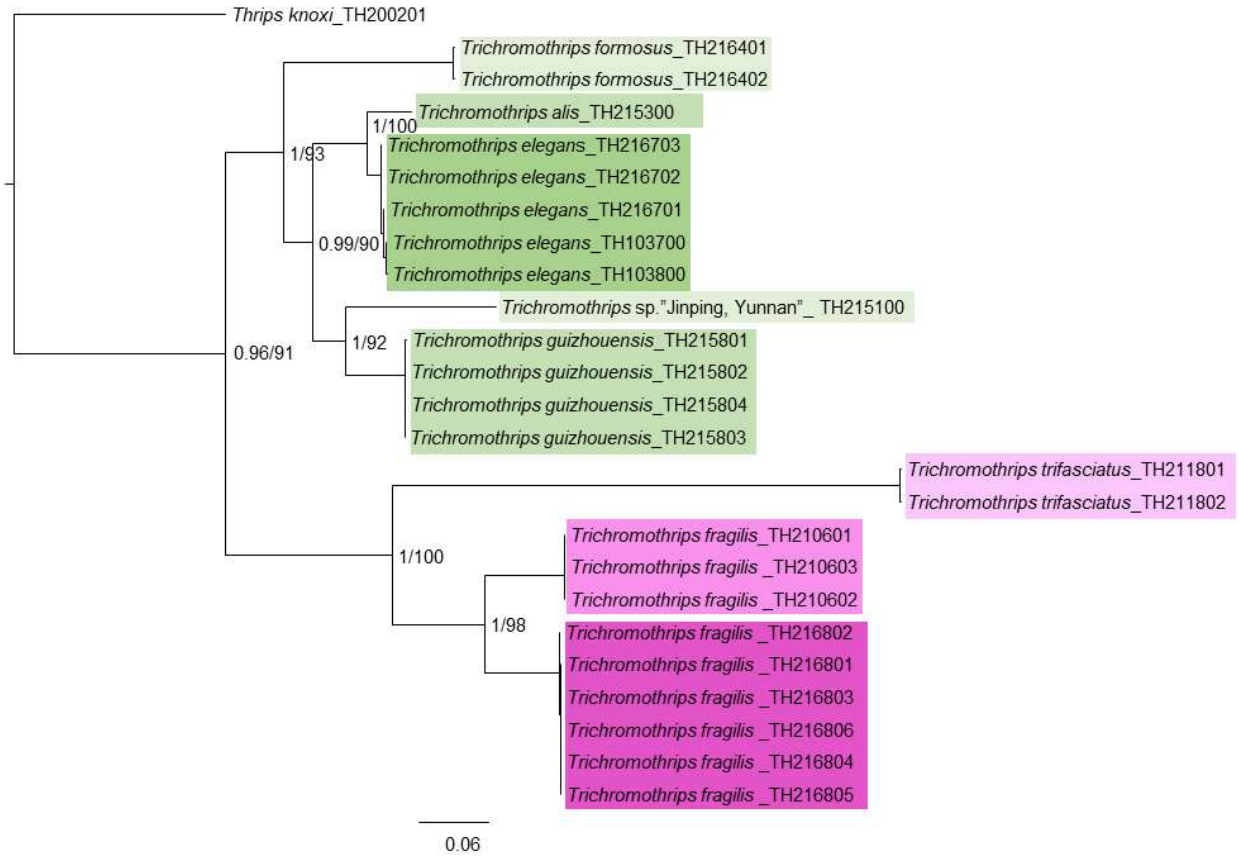

Fig. 2. Multi-locus ML & BI tree of ***Trichromothrips*** was based on three genes (COI, ITS2, and 28S), with ***Thrips knoxi*** as the outgroup. Numbers at nodes indicate the Bayesian posterior probabilities/bootstrap percentage.

forms a monophyletic clade. *T. fragilis* forms two sub-branches with distinct branch length, each forming a single branch, and the support rate of nodes is high, indicating that there may be cryptic species. Although the two *T. fragilis* taxa (lineages *T. fragilis*_TH2106 and *T. fragilis*_TH2168) were so similar that they cannot even be distinguished in morphology, they were divided into two different lineages in the molecular phylogenetic tree.

TAXONOMIC TREATMENT

Trichromothrips alis (Bhatti, 1967)

棘异色蓟马 Fig. 1A, 3A-D

***Specimen examined*: CHINA.** 1 female, Yanzi Cave, Jianshui County, Yunnan Province Yanzi Cave, Jianshui County, Yunnan Province, host on *Poaceae barnhart*, 2 April 2019, leg. *C.Y. Wu*, deposited in Yunnan Agricultural University (YNAU), Kunming.

