

***Telenomus dendrolimi* (Matsumura) (Hymenoptera, Scelionidae) reared from the eggs of *Dendrolimus houi* (Lajonquiere) (Lepidoptera, Lasiocampidae) from China**

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Abstract

The pine moth, *Dendrolimus houi* (Lajonquiere), is a notorious insect pest of coniferous trees in South China. A gregarious egg parasitoid in the family Scelionidae shows great potential to be mass-reared for the biological control of *D. houi*. This parasitoid is identified as *Telenomus dendrolimi* (Matsumura) based on comparison to paratype specimens of *Telenomus dendrolimusi* Chu, a junior synonym of *T. dendrolimi*. *Telenomus dendrolimi* is redescribed and included in a molecular analysis. In addition to parasitizing *D. houi*, *T. dendrolimi* was found to successfully parasitize six other species under laboratory conditions, with *Antheraea pernyi* (Guérin-Ménéville) the most suitable host for mass-rearing.

Keywords

Biological control, egg parasitoid, gregarious, taxonomy

Introduction

The pine moth, *Dendrolimus houi* (Lajonquiere) (Lepidoptera, Lasiocampidae), is a notorious insect pest of several important conifers including *Cryptomeria japonica* var. *sinensis* Miquel (Cupressaceae), *Cupressus funebris* Endl. (Cupressaceae), *Pinus yunnanensis* Faranch. (Pinaceae), and *Pinus kesiya* Royle ex Gordon in South China, India, Myanmar, Sri Lanka, and Indonesia (Lin et al. 2023). Caterpillars of this moth feed on the leaves and branch tips of coniferous trees and have caused thousands of hectares of dead and dying trees of *Cr. japonica* (Zhang 2013), *Cu. funebris* (He et al. 2018), and *P. kesiya* (Yang 2015) in several provinces of South China. In addition to damaging coniferous trees, the venomous setae of the caterpillars have been reported to cause painful allergic reactions in humans, and to contaminate drinking water in rural areas (Luo et al. 1998).

Attempts to control *D. houi* in China have mainly focused on chemical insecticides (Zhang 2013), but more environmentally friendly control methods, such as biological control, have been proposed recently (Yang et al. 2017; Lin et al. 2017; Liang et al. 2018; Lin et al. 2023). To date, 25 parasitoid wasp and fly species have been reported to attack *D. houi* in China (Lin et al. 2017; Liang et al. 2018; Lin et al. 2023), including seven egg parasitoid wasps: *Anastatus gastropachae* Ashmead, *Mesocoms albitarsis* (Ashmead), and *Mesocoms trabalae* (Yao & Yang) (Eupelmidae); *Mesopolobus subfumatus* (Ratzeburg) (Pteromalidae); *Ooencyrtus kuvanae* (Howard) (Encyrtidae); *Trichogramma dendrolimi* Matsumura (Trichogrammatidae); and *Telenomus dendrolimi* (Matsumura) (Scelionidae). During a comprehensive survey of natural enemies of *D. houi* across South China in 2021, a gregarious species belonging to the egg parasitoid genus *Telenomus* Haliday (Scelionidae) was reared from the eggs of *D. houi* in Xiapu County of Fujian Province. Morphological examination and molecular analyses confirmed that it was *T. dendrolimi* (Matsumura) and a colony was established and maintained using eggs of *Antheraea pernyi* (Guérin-Méneville) (Saturniidae) in the laboratory at Fujian Agriculture and Forestry University. This parasitoid species shows potential to be mass-reared for the biological control of *D. houi*. This study aims to redescribe this species to facilitate future work on the taxonomy of the genus *Telenomus* and applications of this egg parasitoid in biological control programs.

Materials and methods

Insect sampling and rearing

A survey for parasitoid species that attack *D. houi* was conducted in five provinces of China in 2021 (Lin et al. 2023). Some parasitized eggs of *D. houi* on *Cryptomeria japonica* var. *sinensis* Miquel trees (Fig. 1) were collected in Xiapu County, Fujian Province (26°49'45.76"N, 119°57'55.3"E). The parasitized eggs were transported to the laboratory at Fujian Agriculture and Forestry University and placed in a 10 cm glass tube maintained at 26 ± 1 °C, 50 ± 10% relative humidity (RH) and 12:12 h light/dark (LD) photoperiod. The eggs were checked daily for the emergence of parasitoids.

