2002

A Taxonomic study of the *Trichogramma westwood* (Trichogrammatidae) of Japan

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DOI: <https://doi.org/10.31979/etd.zz3e-5q7n>
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**A TAXONOMIC STUDY OF THE *TRICHOGRAMMA*
WESTWOOD (TRICHOGRAMMATIDAE)
OF JAPAN**

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Josephine M. Jose

May 2002

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ABSTRACT

A TAXONOMIC STUDY OF THE *TRICHOGRAMMA* WESTWOOD (TRICHOGRAMMATIDAE) OF JAPAN

By Josephine M. Jose

Four species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) are known from Japan: *Trichogramma japonicum* Ashmead, *T. papilionis* Nagarkatti, *T. dendrolimi* Matsumura and *T. chilonis* Ishii and two new species described herein as *T. cultella* Jose and *T. umera* Jose. The taxonomic history of *Trichogramma* in Japan is assessed along with the taxonomic difficulties prevalent to the genus. Previously known species are reviewed with respect to hosts, biological control uses, and distinguishing characters of the male genital capsule. Definitive anatomical features of the male genitalia were captured for *T. papilionis*, *T. dendrolimi*, and *T. chilonis* with scanning electron microscope (SEM) photographs, available for the first time. A key to the species extant in Japan (at the time of this writing) is provided.

ACKNOWLEDGMENTS

I want to thank Dr. Jeffrey Honda who gave me the opportunity to study these organisms. In 1998 he saw that I had the patience and temperament for taxonomic work and encouraged me to pursue it. I am profoundly indebted to Gary R. Platner (UC Riverside) who generously shared his valuable expertise with me for the protocols dealing with specimen preparation for the scanning electron microscope (SEM). Additionally, I am exceptionally grateful to Beth Dawson, Dr. Michael Sneary, Melissa Carter (CSU Hayward), Dr. Wayne Savage, and Albert Boot for use of the SEM at CSU Hayward and SJSU. Further appreciation is extended to Laurel Taylor who not only beautifully prepared the new species diagrams, but also revised the early drafts of this thesis. Also, I want to express thanks to Dr. John D. Pinto (UC Riverside) for his critique and helpful suggestions with the slide mounts. I am further obliged to Dr. Michael Kutilek and Curtis Takahashi (CDFA) for serving on my committee and improving my thesis. Additional gratitude is given to Zia Nisani, who always challenged me and made sure that I was on task and heading in the right direction. Special thanks goes to Dr. J. Gordon Edwards who first inspired me to study entomology. I am also grateful for the endless generosity and support of the Rodriguez family: Joey, Carol and Melissa.

This is dedicated with love to Lorenzo Rodriguez.

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INTRODUCTION

Egg parasitoids of the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) parasitize pestiferous insects in over 32 million hectares of agricultural land and forests yearly (Li 1994). Of the approximately 180 species described worldwide, about 70 species have been employed in agricultural pest control (Pinto 1998, Li 1994). Biological control efforts exploiting *Trichogramma* concentrate on forage crops (corn, rice, wheat, sorghum); crops of primary industries (sugarcane, sugar beet, cotton, soybean); vegetables fruit and forest trees (apple, plum, citrus, avocado, vineyards, pines, spruce) (Li 1994). The deployment of *Trichogramma* for the biological control of lepidopterous pests has been utilized for more than 100 years (Smith 1996). *Trichogramma* may be an economic alternative to expensive insecticides, reducing potential human exposure to insecticide residue and contamination (Andow and Prokrym 1991).

Despite the considerable attention *Trichogramma* has received as an important biological control agent, its taxonomy remains inadequately understood. Immense taxonomic problems must be resolved if applied work in this genus is to reach its full potential (Pinto 1998). Taxonomic complexities are partly attributable to difficulties with collecting and curating these fragile and minute (approximately 0.5 mm) parasitic wasps. This has greatly retarded the accumulation of study material (Platner *et al.* 1999). Early in the history of *Trichogramma* taxonomy confusion stemmed from lost, disregarded, or unusable type specimens which serve as the foundation to nomenclatural stability (Pinto 1998, Pinto and Stouthamer 1994). Consequently, species names were applied

