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**A NEW MONOTYPIC GENUS OF THE SUBFAMILY TETRASTICHINAE
(HYMENOPTERA: CHALCIDOIDEA: EULOPHIDAE) FROM CHINA**

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Summary. New genus *Zhijilingia* Xu, Liu et He, **gen. n.** (type species: *Zhijilingia tettigonia* Xu, Liu et He, **sp. n.**) is described from Sichuan, South China. This new genus can be distinguished from other genera of Tetrastichinae by following characters: antenna with 3 anelli and 4 funicular segments, only one clava segment, all funicular segments slender and longer than broad, funicular segments almost same in length, mesoscutum without a median line, propodeum with a distinct median carina, the whole rim of propodeal spiracle exposed, postmarginal vein absent. The new species probably parasitize eggs of the katyids *Prohimerta dispar* (Bey-Bienko, 1951) (Orthoptera: Tettigoniidae).

Key words: chalcid wasp, taxonomy, new taxa, molecular study, 28S, COI, Sichuan, South China.

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Резюме. Из китайской провинции Сычуань описан новый род *Zhijilingia* Xu, Liu et He, **gen. n.** (типовой вид – *Zhijilingia tettigonia* Xu, Liu et He, **sp. n.**). От других родов подсемейства Tetrastichinae новый род отличается следующими признаками: усик с 3 колечками и 4 жгутиковыми сегментами, булава с одним сегментом, все сегменты жгутика тонкие и длиннее своей ширины, сегменты жгутика почти одинаковой длины, мезоскутум без медианной линии, пропodeум с отчетливым срединным килем, окаймление пропodeального дыхальца выступающее, постмаргинальная жилка отсутствует. Новый вид, по-видимому, является паразитом яиц кузнечика *Prohimerta dispar* (Bey-Bienko, 1951) (Orthoptera: Tettigoniidae).

INTRODUCTION

Tetrastichinae is a subfamily belonging to Eulophidae, with 112 genera and over 2000 species (Domer *et al.*, 2020; Hansson, 2020; Noyes, 2021). Hosts for Tetrastichinae are found in 10 different orders of insects, including Hemiptera, Lepidoptera, Hymenoptera, Diptera, Coleoptera, Orthoptera, Odonata, Blattodea, Thysanoptera and Dictyopteran (LaSalle,

They can also parasitize a few spiders (LaSalle, 1990), mites (Vereshchagina, 1961) and even nematodes (van den Berg *et al.*, 1990). Some species are phytophagous, inquilines, or gall-formers (LaSalle, 1994). In China, 24 genera and 165 species of this subfamily are reported (Perkins, 1912; Timberlake, 1921; Miwa *et al.*, 1935; Yoshimoto, 1970; Yang & Wei, 2003; Yang *et al.*, 2003a,b; 2005; Wu *et al.*, 2001; Li, 2013; Li *et al.*, 2014; Yang *et al.*, 2014; Song *et al.*, 2014; Li *et al.*, 2020; Song *et al.*, 2020; Li, 2021).

In the biodiversity surveys conducted in Sichuan province, we found an unknown Tetrastichinae species staying on the forewing of katydid *Prohimerta dispar* (Bey-Bienko, 1951). The placement of this species is precluded into any of the known genera, and therefore a new genus and a new species is erected herein.

MATERIAL AND METHODS

The type specimens are deposited in the Museum of Biology, East China Normal University (ECNU). Specimens were preserved in 75% ethanol and photographed by a Leica M125 stereo microscope and a SC2000 digital CMOS Camera in the lab. One specimen was used for critical point drying; it was examined by a Hitachi S-4800 scanning electron microscope. Morphological terminology used in this paper is taken from Graham (1987): OOL – ocellar-ocular distance; POL – post-ocular distance; F – funicular segments.

DNA extraction and amplification of *COI* & *28S* genes: The genomic DNA was extracted from a whole specimens stored in 75% ethanol by AxyPrep Genomic DNA Miniprep Kit (AXYGEN), according to the manufacturer's instructions. The sequences of the *28S* rDNA D2 region were analyzed for phylogenetic research. Primers 28S D2-3551 CGTGTGCTTGA TAGTGACG and 28S D2-4057 TCAAGACGGGTCCTGAAAGT were used (Hancock *et al.*, 1988) for *28S* genes. For *COI* genes, primers LCO 1490 GGTCAACAAATCATAAAGAT ATTGG and HCO 2198 TAAACTTCAGGGTGACCAAAAAATCA and were used (Folmer *et al.* 1994).