***Remark*.** The species was first described from Sibpore, India from grass (Bhatti, 1967). This species is newly recorded from Yunnan Province, China.

***Diagnosis*.** Long-winged, body bicolored, yellow legs. Antennae dark brown, 8 segments, segments I-II dark brown, III brown, IV-V base yellow-white and end brown, VI-VIII light brown. Head brown, slightly lighter near the posterior margin, its longer than wide, a strong constriction behind the compound eyes, transverse stripes behind the compound eyes. Mouth short and round. The pronotum is brown with a pale area in the center and brown areas on both sides that converge. The dorsal plates of abdominal segments I-VII are yellow, while those of segments VIII-IX are brown. The forewings are brown.

Trichromothrips formosus Masumoto & Okajima, 2005

美丽异色蓟马 Fig. 1C, 4A-C

***Specimen examined*: CHINA.** 2 female, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science, Yunnan Province, host on *Bischofia polycarpa*, 18 November 2018, leg. *C.Y. Wu*, deposited in Yunnan Agricultural University (YNAU), Kunming.

***Remark*.** This species was first time recorded from China mainland.

***Description*.** Long-winged, body yellowish brown, yellow-white legs. Antennae brown, 8 segments, segments I-II dark brown, III-VI base yellow and end brown, V-VII light brown. Head brown with a small light area at the posterior edge only; back plate of the head has transverse stripes behind the single eye and compound eye, slightly sunken behind the compound eye. Mouth short and round. The pronotum has brown bands on both