incorrectly and inconsistently to specimens (Pinto and Stouthamer 1994, Pinto *et al.* 1978). *Trichogramma* taxonomy additionally suffered from an absence of consistent distinguishing morphological characters (Pinto 1998). This dilemma was partially solved when Nagarkatti and Nagaraja (1971) established the importance of the male genitalia as a diagnostic character, offering taxonomically reliable features for the first time (Pinto and Stouthamer 1994). Although the male genitalia provides dependable taxonomic traits, the genus remains relatively morphologically homogeneous and taxonomists are sometimes forced to use minor differences to separate species (Pinto and Stouthamer 1994, Pinto *et al.* 1989). Unfortunately, if negligible and insignificant variation is utilized as new species criteria a “typological species concept” ensues (Pinto *et al.* 1989). New taxa based on minor and insufficient differences eventually complicate the literature with names that can only be linked with the type material (Pinto *et al.* 1989). New species descriptions should measure and report ranges of intraspecific variation with the morphological data. This assures that future authors do not adopt any minor phenotypic differences as new species criteria (Pinto *et al.* 1989). If necessary, molecular evidence can be incorporated to solidify new species barriers and definitions (Pinto *et al.* 1997).

The history of *Trichogramma* taxonomy in Japan exemplifies many of the taxonomic difficulties prevalent in the genus. Yasumatsu and Watanabe (1964) published “A Tentative Catalogue of Insect Natural Enemies of Injurious Insects in Japan,” and cited the following *Trichogramma* species from Japan: *Trichogramma australicum* Girault, *Trichogramma chilonis* Ishii, *Trichogramma dendrolimi* Matsumura, *Trichogramma evanescens* Westwood, *Trichogramma japonicum* Ashmead and

Trichogramma jezoensis Ishii (Yasumatsu and Watanabe 1964). Ten years later, the description of *Trichogramma papilionis* Nagarkatti from Hazozaki, Fukuoka Prefecture brought the total to seven species (Nagarkatti 1974). Numerous errors to the tentative list have since surfaced, warranting a revision of all known Japanese species. Nagarkatti and Nagaraja (1979) concluded that populations originally determined as *T. australicum* were actually *T. chilonis*, subsequently these populations have been synonymized with *T. chilonis* (Nagarkatti and Nagaraja 1979). Pinto *et al.* (1982) designated lectotypes for *T. australicum* and stated it remains *only* known from Girault's original material collected in Queensland, Australia (Pinto *et al.* 1982). Moreover, the listing of *T. evanescens* may represent a misidentification. It may be impossible to accurately assign this name to a specimen since the holotype of this species is a damaged female (Pinto 1998, Sugonyaev 1986). Additionally, existing descriptions and diagrams of *T. jezoensis* Ishii (1941) lack the morphological data and detail necessary to confidently identify this species. Complicating matters further, Ishii's type specimens have been missing since World War II and are presumed destroyed (J. Honda personal communication, San Jose State University). Thus, the remaining four species: *T. japonicum*, *T. dendrolimi*, *T. chilonis* and *T. papilionis* constitute the known *Trichogramma* fauna of Japan.

Recent collections of *Trichogramma* in Japan (1997-1998) conducted by Dr. Jeffrey Honda (San Jose State University, San Jose, CA) in collaboration with the Institute of Biological Control (Faculty of Agriculture, Kyushu University, Fukuoka) yielded several unidentifiable species. The genital capsules of these *Trichogramma* differed significantly from known Japanese species. Subsequent molecular analysis of

the suspected new species (see Silva *et al.* 1999) based on the size of the ITS-2 rDNA PCR products, further supported the hypothesis that these were indeed new species.

This thesis describes two new *Trichogramma* species from the Okinawa Prefecture, Japan. Diagrams of wings, antennae and genital capsules along with scanning electron microscope (SEM) photographs of the male genital capsule accompany the new species descriptions. Additionally, the extant *Trichogramma* of Japan are reviewed with respect to distinguishing characters of the male genitalia, known hosts and biological control utilization. SEM photos of *T. chilonis*, *T. dendrolimi* and *T. papilionis* are available for the first time in this paper. The SEM was utilized to further investigate anatomical features previously unresolved by the limitations of the compound microscope. A key to differentiate the known species occurring in Japan (at the time of this writing) was developed and is provided in the Appendix B.

METHODS

Specimen Preparation for Slide Mounts

New species descriptions were based on measurements taken from slide mounted specimens. This necessary curation (mounted on microscope slides with Canada balsam) is complicated by specimen fragility, minute size and the necessity to precisely place the male genital capsule (about 0.1 mm in length) in a dorsoventral position for examination under the light microscope. As the most reliable and important structure used in *Trichogramma* species differentiation and identification, specific placement and positioning is crucial (Nagarkatti and Nagaraja 1971).