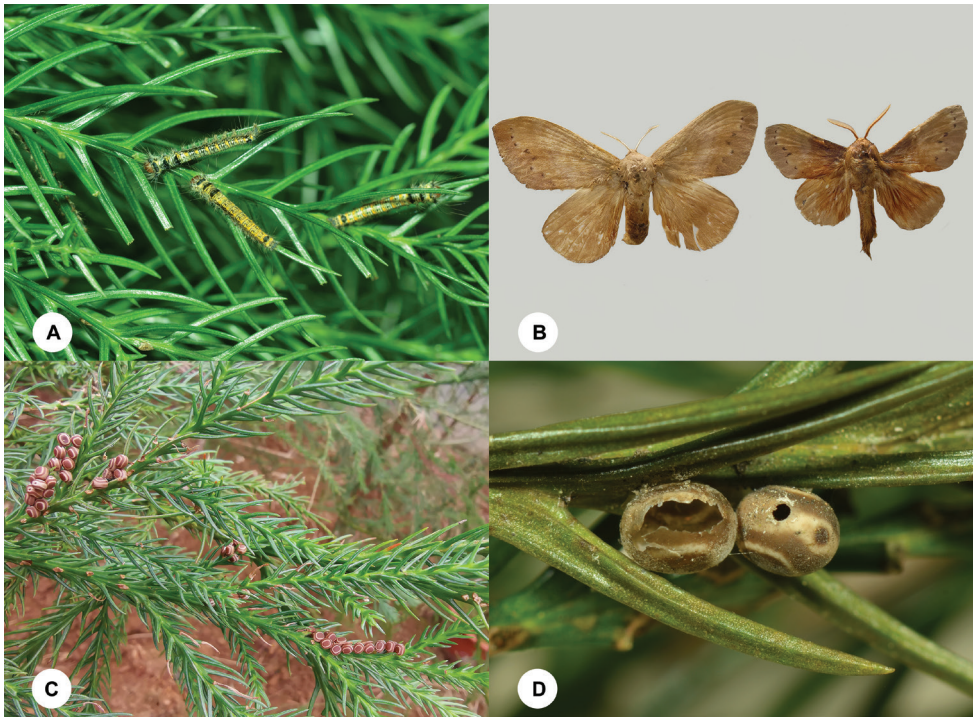


Figure 1. *Dendrolimus houi* (Lajonquiere) **A** larvae feeding on the leaves of *Cryptomeria japonica* var. *sinensis* **B** adult female (left), male (right) **C** parasitized egg masses on leaves **D** parasitized eggs with a parasitoid emergence hole.

All parasitoids were collected and fed with 20% honey water in a screened plastic box (29 × 17 × 9 cm) held at 25 °C and 12:12 h LD after emerging from the eggs. Fresh eggs of *A. pernyi* were provided to establish a colony in the laboratory. Additional eggs of the lepidopteran families Lasiocampidae (*Dendrolimus kikuchii* Matsumura, *Dendrolimus punctatus* Walker), Noctuidae (*Spodoptera frugiperda* (J.E. Smith), *Spodoptera litura* (Fabricius)), Plutellidae (*Plutella xylostella* (Linnaeus)), and Saturniidae (*Actias sinensis* (Walker), *Antheraea pernyi* (Guérin-Méneville), *Caligula japonica* Moore, *Eriogyna pyretorum* Westwood) were used to test their suitability as hosts.

Some parasitoid specimens were preserved in 100% ethanol for further morphological and molecular studies. All voucher specimens are deposited in the Insect Collection of South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China (SCBG).

Morphological terms

Abbreviations and morphological terms used in text: **A1–A12**: antennomere 1–12; **T1–T7**: metasomal tergite 1–7; **S1–S7**: metasomal sternite 1–7. Morphological terminology otherwise generally follows Mikó et al. (2007).

DNA extraction and sequencing

Genomic DNA was extracted from a female and a male of *T. dendrolimi*, *T. remus* Nixon, and *T. dignus* (Gahan) using a TIANamp Micro DNA Kit (Tiangen Biotech (Beijing), Co., Ltd.), following an established non-destructive DNA extraction protocol (Taekul et al. 2014). Two molecular markers were amplified: mitochondrial DNA (mtDNA) cytochrome c oxidase 1 (COI) and nuclear 28S rDNA D2–3 (28S). Polymerase chain reactions (PCRs) were performed using Tks Gflex™ DNA Polymerase (Takara) with primer pairs LCO1490/HCO2198 (Folmer et al. 1994) for COI and D23F/28Sb (Whiting et al. 1997) for 28S. Thermocycling was conducted in a T100™ Thermal Cycler (Bio-Rad) following the conditions of Yan et al. (2022). Both DNA strands of amplicons were directly sequenced on an Applied Biosystems (ABI) 3730XL using the same primers used for the PCR by Guangzhou Tianyi Huiyuan Gene Technology Co., Ltd. (Guangzhou, China). Forward and reverse sequences were assembled with Geneious 11.0.3. The assembled sequences of each gene were compared via BLAST against the GenBank non-redundant nucleotide database to check for contamination and stop codons in the open reading frame of COI that would indicate pseudogenetic DNA (e.g., nuclear mitochondrial DNA, NUMT). The sequences generated from this study are deposited in GenBank (accession numbers are shown in Suppl. material 1).