Molecular phylogenetic analyses based on *28S* gene: Other Tetrastichinae molecular samples were acquired for *28S* gene (Gauthier *et al.* 2000; Burks *et al.* 2011). Each gene was aligned in MEGA X following MUSCLE method. The best-fit evolutionary model TIM3+I+G4+F was derived from ModelFinder embedded in PhyloSuite (Kalyaanamoorthy *et al.* 2017; Zhang *et al.* 2019) according to AIC criterion. Maximum likelihood (ML) analysis was conducted in IQ-Tree that was also integrated within PhyloSuite with 20000 ultrafast bootstraps replicates ((Minh *et al.* 2013; Nguyen *et al.* 2015). Tree topologies were tested with Approximate SH-aLRT test (Guindon *et al.* 2010).

RESULTS OF MOLECULAR STUDY

In total, twenty two *28S* concatenated sequences from Tetrastichinae were obtained, including our newly reported *Z. tettigonia* sp. n., as well as *Ceranisuus menes* (Entedoninae) as the outgroup. In general, our results made consensus with previous molecular studies. According to our genealogical tree, *Z. tettigonia* sp. n. has close relationship with *Galeopsomyia* sp. 2 and *Galeopsomyia fausta* (Fig. 1).

DESCRIPTIONS OF NEW TAXA

Family Eulophidae Westwood, 1829

Subfamily Tetrastichinae Graham, 1987

Genus *Zhijilingia* Xu, Liu et He, gen. n.

<http://zoobank.org/NomenclaturalActs/856364AA-E379-4136-B350-24AC8CBE64D7>

Type species: *Zhijilingia tettigonia* sp. n., here designated.

DESCRIPTION. Antenna with 3 anelli and 4 funicular segments, only one clava segment, all funicular segments slender and longer than broad, and funicular segments almost the same length, scape and pedicel without rasp-like reticulation, malar sulcus present and slightly curved, mesoscutum without a median line, petiole present, propodeum with a distinct median carina, the whole rim of propodeal spiracle exposed, cercal setae unequal in length, with one being distinctly longer than the others and sinuate, postmarginal vein absent, submarginal vein with 2–3 dorsal setae.

DIAGNOSIS. This new genus can be distinguished from other genera by the characters given above, in particular the slender antenna and antenna with 4 funicular segments and 1 clava. Except this antennal feature, this new genus is similar to *Aprostocetus* (*Ootetrastichus*) Perkins, 1833, but it differs from latter in propodeal spiracle with its entire rim exposed. This new genus is also similar to *Minotetrastichus* Kostjukov, 1977, but it differs in one of the cercal setae distinctly longer than the others, and generally slightly curved or sinuate.

ETYMOLOGY. This genus name *Zhijilingia* is a Chinese headwear with long ornament, which refers to its long antennae.