The following was modified from protocols established by Noyes (1982) and Platner *et al.* (1999). Dead specimens (stored in either 95% ethanol or cotton) were placed in a watch glass with 10% KOH and then on a slide warmer for at least 1 hour (duration varies with color of the insect) at 40° C. After the wasps "cleared" to a light pale brown the genital capsule was visible through the abdomen. Next, the wasps were transferred with a size 000 nylon paint brush (made thinner with ½ the bristles cut away) into a ceramic depression plate with 10ml of 10% ethanol and 1 drop of Triton X-100®. The ceramic plate was then placed back in a 40° C slide warmer for 1 hour after which, the 10% ethanol and Triton X-100® solution was pipetted off and replaced with 20% ethanol. The ceramic depression plate was removed from the slide warmer and left at room temperature overnight. Alcohol dehydration was performed using a series of 40, 60, 80, 95 and 100% ethanol. Specimens were dehydrated for 45-60 minutes in each step. Clove oil replaced the 100% ethanol after the final dehydration step, and the

specimen remained in clove oil for at least 2 hours. The wasps were mounted on glass microscope slides with Canada balsam, and prepared by dipping the head of a (#1) insect pin into Canada balsam and then placing a small “dot” of balsam in the center of the microscope slide. Wasps were transferred on to the prepared slide, with a 000 nylon paintbrush with all but two bristles cut away, into the small dot of balsam. With the ventral side up, wasps were positioned/manipulated with the fine probes using a dissecting scope at 100X magnification. The antennae, legs and wings were positioned in a single plane and clear of any obstruction from other body parts to ensure proper views for accurate measurements. Most importantly, the male genital capsule or female ovipositor was oriented in a dorsoventral position to ensure a complete view of the genitalic structures. A small amount of xylene was applied with the 2-bristle paintbrush when the Canada balsam became too “tacky” and needed to be liquefied. After positioning was complete, the slide was placed in a covered dust free slide warmer at 30° C for at least one week, before the 5 mm round cover slip was placed. A “dot” of Canada balsam equal in size to the amount of Canada balsam surrounding the specimen on the slide was placed on the cover slip using the head of an insect pin. The Canada balsam was “mixed” with a 2-bristle paintbrush dipped in xylene. With fine forceps, the cover slip was then carefully placed over the specimen, making sure there were no trapped air bubbles. The covered slide was placed on the slide warmer at 30° C for at least one week before measurements were taken.

Species Descriptions

Species descriptions follow the anatomical terminology, morphological measurements and ratios (relative dimensions) used in Pinto (1998). Linear measurements were reported with means $\pm$ 1 standard deviation measured to the nearest 0.01 mm. Ratios using anatomical measurements were reported with means $\pm$ 1 standard deviation. Descriptions were based on specimens mounted in Canada balsam on glass slides. Measurements were taken with an optical micrometer installed within a compound microscope. Diagnoses were based on morphological data from published species descriptions and/or redescrptions.

Specimen Preparation for the Scanning Electron Microscope (SEM)

The subsequent methods were based on personal communication with Gary R. Platner (UC Riverside). As with the procedures for slide mounts, dry male *Trichogramma* were placed in 10% KOH over a 40° C slide warmer for at least 1 hour. After clearing, the genital capsules were partially dissected from the abdomen such that the genital capsule remained attached to the body by a small amount of cuticle. Wasps were then transferred into a 5% solution of bleach for two minutes, and then transferred into de-ionized water for at least one minute. Specimens were then placed into a ceramic depression plate with 10 ml of 10% ethanol and 1 drop of Triton X-100® on a slide warmer at 40° degrees C for at least 1-2 hours. The specimens were then transferred through the following ethanol series: 20, 40, 60, 80, 95 and 100%. Wasps were left in each concentration for at least 45-60 minutes (not exceeding 60 minutes in 95% or 100% ethanol). While in the 100% ethanol, the genital capsules were completely severed from