Phylogenetic analyses

The 28S rDNA and COI sequences from *T. dendrolimi*, *T. remus*, and *T. dignus* were combined with the *Telenomus* 28S rDNA and COI dataset of Polaszek et al. (2021) (excluding *Trissolcus thyanthae* Ashmead), in addition to a COI sequence of *Telenomus gregalis* Rajmohana (Rajmohana et al. 2024) downloaded from GenBank ([KT896659.1](#)). Multiple sequence alignments for each gene were performed separately with MAFFT v7.450 (Katoh and Standley 2013) using the E-INS-i algorithm for 28S and the L-INS-i algorithm (Translation Align feature in Geneious Prime 2024.0.4) for COI. Phylogenetic analyses were conducted in IQ-TREE v. 2.3.6 (Minh et al. 2020) for 28S, COI, and 28S+COI following the methodology of Chen et al. (2021). No partitions were specified in the 28S analysis. Three partitions (one for each codon position) were specified in the COI analysis. Four partitions (28S and one for each codon position of COI) were specified in the combined analysis (Chernomor et al. 2016). In each analysis, the best nucleotide substitution model for each partition was determined with ModelFinder (Kalyaanamoorthy et al. 2017), with the merge option enabled. Branch support was estimated with 1000 ultrafast bootstrap replicates (Hoang et al. 2018) and 1000 replicates of the SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010). Each analysis consisted of 100 independent runs, and *Trissolcus basalis* (Wollaston) was selected as the outgroup.

Imaging

Multifocal images of mounted specimens were made using a Nikon SMZ25 microscope with a Nikon DS-Ri 2 digital camera system and a Keyence VHX-970F imaging system. Scanning electron micrographs were produced using a JSM-6360LV scanning electron microscope (JEOL, Tokyo, Japan). Images were post-processed with Adobe Photoshop CS6 Extended.

Results and discussion

Biology

Telenomus dendrolimi is a gregarious species, with more than one wasp emerging from a single egg of *D. houi*. Under laboratory conditions, it successfully parasitized two species of Lasiocampidae: *D. kikuchii* and *D. punctatus*; and four species of Saturniidae: *A. sinensis*, *A. pernyi*, *C. japonica*, and *E. pyretorum*. Similarly, more than one wasp emerged from a single host egg, indicating gregarious behavior for all of these hosts. Among the six alternative host species, *A. pernyi* was the most promising for mass-rearing of *T. dendrolimi*, with up to 20 wasps (female to male ratio was about 4:1) emerging per egg. Related biological parameters are under investigation and will be reported in future studies.

Molecular analyses

Six *Telenomus* specimens belonging to three species were subjected to non-destructive DNA extraction followed by amplification and sequencing of the D2 and D3 regions of the 28S rDNA and the barcode region of COI. Multiple sequence alignments for 28S and COI were 1,134 and 845 characters (nt + gaps), respectively. In the COI single gene (Suppl. material 3) and combined gene analyses (Suppl. material 4), *T. dendrolimi* was recovered as the sister taxon to *T. dalmanni* with weak support. *Telenomus dendrolimi* was the sister taxon to an undescribed species of the *Telenomus californicus* complex in the 28S single gene analysis (Suppl. material 2), also with weak support. The COI single gene analysis revealed a moderately supported clade of species with gregarious life histories that includes taxa with known biologies, such as *T. gregalis*, *T. moricolus*, and *T. dendrolimi*. Also included in this clade is *T. dalmanni*, a solitary egg parasitoid of *Orgyia antiqua* (Linnaeus) (Lepidoptera: Lymantriidae) based on the published literature and rearing records of specimens accessioned in the Museum of Biological Diversity database (The Ohio State University). The newly sequenced *T. remus* (Note: The GenBank specimen [MW452546.1](#), labeled as *Telenomus remus* c in Fig. 7, Suppl. material 4) is misidentified as *T. remus* and represents a closely related, potentially undescribed species) and *T. dignus* specimens formed monophyletic groups with other material of the same species deposited in GenBank (Fig. 7).

***Telenomus dendrolimi* (Matsumura)**

Figs 2–6

Holcaerus (?) *dendrolimi* Matsumura, 1925: 44 (original description).*Telenomus dendrolimi* (Matsumura): Tachikawa 1965: 284, plate 142 (description); Ryu and Hirashima 1985: 31, 47 (description, keyed); Johnson 1992: 584 (cataloged).*Telenomus (Aholcus) dendrolimi* (Matsumura): He et al. 2004: 307 (description).*Telenomus dendrolimusi* Chu, 1937: 60 (original description); Ishii 1938: 105 (junior synonym of *Telenomus dendrolimi* (Matsumura)); Peng and Liu 1992: 1473 (description, distribution).*Telenomus (Aholcus) dendrolimusi* Chu: Wu and Chen 1980: 79, 81 (keyed); Chen and Wu 1981: 109, 110 (description, keyed); Chen and Tong 1980: 311 (description).**Description.** Female body length: 1.07–1.21 mm (n = 10). Male body length 0.8–1.0 mm (n = 8).

Color: female body entirely black; antenna dark brown to black, with ventral side paler; coxae and femora of all legs dark brown to black, remainders of legs brown to yellow, with distal tarsomere darker; wings hyaline. Male similar to female, but antenna brown to dark brown, tibiae and tarsi of all legs paler.