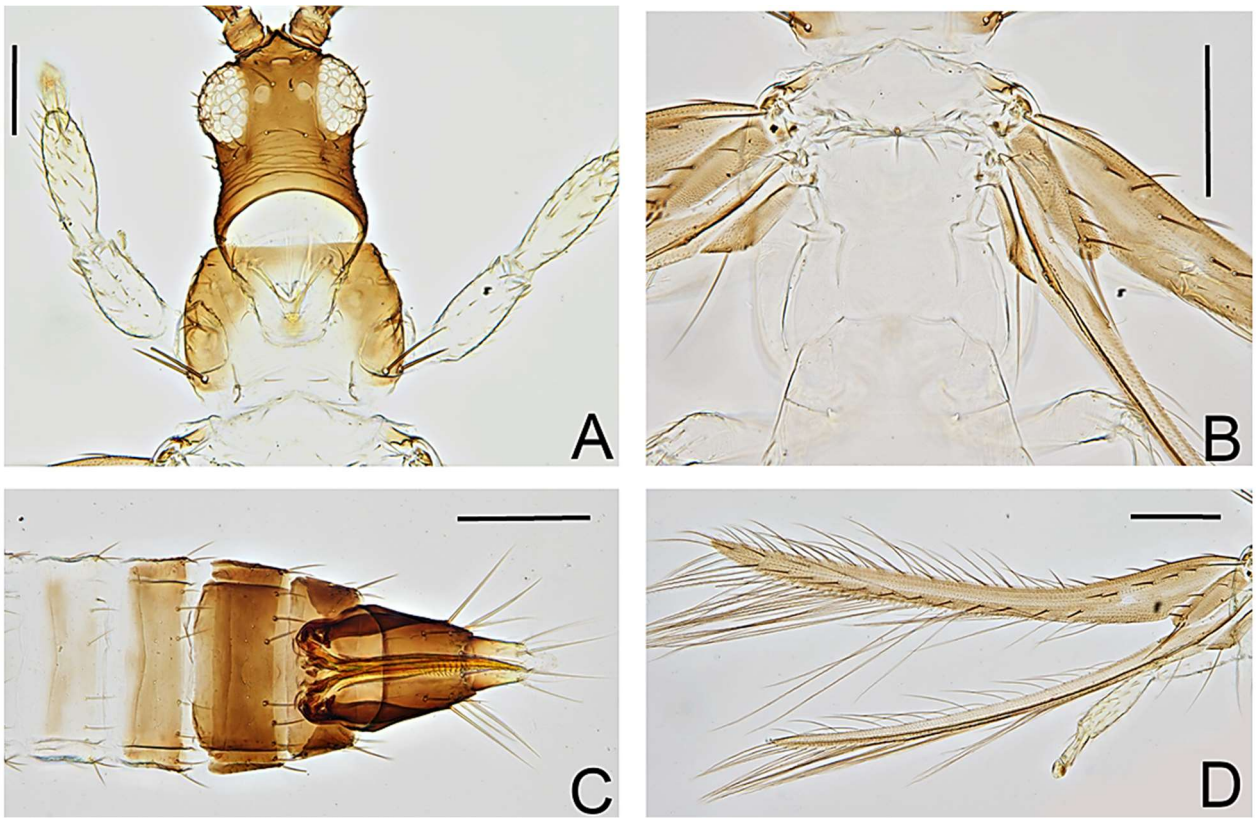

Fig. 3. ***Trichromothrips alis***, female. **A.** Head and prothorax; **B.** Meso- and metanotum; **C.** Abdominal tergites V–X; **D.** Wings. Scale bar = 50 μm.

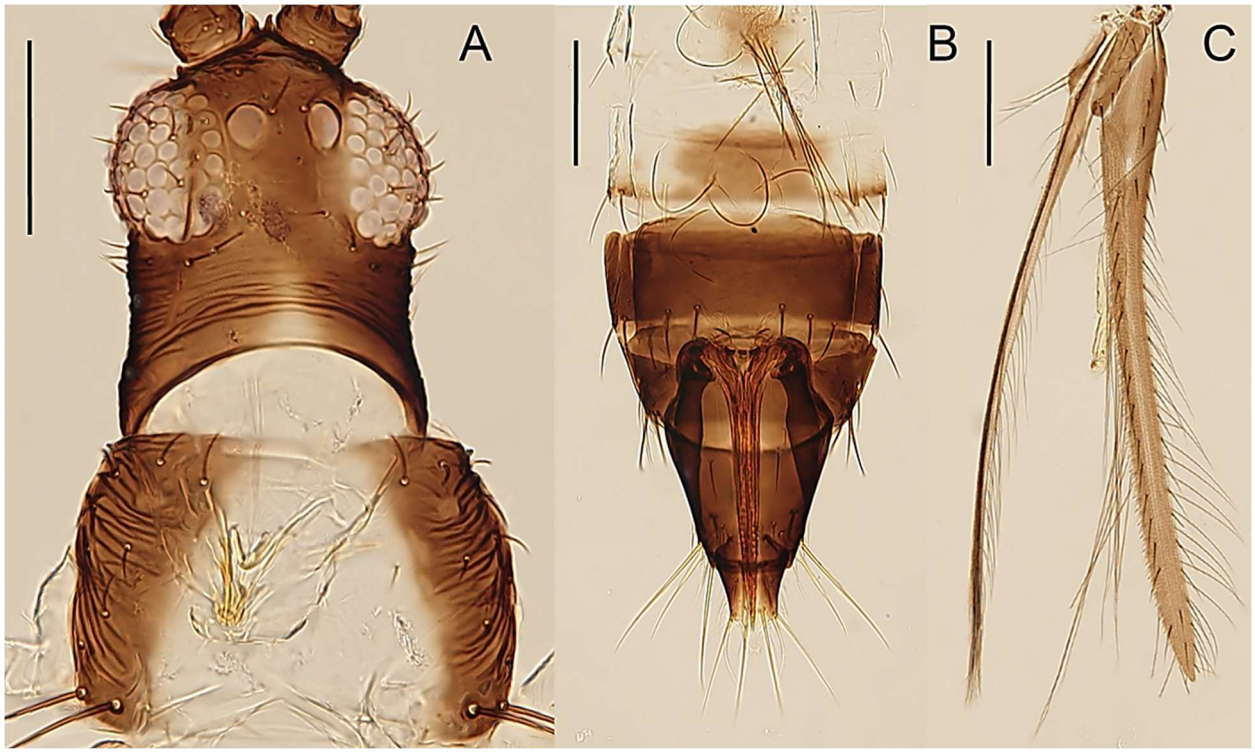

Fig. 4. ***Trichromothrips formosus***, female. **A.** Head and prothorax; **B.** Abdominal tergites V–X; **C.** Wings. Scale bar = 50 μm.

sides and a triangular pale area in the middle that extends to the anterior margin. The mesonotum has a pair of bristles before the submedian suture. The mesoscutum is smooth in the middle. The dorsal plate of the abdomen is smooth in the middle with transverse stripes on both sides. The tergites VI-IX are brown, tergite X has a brown base and yellow apex. The forewings are dark brown with cilia on the posterior margin wavy.