the body, and any extraneous abdominal tissue was removed using fine forceps and a probe. With a 2-bristle paintbrush the capsules were placed in a small watch glass with a few drops of hexamethyldisilazane (HMDS) under a fume hood. The HMDS was allowed to evaporate completely. With the aid of a very fine probe and a dissecting microscope (up to 200X), the genital capsules were carefully placed on a carbon conductive tab affixed to a scanning electron microscope (SEM) stub. The specimens were then sputter coated with gold-palladium using a Hummer II (Technics, Inc.) to a depth of 250-300 Ångstroms. Images were captured with a Philips XL 40 SEM at the M.A.G.I.C. Laboratory facility, California State University at Hayward. Magnification and acceleration voltage (10 kV) used was printed on the photographs. Images of the male genital capsules of *T. dendrolimi*, *T. chilonis* and *T. papilionis* were captured to reveal and explore characters beyond the capabilities of light microscopy. Specimens of *T. japonicum* were unavailable for this study, however SEM photos of *T. japonicum* are available in Pinto (1998). Samples of *T. dendrolimi* and *T. chilonis* from the Shimane Agricultural Experiment Station, Shimane Prefecture, Japan and *T. papilionis* specimens from Fukuoka, Fukuoka Prefecture, Japan were used in this study.

RESULTS

Trichogramma cultella Jose, new species (Figs. 1-4)

Diagnosis:

Trichogramma cultella appears similar to the Nearctic *T. thalense* Pinto and Oatman. *Trichogramma thalense* narrows much more apically, with an apical width measuring only 0.51 ± 0.04 the genital width (Pinto 1998). The volsellae in *T. thalense* attain a greater portion (0.7 – 0.8) of the apical distance (Pinto 1998). Flagellar setae are shorter with this species, only 2.91 ± 0.22 times greater than the flagellum width (Pinto 1998). Forewing setal densities are dramatically denser in *T. thalense* with 30 – 60 setae between the 4th and 5th setal tracks (Pinto 1998). Basiconic peg sensilla formulas vary between the two species with *T. thalense* 0(1)-1-1-0-1-1 (Pinto 1998). Ovipositor lengths are clearly shorter in *T. thalense*, only 0.79 ± 0.01 the length of the hind tibia (Pinto 1998).

Trichogramma cultella may also be confused with the Palearctic *T. elegantum* Sorokina. Flagellar setae of *T. elegantum* are considerably shorter, only 2.2 times greater than the flagellum width (Sorokina 1984, Pinto 1998). *Trichogramma elegantum* females also have relatively shorter ovipositors, attaining 0.95 the hind tibial length (Sorokina 1984, Pinto 1998).

Two additional morphologically similar congeners include the Taiwanese species *T. choui* Chan and Chou and *T. taiwanese* Chan and Chou. The genital capsules of the two are much wider in comparison, 0.36-0.40 and 0.41-0.45 as wide as long in *T. choui* and *T. taiwanese*, respectively (Chan and Chou 2000). The volsellae in *T. choui* attain a

greater fraction (0.90-1.0) of the apical distance (Chan and Chou 2000). Wing setation patterns vary, with *T. choui* having higher forewing setal densities of 16-27 setae between the 4th and 5th setal tracks and 7-10 setae in the posterior track of the hind wing (Chan and Chou 2000).

Description:

Unless otherwise indicated, quantitative data was taken from 7 males with a hind tibial length (HTL) of 0.14-0.16 mm and 2 females (HTL = 0.14-0.15 mm). Color was recorded from dried specimens wrapped in tissue collected 4 November 1997 by Y. Nirai and Itagaki (first initial unknown) from Iriomote Island, Okinawa, Japan from *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, placed on green pepper. Males and females have metasomas with extensive brown suffusion and a dull yellow to tan mesosoma.

Forewings are relatively narrow, 0.25 ± 0.01 mm wide (N=5); forewing width (FWW) 0.52 ± 0.02 as long as the forewing length (FWL) (N=5); setation relatively sparse, 7-13 (N=4) setae between 4th and 5th setal tracks; longest fringe setae 0.13 ± 0.03 (N=5) the FWW, approximately 1.5-2.1 times greater than the hind tibial width (HTW). Hind wings with 0 (N=2) and 3-5 (N=2) setae in the anterior and posterior tracks, respectively, the latter tract occupying 35-36% of the distance from the hamuli to wing apex. Scutellum with anterior pair of setae equaling 91-95% (N=2) the length of the posterior pair of setae.