Head. Female antenna 10-merous; claval formula 1-2-2-2; A2 distinctly longer than wide, A3 and A10 slightly longer than wide, A4 as long as wide, A5–A9 wider than long.Male antenna 12-merous; A2 slightly longer than wide; A4 and A5, slightly dilated, but A5 modified, with tyloid projecting anteriorly; A6–A11 transverse; A12 $1.60 \times$ as long as wide.

Mandible with 3 teeth; clypeus with 4 setae; labrum, medially pointed, flanked by arched concavities; interantennal process present; frons coriaceous, becoming smooth around toruli and malar sulcus; frontal depression indicated by area of effaced microsculpture; gena coriaceous behind eye, becoming smooth ventrally and above occipital carina; eyes setose; vertex smoothly rounded onto occiput, without hyperoccipital carina; vertex coriaceous, becoming smooth above occipital carina; occiput entirely smooth; occipital carina present, higher and weaker medially, ventrally extending to posterior articulation of the mandible.

Mesosoma. Pronotum: pronotal cervical sulcus present as a smooth furrow; netrion smooth; netrion sulcus complete and distinct.**Mesonotum:** mesoscutum evenly rounded in lateral view, with reticulate microsculpture except smooth narrow area along mesoscutal humeral sulcus; mesoscutal supra-humeral sulcus absent; mesoscutal humeral sulcus indicated by a mostly smooth furrow; mesoscutellum smooth, sparsely setose; axilla smooth, setae present; scutoscuteellar sulcus more or less smooth medially, foveate laterally; posterior mesoscutellar sulcus foveate.**Metanotum:** metascutellum rugose, slightly protruding posteriorly; metascutellar carina present; metanotal trough smooth.**Propodeum:** metapostnotum medially tapering to a slender point, creating a gap between posterior margin of metanotum and anterior margin of propodeum; lateral



Figure 2. *Telenomus dendrolimi* (Matsumura) **A–D** female (OSUC 0071700) **A** dorsal habitus **B** lateral habitus **C** head and mesosoma, dorsal view **D** head and mesosoma, lateral view **E, F** male (OSUC 0071702) **E** lateral habitus **F** antenna.

propodeal carina present, fusing with metapleural carina to form perimeter of metasomal depression; metasomal depression mostly smooth, with short rugae radiating from propodeal foramen.

Mesopleuron: acropleural sulcus present, continuous with prespecular sulcus, forming a furrow extending ventrally to mesopleural pit; speculum weakly rugose; mesepimeral sulcus complete, foveate; mesopleural pit deep, slightly transverse; ventral mesopleuron smooth; episternal foveae absent; postacetabular patch clearly indicated, without setae; acetabular carina present; postacetabular sulcus indicated by a smooth