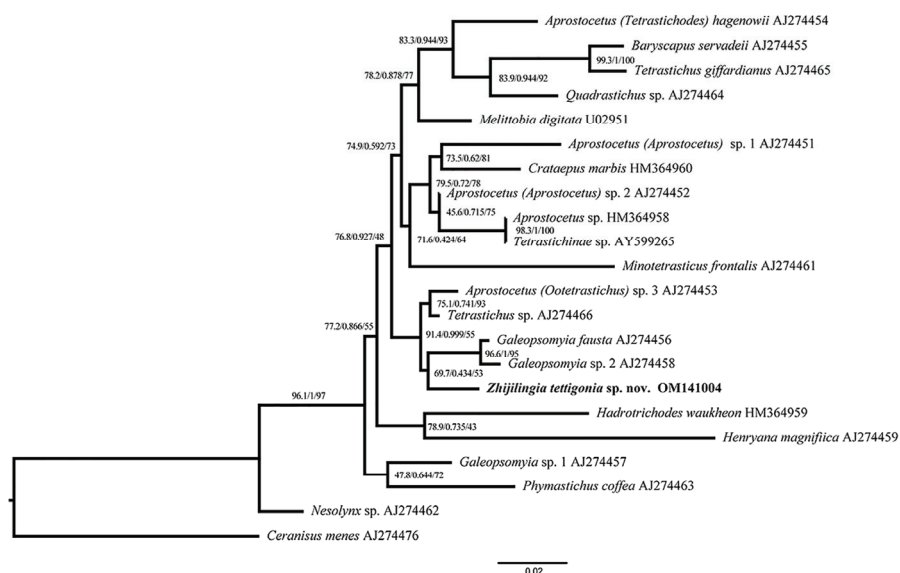


Fig. 1. The phylogenetic relationship among Tetrastichinae species inferred from the 28S concatenated fragments. The tree was constructed by Maximum likelihood (ML) with TIM3+I+G4+F model and rooted by *Ceranisis menes* (Entedoninae). Topology supports of all major nodes are indicated amidst the branches by SH-aLRT value and the bootstrap value.

Zhijilingia tettigonia Xu, Liu et He, sp. n.

<http://zoobank.org/NomenclaturalActs/01CEB5D9-BDCE-4C5D-A59F-109AE88F6824>

Figs 2, 3

COI gene number of NCBI: OM089661

28S gene number of NCBI: OM141004

TYPE MATERIAL. Holotype: ♀, **China**: Sichuan, Yaan, Tianquan, Erlangshan, Labahe, 30.08° N, 102.42° E, 18.VII 2020, coll. He Zhu-Qing (ECNU). Paratypes: 3 ♀, same data as holotype (ECNU).

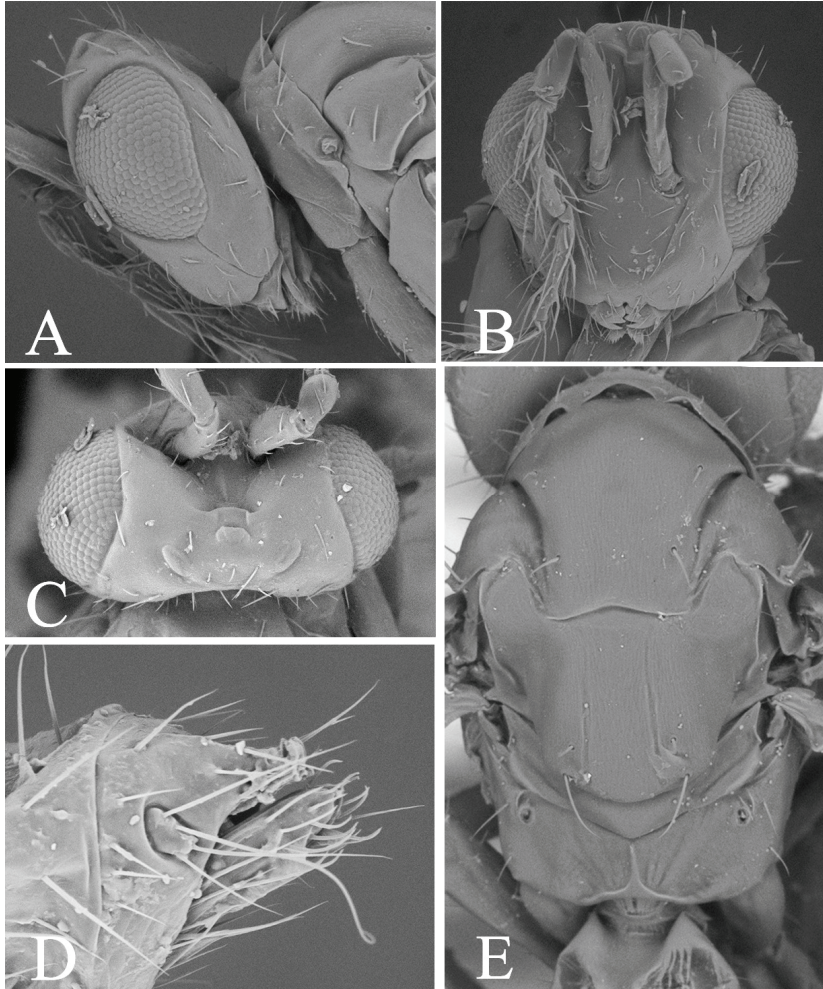


Fig. 2. Female of *Zhijilingia tettigonia* sp. n. A – head, lateral view; B – head, frontal view; C – head, dorsal view; D – apex of gaster showing cercal setae, lateral view ; E – thorax, dorsal view.