Male:

Flagellum length (FL) 0.16-0.19 mm long, approximately 2.4 times longer than the scape length, FL 6.57 ± 0.63 times greater than the flagellum width (FW), FL 1.22 ± 0.11 times greater than the HTL; flagelliform setae long, tapering toward apex, the longest flagelliform setae 3.53 ± 0.57 times longer than the FW; lacking unsocketed setae; basiconic peg sensilla (BPS) relatively small and only slightly inflated, with the formula 2-2-2-1-1-1 (N=6); terminal placoid sensilla (PLS) with 0.23-0.34 extending beyond flagellum apex.

Genital capsule (GC) relatively narrow, only 0.30 ± 0.01 as wide as long; apical distance (AD) 0.19 ± 0.01 as long as the genital length (GL); apical width (AW) measuring 0.68 ± 0.04 the genital width (GW); dorsal aperture length (DAL) comprising 0.49 ± 0.02 the GL; dorsal lamina (DLA) arising in the anterior half of the GC without approaching the GC sides, the slender posterior extension of the DLA narrows and sharply tapers toward the apex occupying 0 – 0.42 of the AD (see Variation), width at the level of the intervolsellar process (IVP) much smaller than the aedeagus; DLA 1.68 ± 0.34 (see Variation) as long as wide and 0.47 ± 0.04 the length of the GC; IVP short, subtriangular, occupying only 0.11- 0.17 the AD; volsellae (VS) relatively straight, occupying 0.42-0.61 the AD; ventral ridge (VR) narrow and abruptly widening at base, occupying 0.45 – 0.58 the basal distance (BD); ventral processes (VP) not obviously protuberant, only slightly laterally displaced to the VR and positioned distinctly anterior to the IVP. Aedeagus length (AL) approximately equal to GL, attaining 0.76 ± 0.07 (N=6) the HTL; apodemes occupying approximately 0.58 the AL.

Female:

Funicle of antennae with 1 BPS on the first funicular segment (F1) and 2 on the second funicular segment (F2). Ovipositor length (OL) 1.04 – 1.07 times greater than the hind tibial length.

Types:

Holotype ♂ and allotype ♀. JAPAN. *Okinawa Prefecture*: Iriomote Island; 4 November 1997; *Ephestia kuehniella*; Y. Nirai/Itagaki. Seven paratypes (6 ♂, 1 ♀),

Etymology:

Feminine form of the Latin word *cultellus* meaning small knife, referring to the knife-like appearance of the posterior extension of the DLA.

Variation:

The posterior extension of the DLA usually occupied 0.22 – 0.42 of the AD, although the posterior extension of the DLA of one of the paratypes failed to reach the level of the AD, only measuring 0.97 as wide as long.

Geographic Distribution:

Known only from Japan.

***Trichogramma umera* Jose, new species**
(Figs. 5-8)

Diagnosis:

Trichogramma umera closely resembles *T. pretiosum* Riley. In that species dorsal lamina width to length ratios vary with a mean of 1.67 ± 0.11 with the dorsal lamina comprising 0.26 ± 0.01 the genital length (Pinto 1998). *Trichogramma pretiosum*

females have longer ovipositors, measuring 0.99 ± 0.03 the hind tibial length (Pinto 1998).

Trichogramma umera might be confused with *T. minutum* Riley and *T. platneri* Nagarkatti. The dorsal lamina length to genital capsule length ratios are greater in *T. umera*, with the two known species *T. minutum* and *T. platneri*, equaling only 0.29 ± 0.03 and 0.28 ± 0.02 (Pinto 1998). Forewings are relatively wider for the two previously known species, more than 50% as wide as long (Pinto 1998). The flagellum lengths of the comparative species are shorter, only 5.76 ± 0.37 and 5.92 ± 0.30 times longer than the flagellum width for *T. minutum* and *T. platneri*, respectively (Pinto 1998). *Trichogramma minutum* and *T. platneri* have longer ovipositors measuring 1.14 ± 0.04 and 1.08 ± 0.05 times longer than the hind tibial length, respectively (Pinto 1998).

The Australian *T. australicum* Girault also bears resemblance to *T. umera* but the former has a slightly wider genital capsule, equaling 0.36 as wide as long (Pinto 1982). Flagellar setae are significantly shorter in *T. australicum*, only 1.44 times longer than the flagellum width (Pinto 1982). The dramatically longer ovipositor of *T. australicum* (1.22 times greater than the hind tibial length) (Pinto 1982) further differentiates the two.