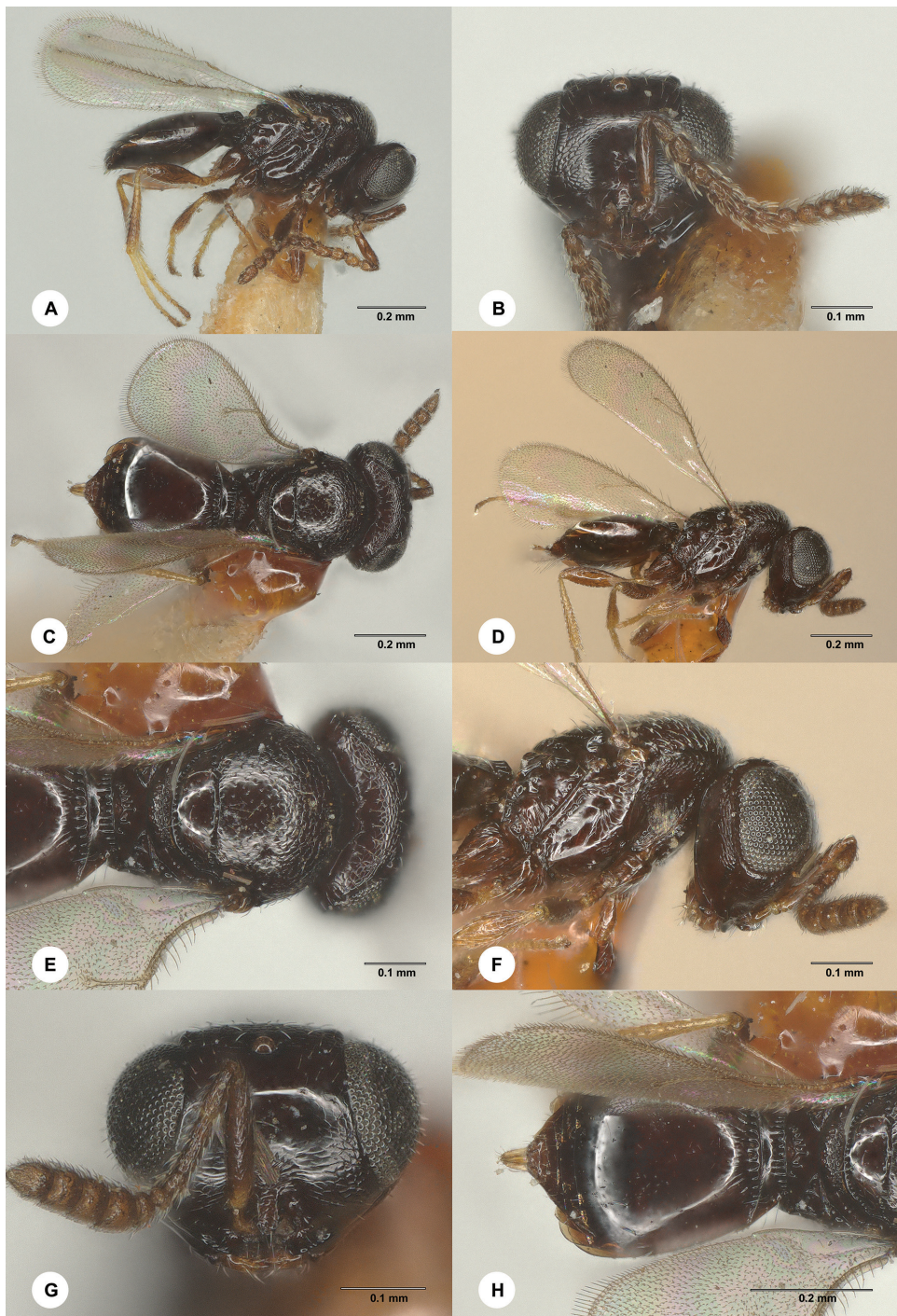


Figure 3. *Telenomus dendrolimusi* Chu **A, B** allotype, male **A** lateral habitus **B** head, anterior view **C–H** paratype, female **C** dorsal habitus **D** lateral habitus **E** head and mesosoma, dorsal view **F** head and mesosoma, lateral view **G** head, anterior view **H** metasoma, dorsal view.



Figure 4. *Telenomus dendrolimi* (Matsumura) from Fujian, female (SCBG_E0010794) **A** dorsal habitus **B** lateral habitus **C** head and mesosoma, dorsal view **D** head and mesosoma, lateral view **E** head, anterior view **F** metasoma, dorsal view.

furrow; length of intercoxal space longer than fore coxae; mesopleural epicoxal sulcus indicated by shallow foveae.

Metapleuron: Metapleural carina interrupted by propodeal spiracle; dorsal metapleural area smooth; ventral metapleural area weakly rugose along anterior and posterior margins; metapleural pit present; metapleural sulcus indicated as a weak, shallow groove posterior to the metapleural pit; metapleural epicoxal sulcus indicated by crenulae.