***Trichromothrips* sp**. "Yunnan, Jinping"

Fig. 1H

***Specimen examined*: CHINA.** 1 male, Jinping county, Yunnan province, host on *Isodon amethystoides*,1 April 2019, leg. *C.Y. Wu*, deposited in Yunnan Agricultural University (YNAU), Kunming.

***Remark*.** The only one specimen of this *Trichromothrips* sp. was a male collected from Jinping County, Honghe Hani and Yi Autonomous Prefecture, Yunnan Province, China in April, 2019, and its host was *Isodon amethystoides*. This potential new species could not be published at present because of the lack of specimens.

DISCUSSION

In the present study, phylogenetic relationships were established based on single genes and multiple loci by using three gene sequences in 24 individuals from 7 species of the genus *Trichromothrips*. Although some important and valid morphological studies have been conducted on thrips of the genus *Trichromothrips* (Mound *et al.*, 2004; Masumoto *et al.*, 2005; Li *et al.*, 2019; Bhatti *et al.*, 2000; Masumoto *et al.*, 2012; Tyagi *et al.*, 2017b), there is a gap in molecular studies. In this study, DNA data were used, which also provided new ideas for future molecular studies of other genera and a more robust classification system for higher taxa. Therefore it further provides important theoretical basis and practical guidance for fields such as taxonomy, ecology, and evolutionary biology.

This study added barcoding data of multiple species of the genus *Trichromothrips* to the NCBI (National Center for Biotechnology Information). The study collected lineages *T. fragilis*_TH2106 and *T. fragilis*_TH2168 in different locations and different hosts (**Table 1**) but were both within *T. fragilis*. The distinguishing features of *T. fragilis* are a brown head, well-developed postocular setae, and the presence of metanotal campaniform sensilla. The two lineages were separated in both single-gene trees and multi-locus trees, and in the COI gene, the molecular genetic distance was greater than 2% (**Table S1**). Therefore, the lineages could be considered distinct species. The study suggests that variations in the three genes of *T. fragilis* did not affect morphological characteristics. Morphological evolution is generally slower than molecular evolution. What is clear from this study is that the morphologically same insect species collected from different locations and different host plants may differ genetically.

Many studies have shown that Yunnan Province of China has a very rich species diversity and is a hotspot of biodiversity research (Yu *et al.,* 2022; Agung *et al.,* 2022; Zhang *et al.,* 2023). In fact, the potential new species *T.* sp. "Yunnan, Jinping" and two newly recorded species *T. alis* and *T. formosus* in China of the genus *Trichromothrips* used in this study were found in Yunnan Province. In the background of the sixth mass extinction, further in-depth research and exploration of insect diversity in the region is very important work. In addition to morphological identification, molecular tools will be a faster way to investigate new species. Morphological identification is basic and necessary work that requires a skilled professional. However, even experts may not be able to accurately identify females, males, or larvae with less obvious characteristics of similar species or incomplete specimens (Austen *et al.*, 2016; Gooliaff *et al.*, 2018). For thrips, size of individual is so small and varied that collecting different species is very difficult, which has hindered identification, classification, and phylogenetic research. However, effective molecular markers could help obtain information for molecular classification of thrips and other microscopic insects (Mehle *et al.*, 2012; Tyagi *et al.,* 2017a; Oktarianti *et al.*, 2021; Xie *et al.,* 2022), allowing greater detail in identification and classification.

This study provides an approach to use molecular information to identify microscopic insects and examine phylogenetic relationships among species. The genes COI, ITS2, and 28S successfully separated specimens into the correct species in the genus *Trichromothrips* in separate phylogenetic trees to reflect relationships among the seven species. So the three genes were good barcoding markers for thrips. In general, more genetic information leads to better study of relationships among species. With collection of additional samples from different species and expansion of the number genes, deeper insight into the phylogenetic relationships of thrips may be possible. In future research, the number of species of the order Thysanoptera will be expanded based on this study, and in particular, phylogenetic relationships will be examined between species that have only been studied morphologically and not at the molecular level. Additional genes should be selected, including mitochondrial and nuclear genes, and integrated with morphological data to conduct phylogenetic research on thrips. With such an approach, phylogenetic relationships can be obtained that are more consistent with evolutionary reality, providing a basis for further research on phylogenetic relationships among species of thrips.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China [31860614], and the Yunnan Tobacco Company of China National Tobacco Corporation [2022530000241021].