Another virtually identical congener, *T. chilotraeae* Nagaraja and Nagarkatti, is separated from *T. umera* by a relatively longer aedeagus that roughly equals the hind tibial length (Nagaraja and Nagarkatti 1969). Additionally, the margins of the posterior extension of the dorsal lamina vary; they are curved in *T. umera* where as almost straight in *T. chilotraeae* (Sorokina 1993).

Trichogramma ostrinae Pang and Chen also resembles *T. umera*, but they are differentiated as the ventral processes are adnate to the intervalsellar process in *T. ostrinae* (Pinto 1998). Fringe setae of the *T. ostrinae* forewing are relatively shorter, only 1.51 ± 0.01 times greater than the width of the hind tibia (Pinto 1998). *Trichogramma ostrinae* also have shorter flagellar setae, only 2.25 ± 0.04 times longer than the width of the flagellum and longer ovipositors 1.18 ± 0.01 times greater than the hind tibial length (Oatman *et al.* 1982).

Trichogramma umera can also be confused with the Japanese *T. papilionis* Nagarkatti but *T. papilionis* has a longer aedeagus, 0.90 the hind tibial length (Oatman *et al.* 1982). The papilliform ventral processes adnate to the intervalsellar process accompanied with truncate and rectangular volsellae help distinguish *T. papilionis* (Fig. 17) from *T. umera*. The setae in the posterior track of the hind wing in *T. papilionis* occupy a greater portion (75%) of the distance from the hamuli to wing apex (Oatman *et al.* 1982). Lastly, *T. papilionis* have much longer ovipositors, 1.20 times greater than the hind tibial length (Oatman *et al.* 1982).

Description:

Unless otherwise indicated, quantitative data was taken from 8 males with a hind tibial length (HTL) of 0.13-0.16 mm and 3 females (HTL = 0.14-0.17 mm). Specimens were originally collected 13 March 1997 by Y. Narai and Kajitani (first initial unknown) on Ishigaki Island, Okinawa, Japan from *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae). Males and females appear yellow with brown suffusion in the mesosoma and almost an entirely brown metasoma.

Forewings are narrow, 0.21 ± 0.02 (N=7) mm wide; the forewing width (FWW) only 0.47 ± 0.03 (N=7) the forewing length (FWL); 9-21 setae between 4th and 5th setal tracts (N=5); the longest fringe setae measuring 0.19 ± 0.02 (N=7) the FWW, about 2.38 times greater than the hind tibial width (HTW) (N=7). Hind wing with 1-3 and 4-8 setae in the anterior and posterior tracts, respectively, the latter tract occupying approximately 52% of the distance from the hamuli to wing apex. Scutellum with posterior pair of setae measuring 86 – 91% the anterior pair of setae (N=2).

Male:

Flagellum length (FL) 0.15 – 0.18 mm, 1.92 – 2.42 times longer than the length of the scape, FL measuring 6.85 ± 0.44 times greater than the flagellum width (FW), FL 1.16 ± 0.09 times longer than the HTL; flagelliform setae long, 3.0 ± 0.12 times greater than the FW; without unsocketed setae; basiconic peg sensilla (BPS) relatively small, only slightly inflated, formula 1(2)-2-2-0-1-1, terminal placoid sensilla (PLS) with apical $0.09 - 0.16$ (N=6) extending beyond flagellum apex.

Genital capsule (GC) 0.33 ± 0.01 as wide as long; sides not constricted at level of the intervalsellar process (IVP); parameres (PM) relatively straight, slightly convergent at apex; apical distance (AD) measuring 0.26 ± 0.01 the entire genital length (GL); apical width (AW) amounting to 0.60 ± 0.03 the genital width (GW); dorsal aperture length (DAL) 0.56 ± 0.02 the GL; dorsal lamina (DLA) originating posterior to the middle of the GL, moderately notched at base with moderately developed shoulders, not reaching sides of GC; sides of DLA gradually narrowing posteriorly to form a sublinguiform posterior extension, distinctly acuminate at apex, whose width is approximately equal to

the aedeagus at the intervalsellar process (IVP) level; DLA measuring 1.38 ± 0.05 as long as wide, equaling 0.39 ± 0.02 the GL, occupying 0.42-0.57 the AD; volsellae (VS) slightly bowed occupying 0.42-0.57 the AD; IVP elongate, narrowing apically toward a pointed apex, occupying 0.30 – 0.43 the AD; ventral processes (VP) not obviously protuberant in shape, positioned immediately basal to the IVP and slightly anterior to the ventral ridge (VR); VR occupying 0.24 – 0.34 the basal distance (BD). Aedeagus length (AL) approximately equal to GL, attaining only 0.77 ± 0.05 the HTL; apodemes about 0.49 the AL.