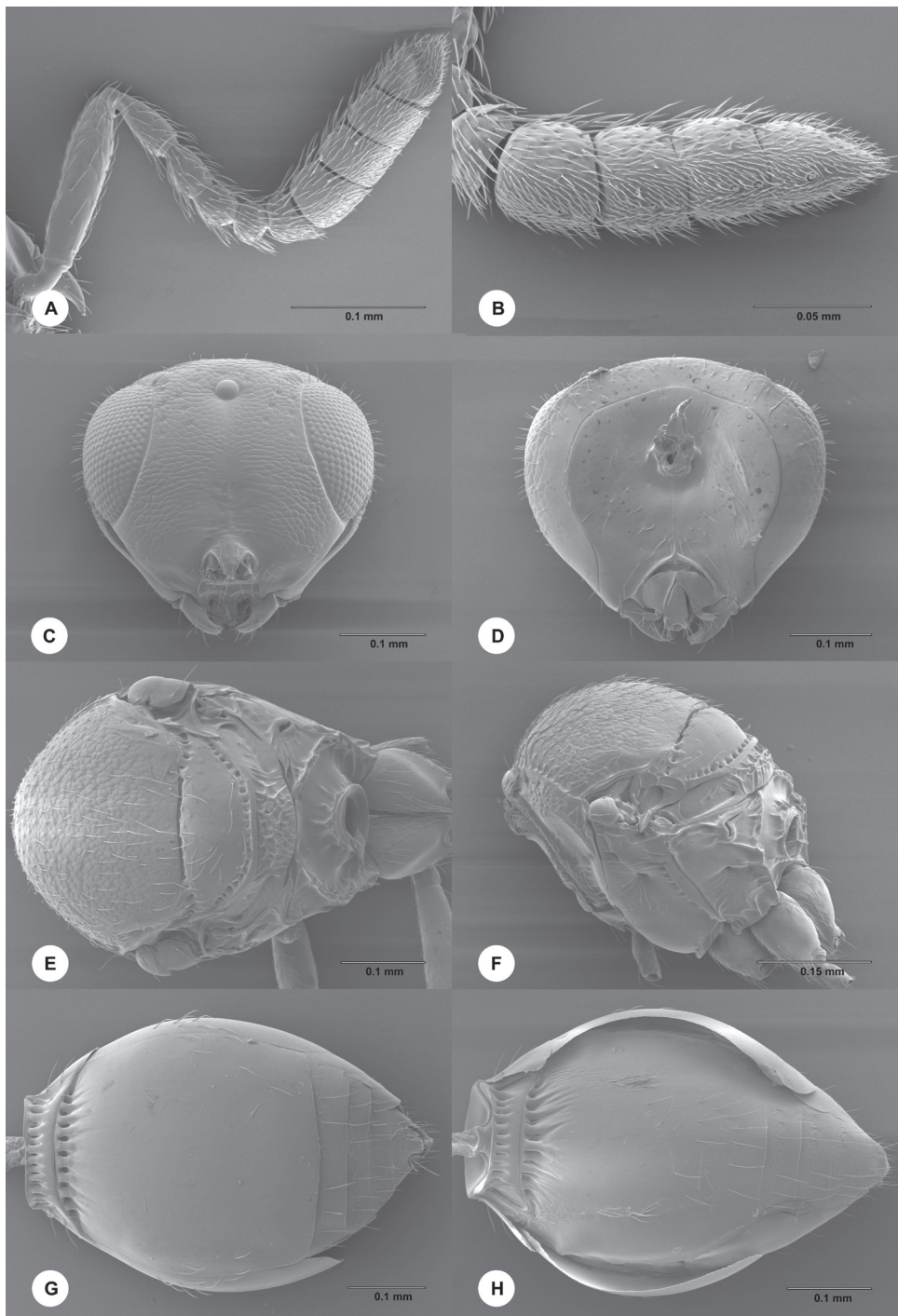


Figure 5. *Telenomus dendrolimi* (Matsumura) from Fujian, female (SCBG_E0010790) **A** antenna, lateral view **B** clava, ventrolateral view **C** head, anterior view **D** head, posterior view **E** mesosoma, dorsal view **F** mesosoma, dorsolateral view **G** metasoma, dorsal view **H** metasoma, ventral view.

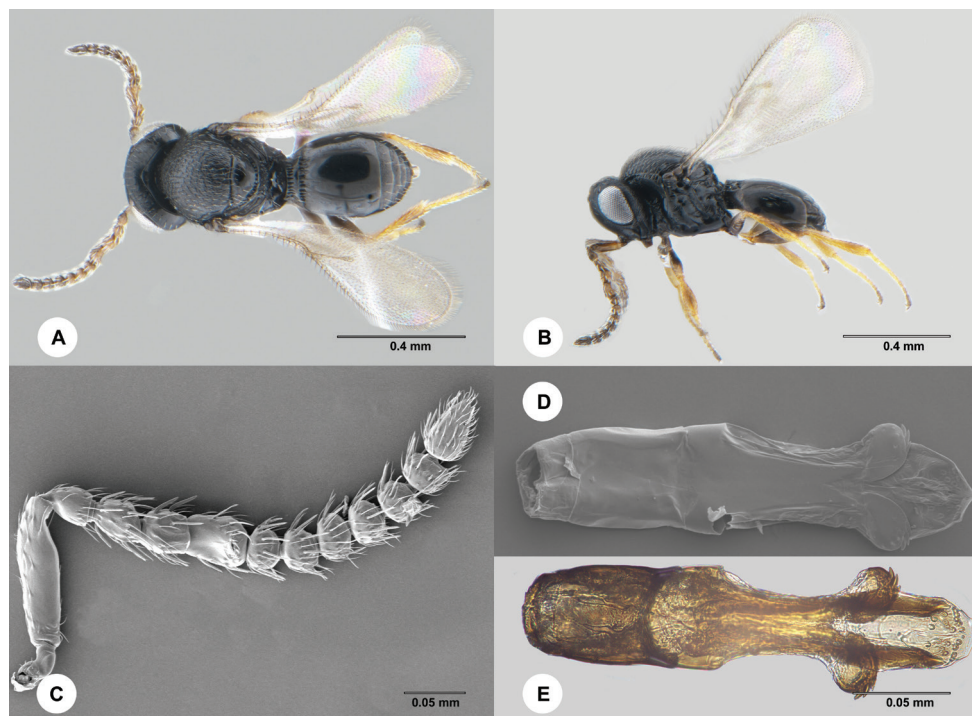


Figure 6. *Telenomus dendrolimi* (Matsumura) from Fujian, male (SCBG_E0010786) **A** dorsal habitus **B** lateral habitus **C** antenna, lateral view **D** genitalia, ventral view **E** genitalia, dorsal view.

Wings. Length of postmarginal vein about twice as long as stigmal vein. Fore wing apex reaching beyond T6.

Metasoma. T1 with 1 pair of sublateral setae, 2 pairs of lateral setae; foveae along anterior T1 distinct, elongate; foveae along anterior T2 distinct, with short costae extending less than $\frac{1}{4}$ the length of T2; T2 with sparse setae on lateral part of mediotergite and dorsomedial part of laterotergite; T3–T6 with a transverse line of long setae, setae absent along midline; laterotergites 3–6 glabrous; foveae along anterior S1 distinct, with costae extending to mid-length; foveae along anterior S2 distinct, with short costae extending less than $\frac{1}{4}$ the length of S2; S2 felt field indicated by coriaceous sculpture and setal patch; sparse setation present in posteromedial portion of S2; S3–S5 with a transverse line of long setae, setae absent along midline; S6 setose laterally, without setae along midline.