LITERATURE CITED

Agung, A.P., Chornelia, A., Grismer, L.L., Grismer J.L., Quah, E.S.H., Lu, J.M., Tomlinson, K.W., Hughes, A.C. 2022 Description of two new species of Hemiphyllodactylus (Reptilia: Gekkonidae) from karst landscapes in Yunnan, China, highlights complex conservation needs. Zool. Res. **43(5):** 767–786.

Austen, G.E., Bindemann, M., Griffiths, R.A., Roberts, D.L. 2016 Species identification by experts and non-experts: comparing images from field guides. Sci. Rep. **6(1):** 33634.

Bhatti, J.S. 2000 Revision Of *Trichromothrips* and related genera (Terebrantia: Thripidae). Orient. Insects **34(1):** 1–65.

Bianciardi, A., Boschi, M., Swanson, E.E., Belloni, M., Robbins, L.G. 2012 Ribosomal DNA Organization Before and After Magnification in *Drosophila melanogaster*. Genetics **191(3):** 703–723.

Buckman, R.S., Mound, L.A., Whiting, M.F. 2013 Phylogeny of thrips (Insecta: Thysanoptera) based on five molecular loci. Syst. Entomol. **38(1)**: 123–133.

Chen, B., Butlin, R.K., Harbach, R.E. 2003 Molecular phylogenetics of the Oriental members of the Myzomyia Series of Anopheles subgenus Cellia (Diptera: Culicidae) inferred from nuclear and mitochondrial DNA sequences: Phylogenetics of Oriental Myzomyia. Syst. Entomol. **28(1):** 57–69.

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

Glover, R.H., Collins, D.W., Walsh, K., Boonham, N. 2010 Assessment of loci for DNA barcoding in the genus *Thrips* (Thysanoptera:Thripidae). Mol. Ecol. Resour. **10(1):** 51–59.

Gooliaff, T., Hodges, K.E. 2018. Measuring agreement among experts in classifying camera images of similar species. Ecol. Evol. **8(22):** 11009–11021.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O. 2010 New algorithms and methods to estimate Maximum-Likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. **59(3):** 307–321.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R. 2003a Biological identifications through DNA barcodes. Proc. R. Soc. B: Biol. Sci. **270(1512):** 313–321.

Hebert, Paul D.N., Ratnasingham, S., de Waard, J.R. 2003b Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proc. R. Soc. B: Biol. Sci. **270((Suppl 1):** S96–S99.

Hoddle, M.S., Heraty, J.M., Rugman-Jones, P.F., Mound, L.A., Stouthamer, R. 2008 Relationships among species of *Scirtothrips* (Thysanoptera: Thripidae, Thripinae) using molecular and morphological data. Ann. Entomol. Soc. Am. **101(3):** 491–500.

Inoue, T., Sakurai, T. 2007 The phylogeny of thrips (Thysanoptera: Thripidae) based on partial sequences of cytochrome oxidase I, 28S ribosomal DNA and elongation factor-1 α and the association with vector competence of tospoviruses. Appl. Entomol. Zool. **42(1):** 71–81.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. 2018 MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. **35(6):** 1547–1549.

Kumar, V., Dickey, A., Seal, D., Shatters, R., Osborne, L., McKenzie, C. 2017 Unexpected high intragenomic variation in two of three major pest thrips species does not affect ribosomal internal transcribed spacer 2 (ITS2) utility for thrips identification. Int. J. Mol. Sci. **18(10):** 2100.

Kumar, V., Seal, D.R., Osborne, L.S., McKenzie, C.L. 2014 Coupling scanning electron microscopy with DNA bar coding: a novel approach for thrips identification. Appl. Entomol. Zool. **49(3):** 403–409.

Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S. 2012 PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. **29(6):** 1695–1701.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B. 2017 PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. **34(3):** 772–773.

Li, Y.J., Li, Z., Zhang, H. 2019 *Trichromothrips* genus-group (Thysanoptera, Thripidae) from China, with descriptions of three new species and ten new records. Zootaxa **4544(1):** 79.

Marullo, R., Mercati, F., Vono, G. 2020 DNA barcoding: A reliable method for the identification of Thrips Species (Thysanoptera, Thripidae) collected on sticky traps in onion fields. Insects **11(8):** 489.

Masumoto, M., Okajima, S. 2005 *Trichromothrips* priesner (Thysanoptera, Thripidae) of Japan and Taiwan, with descriptions of four new species and a review of the *Trichromothrips* group of genera. Zootaxa **1088(1):** 1.