DESCRIPTION. Female. Body length 1.62–1.94 mm. Head (Fig. 2 A–C): POL about 1.8 times as long as OOL. Antennal torulus placed above level of ventral margin of eye. Malar sulcus present and slightly curved. Scape reaching to vertex, but not extending over vertex.

Antenna (Fig. 3 D–F): with 3 anelli and 4 funicular segments. All funicular segments slender and longer than broad. F1, F2 and F3 are almost the same length; F4 the shortest (F1: F2: F3: F4 = 0.9: 1.0: 1.0: 0.85). Only one clava segment; clava segment 5.0 times as long as broad, nearly as long as funicular segments, clava with distinct terminal spine. Scape and pedicel without rasp-like reticulation.

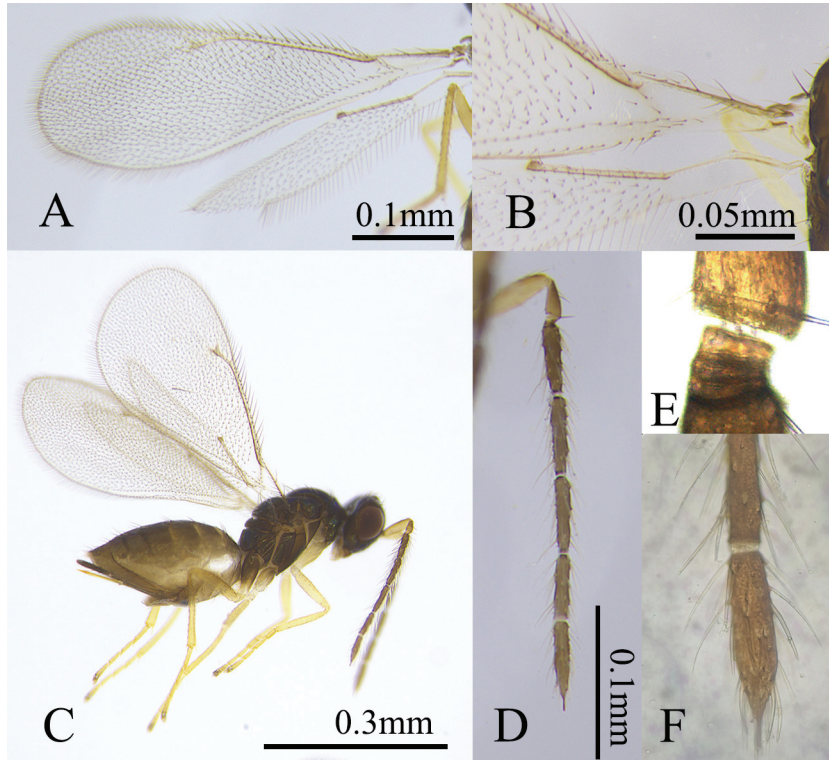


Fig. 3. Female of *Zhijilingia tettigonia* sp. n. A – wing; B – submarginal vein; C – habitus, lateral view; D – antenna; E – anelli; F – clava.

Thorax (Fig. 2 E): Mesosoma about 1.50 as long as broad in dorsal view. Mesoscutum without a median line; with 2 adnotaular setae. Scutellum with distinct submedian lines; 2 pairs of setae on scutellum, anterior pair of setae located below the middle. Dorsellum about 0.6 times as long as propodeum in dorsal view. Propodeum with a distinct median carina; without paraspiracular carina. The whole rim of propodeal spiracle exposed. Callus with 2 setae.

Gaster (Fig. 2 D): Almost as long as head plus thorax. Apex of hypopygium extending about 0.17–0.21 length of gaster and reaching almost up to the posterior margin of G4. Ovipositor sheaths slightly exerted. Cercus with 4 setae; Cercal setae unequal in length, with one being distinctly longer than the others and sinuate.

Wing (Fig. 3 A–B): Submarginal vein with 2–3 dorsal setae; Postmarginal vein absent. Costal cell: marginal vein: stigmal vein = 2.9–3.3: 4.7–5.3: 1.0. Forewing with cubital line of setae starting at the level of basal vein. Subcubital line of setae reaching the level of basal vein.

Coloration. Body mainly black, dorsum of thorax mainly black with slightly metallic coloration. Head black with slightly metallic coloration. Scape mainly brownish yellow, but other antennal segments brown. Gaster mainly brownish yellow. Legs mainly brownish yellow. Wing veins light brown.