Male genitalia. Length of aedeagal lobe about $0.3 \times$ length of aedeago-volsellar shaft, distally pointed; digiti large, about half maximum length of aedeagal lobe, with 3 digital teeth, basal ring about $0.4 \times$ length of aedeago-volsellar shaft.

Species-group placement. *Telenomus californicus*-complex (Johnson 1984).

Host. *Telenomus dendrolimi* was reared from field-laid eggs of *D. houi* and *D. punctatus* in China, and *Dendrolimus spectabilis* Butler in Japan. Under labora-

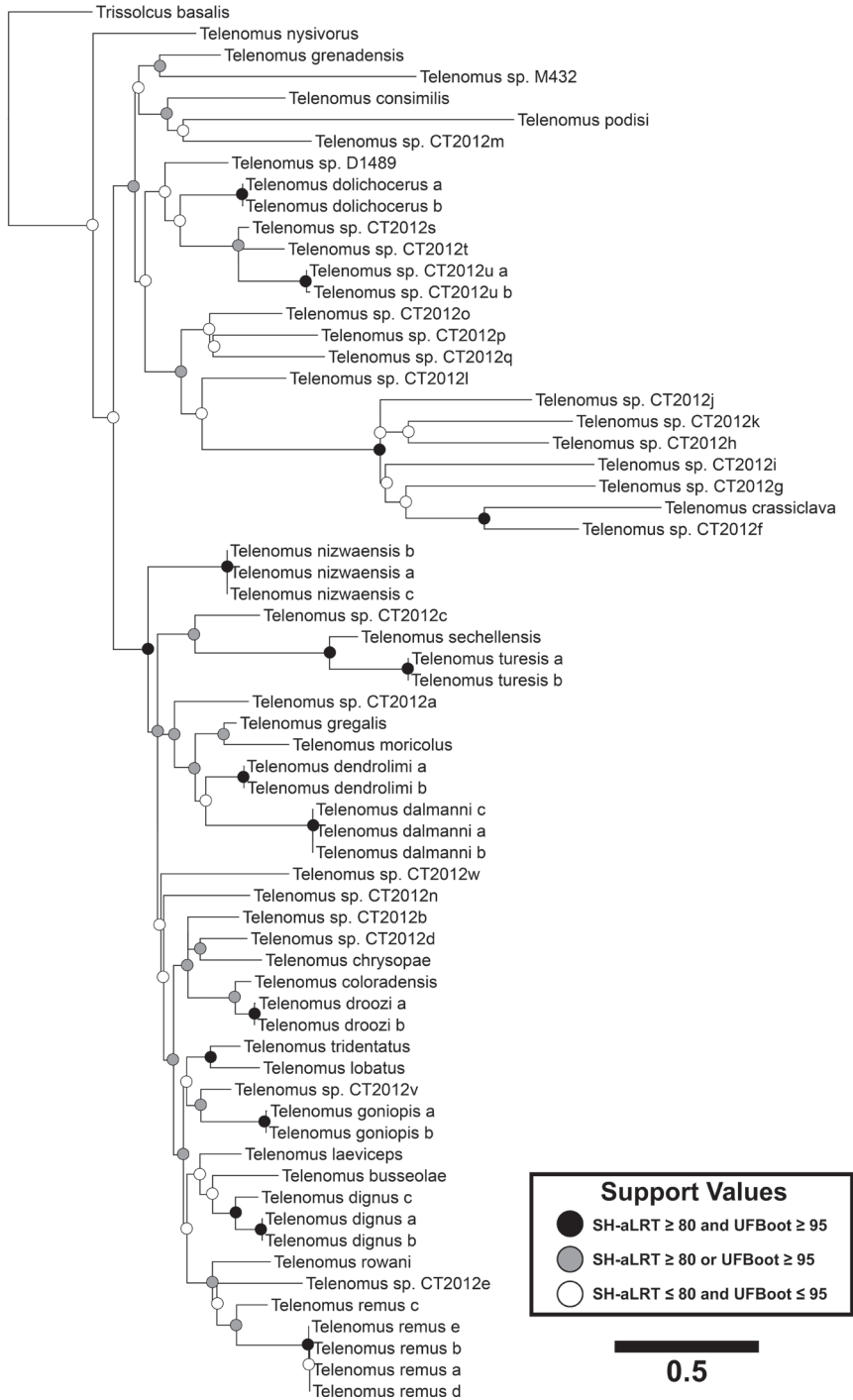


Figure 7. Combined 28S and COI maximum likelihood phylogenetic analysis of *Telenomus* using a modified dataset of Polaszek et al. (2021). The number under the scale bar indicates the number of expected nucleotide substitutions per site. Support values (SH-aLRT and UFBoot) are indicated by colored circles at nodes. Terminal labels correspond to the taxa listed in the supplementary table (Suppl. material 1).

tory conditions, its alternative hosts include *D. kikuchii* and *D. punctatus*, *A. sinensis*, *A. pernyi*, *C. japonica*, and *E. pyretorum*.