Masumoto, M., Okajima, S. 2012 Trichromothrips genus-group (Thysanoptera, Thripidae) from Vietnam, with descriptions of new species in both *Okajimaella* and *Paithrips*. Zootaxa **3313(1):** 1.

Mehle, N., Trdan, S. 2012 Traditional and modern methods for the identification of thrips (Thysanoptera) species. J. Pest. Sci. **85(2):** 179–190.

Minh, B.Q., Nguyen, M.A.T., von Haeseler, A. 2013 Ultrafast approximation for phylogenetic bootstrap. Mol. Biol. Evol. **30(5):** 1188–1195.

Moritz, G., Paulsen, M., Delker, C., Picl, S., Kumm, S. 2002 Identification of thrips using ITS-RFLP analysis In R. Marullo and L.A. Mound (eds.), Thrips and Tospoviruses Proceedings of the 7th International Symposium on Thysanoptera, Australian National Insect Collection, Canberra, Australia, pp. 365–367.

Mound, L.A., Marullo, R., Trueman, J.W.H. 2001 The greenhouse thrips, *Heliothrips haemorrhoidalis*, and its generic relationships within the subfamily Panchaetothripinae (Thysanoptera: Thripidae). Insect Syst. Evol. **32(2)**: 205–216.

Mound, L.A., Masumoto, M. 2004 *Trichromothrips* veversae sp.n. (Insecta, Thysanoptera), and the botanical significance of insects host-specific to Austral Bracken Fern (Pteridium esculentum). Proc. Linn. Soc. N. S. W. **125**, 67–71.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q. 2015 IQ-TREE: A fast and effective stochastic algorithm for

estimating Maximum-Likelihood phylogenies. Mol. Biol. Evol. **32(1):** 268–274.

Oktarianti, R., Sholihah, A., Masruroh, D., Wathon, S., Senjarini, K. 2021 Identification and phylogenetic analysis of *Drosophila melanogaster* based on ITS 2 rDNA Sequences. IOP Conference Series: Earth and Environmental Science. **747(1):** 012038.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P. 2012 MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. **61(3):** 539–542.

Stuart, R.R., Gao, Y., Lei, Z. 2011 Thrips: Pests of concern to China and the United States. Agricultural Sciences in China **10(6):** 867–892.

Sun, Y.B. 2017 FasParser: A package for manipulating sequence data. Zool. Res. **38(2)**: 110–112.

Tyagi, K., Chakraborty, R., Singha, D., Kumar, V. 2017b A new species of *Trichromothrips* (Thysanoptera) from India with four new records. Zootaxa **4363**: 145.

Tyagi, K., Kumar, V., Singha, D., Chakraborty, R. 2015 Morphological and DNA barcoding evidence for invasive pest thrips, *Thrips parvispinus* (Thripidae: Thysanoptera), newly recorded from India. J. Insect Sci. **15(1):** 105.

Tyagi, K., Kumar, V., Singha, D., Chandra, K., Laskar, B.A., Kundu, S., Chakraborty, R., Chatterjee, S. 2017a DNA barcoding studies on Thrips in India: Cryptic species and Species complexes. Sci. Rep. **7(1):** 4898.

Xie, Y.L., Mound, L.A., Lima, É.F.B., He, S.Q., Zhang, H.R., Li, Y.J. 2022 Molecular studies of relationships and identifications among insects of the subfamily Panchaetothripinae (Thysanoptera, Thripidae). J. Insect Sci. **22(5):** 6.

Yu, T.T., Chang, Z., Dong, Z.W., Li, K.Q., Ma, F.Z., Wang, Wen., Li, X.Yan. 2022 A Glimpse into the biodiversity of insects in Yunnan: An updated and annotated checklist of butterflies (Lepidoptera, Papilionoidea). Zool. Res. **43(6):** 1009–1010.

Zhang, D., Gao, F.L., Jakovlić, I., Zou, H., Zhang, J., Li, W.X., Wang, G.T. 2020 PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol. Ecol. Resour. **20(1):** 348–355.

Zhang, Q.Q., Li Y, Lin, Y.C., Li, S.Q., Yao, Z.Y., Zhang, X.Q. 2023 Regression of east tethys resulted in a center of biodiversity: A study of Mysmenidae spiders from the Gaoligong Mountains, China. Zool. Res. **44(4):** 737–738.

Supplementary materials are available from Journal Website