Male. Unknown.

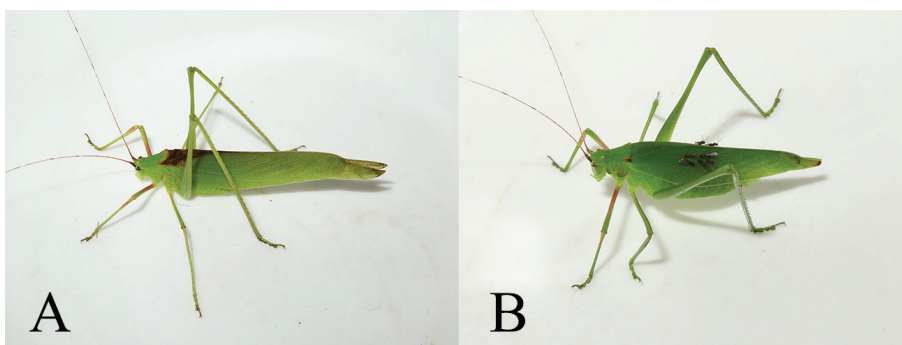


Fig. 4. *Prohimerta dispar* (Bey-Bienko, 1951). A – male; B – female with *Zhijilingia tettigonia* sp. n. on forewing.

BIOLOGY. The new species were collected from the forewing of two female katydids *Prohimerta (Anisotima) dispar* (Bey-Bienko, 1951) belonging to subfamily Phaneropterinae (Orthoptera: Tettigoniidae). There was no individual found on that part of males (Fig. 4). We also collected other katydids in same place such as *Tettigonia chinensis* Willemse, 1933, *Phyllomimus sinicus* Beier, 1954, *Qinlingea brachystylata* (Liu & Wang, 1998), *Paraxantia szechwanensis* Liu, 2014, *Neocyrtopsis fallax* Wang & Liu, 2012, but no chalcid wasp was found.

ETYMOLOGY. The name *tettigonia* refers to its parasitizing katydid eggs.

DISTRIBUTION. China (Sichuan).

DISCUSSION

Gauthier *et al.* (2000) pointed out that there were some sophisticated indel events in *28S* genes, which made it difficult to reasonably assess the homologies with the present set of taxa. We provided *28S* gene, and the result of phylogenetic tree provided the validation of the new genus belonging to subfamily Tetrastichinae. We also provided *COI* genes of the new species for future study, because it hardly has indel by previous studies (Nugnes *et al.* 2015).

Refer to the record of Graham (1987), Tetrastichinae species attack a variety of hosts, including 10 orders of insects. In Orthoptera, Tetrastichinae species are mainly parasitoids of Gryllidae. Graham (1987) recorded 3 species are egg parasitoids of Orthoptera: *Aprostocetus (Ootetrastichus) crino* (Walker, 1838) is egg parasitoids of *Oecanthus pellucens* (Scopoli, 1763), *O. nigricornis* Walker, 1869 and *O. quadripunctatus* Beutenmüller, 1894; *A. (O.) ovivorax* (Silvestri, 1920) and *A. (O.) percaudatus* (Silvestri, 1920) are egg parasitoids of *O.*

pellucens (Scopoli, 1763). There is rare record that Tetrastichinae species is parasitoids of Tettigoniidae. Viggiani (2021) reported a new species *Aprostocetus brevifragiatus* is the egg parasitoids of *Phaneroptera nana* (Fieber, 1853) (Orthoptera: Tettigoniidae). It is the first record of an *Aprostocetus* egg parasitoid of Tettigoniidae in Europe. Our newly reported Tetrastichinae species herein is the first record of an egg parasitoid of Tettigoniidae in China. Based on molecular result, *Galeopsomyia* Girault, 1916 has the close relationship, but with a strong paraspiracular carina, and propodeum strongly reticulate. *Galeopsomyia* species are associated with galls, mostly as parasitoids of Cecidomyiidae and Cynipidae (LaSalle 1994). However, parasitoids of Gryllidae (subgenus *Ootetrastichus*) also have close relationship with the new species, which may indicate the common ancestor parasitizing Orthoptera species. To test this hypothesis, more parasitoids of Orthoptera species in subfamily Tetrastichinae need to be sequenced for phylogenetic studies in the future.

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