Material examined. Allotype of *Telenomus dendrolimusi* Chu, 1937, • male: CHINA: Tangki (Tangxi), Chekiang (Zhejiang), ex. eggs of *Dendrolimus punctatus* Walker, 11.VI.1934, Joo-Tso Chu (deposited in Zhejiang University). Paratype of *Telenomus dendrolimusi* Chu, 1937, CHINA • 1 female, same data as allotype • 1 female, same data except collected on 22.VI.1933 (deposited in Zhejiang University). Other material • 2 females 1 male, JAPAN: (Kyushu) Hakozaiki Fukuoka Jul. 31, 1959 Col. Y. Hirose, host: Egg of *Dendrolimus spectabilis* Butler, OSUC 71700-702 (deposited in OSUC) • 6 females 6 males, CHINA: Fujian, Xiapu County, 26°49'45.76"N, 119°57'55.3"E, ex. eggs of *Dendrolimus houi* (Lajonquiere) feeding on *Cryptomeria japonica* var. *sinensis* Miquel trees, 10.IX.2021, Guang-hong Liang, SCBG-E0010785-0010796 (deposited in SCBG).

Distribution. China (Liaoning, Hebei, Shandong, Henan, Jiangsu, Anhui, Fujian, Zhejiang, Jiangxi, Hubei, Hunan, Sichuan, Fujian, Guangdong, Guangxi, Guizhou, Yunnan), Japan, North Korea (Ryu and Hirashima 1985), Russia (Boldaruev 1969).

Comments. *Aholcus* Kieffer (Kieffer 1913) was erected for telenomines in which females have 10-merous antennae. *Aholcus* has since been treated as a junior synonym of *Telenomus* Haliday, a subgenus, a valid genus, and again as a junior synonym (Nixon 1935, Nixon 1937, Mineo et al. 2011, Taekul et al. 2014). Regardless of the generic value of the 10-merous female antenna, this character is useful for reducing the number of species that must be compared for identification. Nonetheless, there are dozens of species in this lineage, many of which have been described from Asia. *Telenomus dendrolimi* is a generalist that is known from southeastern China to Japan and may also be present much farther to the west. A reliable morphological diagnosis is not possible until the species of the broader region are revised— an effort that is beyond the scope of this project. However, by advancing the taxonomy of one described species, we are contributing toward this goal, and the combination of the description, images and sequence data provide a means to determine if a specimen is *T. dendrolimi*.

In this study, our identification of reared specimens was facilitated by their association with *Dendrolimus* eggs and matching them to paratype specimens of *T. dendrolimusi* and three non-type specimens of *T. dendrolimi* from Japan.

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Supplementary material I

Taxa, GenBank accession numbers, species group placement, collection location, and references associated with the sequence data used in the phylogenetic analyses

Authors: Hua-Yan Chen, Elijah Talamas, Zachary Lahey, Norman F. Johnson, Ci-Ding Lu, Guang-Hong Liang

Data type: xlsx

Explanation note: Species group/complex placement mainly follows Johnson (1984) and Taekul et al. (2013).

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Link: <https://doi.org/10.3897/jhr.97.139056.suppl1>

Supplementary material 2

28S ribosomal DNA cladogram of *Telenomus*

Authors: Hua-Yan Chen, Elijah Talamas, Zachary Lahey, Norman F. Johnson, Ci-Ding Lu, Guang-Hong Liang

Data type: docx

Explanation note: Numbers on branches to the left of the forward slash indicate SH-aLRT values and numbers to the right of the forward slash are ultrafast bootstrap values.

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Link: <https://doi.org/10.3897/jhr.97.139056.suppl2>

Supplementary material 3

Mitochondrial cytochrome oxidase I (COI) cladogram of *Telenomus*

Authors: Hua-Yan Chen, Elijah Talamas, Zachary Lahey, Norman F. Johnson, Ci-Ding Lu, Guang-Hong Liang

Data type: docx

Explanation note: Numbers on branches to the left of the forward slash indicate SH-aLRT values and numbers to the right of the forward slash are ultrafast bootstrap values. Terminal labels correspond to the taxa listed in the supplementary table.

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Link: <https://doi.org/10.3897/jhr.97.139056.suppl3>

Supplementary material 4

Combined 28S and mitochondrial cytochrome oxidase I (COI) cladogram of *Telenomus*

Authors: Hua-Yan Chen, Elijah Talamas, Zachary Lahey, Norman F. Johnson, Ci-Ding Lu, Guang-Hong Liang

Data type: docx

Explanation note: Numbers on branches to the left of the forward slash indicate SH-aLRT values and numbers to the right of the forward slash are ultrafast bootstrap values. Terminal labels correspond to the taxa listed in the Suppl. material 1.

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