Female:

Antennae with 1 and 2 BPS on the first funicular segment (F1) and the second funicular segment (F2), respectively. Ovipositor length (OL) only 0.82 – 0.85 the HTL.

Types:

Holotype ♂ and allotype ♀. JAPAN. *Okinawa Prefecture*: Iriomote Island; 13 March 1997; *Plutella xylostella*; Y. Nirai/Kajitani. 9 paratypes (7 ♂ and 2 ♀).

Etymology:

Feminine form of the Latin word *umerus* meaning shoulder, referring to the distinct shoulders of the DLA present in the type series.

Review of Extant Species in Japan

Due to the extensive nomenclatural instabilities and inaccuracies within this genus much of the wide spread literature addressing *Trichogramma* host records and biological control utilization are of limited value and should be approached carefully (Pinto 1998, Noyes 1994). With this in mind, this paper presents published hosts and biological

control uses for known Japanese species since this information may be useful to ecologists and biological control practitioners.

***Trichogramma japonicum* Ashmead**
(Table 1, 4)

***Trichogramma japonicum* Ashmead** 1904: 165; Nagarkatti and Nagaraja 1971: 22; Pang and Chen 1974: 450; Oatman, Pinto and Platner 1982: 16; Hayat and Viggiani 1984: 36; Sorokina 1993: 38; Pinto 1998: 217; Chan and Chou 2000: 140

Distribution: Japan, India, Vietnam, China, Thailand, Hawaii, Malaysia, Philippines (Sorokina 1993), Canada, Australia (Pinto 1998), and Taiwan (Chan and Chou 2000)

Hosts: Table 1.

Biological Control Uses of *Trichogramma japonicum*: See Table 4.

Distinguishing Characters of Genital Capsule: See Pinto (1998) for SEM photographs. The anatomical features distinguishing *Trichogramma japonicum* include: a uniformly wide dorsal lamina base, elongate nearly parallel parameres and an exceptionally long apical distance (0.34 ± 0.01 the genital length) (Pinto 1998).

***Trichogramma chilonis* Ishii**
(Figs. 9-11; Table 2, 4)

***Trichogramma chilonis* Ishii** 1941: 173; Nagarkatti and Nagaraja 1979: 115; Oatman, Pinto and Platner 1982: 22; Hayat and Viggiani 1984: 34; Sorokina 1993: 68; Chan and Chou 2000: 136

Distribution: India, Japan, China, Hawaii, Guam (Pinto *et al.* 1982), Pakistan, Vietnam, New Guinea, Philippines, Madagascar, Mauritius (Sorokina 1993) and Taiwan (Chan and Chou 2000)

Hosts: Table 2.

Biological Control Uses of *Trichogramma dendrolimi*: Table 4.

Distinguishing Characters of Genital Capsule: The SEM images depict several characters defining *Trichogramma chilonis*: prominent lateral lobes (not extending to the edge of the genital capsule), the dorsal lamina the sublinguiform posterior extension abruptly narrowing above the shoulders which reach the level of the volsellae, an elongate and narrowly subtriangular intervalsellar process and papilliform ventral processes just basal to the intervalsellar process.

***Trichogramma dendrolimi* Matsumura**
(Figs. 12-14: Table 3, 4)

***Trichogramma dendrolimi* Matsumura** 1926: 45; Nagarkatti and Nagaraja 1971: 17;
Pang and Chen 1974: 444; Hayat and Viggiani 1984: 35; Sorokina 1993: 69;
Chan and Chou 2000: 140

Distribution: Japan (Ishii 1941), Russia, Belarus, Ukraine, Moldavia, Kazakhstan,
Western Europe, China (Sorokina 1993) and Taiwan (Chan and Chou 2000)

Hosts: Table 3.

Biological Control Uses of *Trichogramma dendrolimi*: Table 4.

Distinguishing Characters of Genital Capsule: The SEM photographs clearly represent the distinguishing characters of *Trichogramma dendrolimi*: a dorsal lamina with prominent shoulders extending to the edge of the genital capsule with deep notching, and a prominent subtriangular intervalsellar process (equal in length to the volsellae) occupying about 75% of the apical distance.

***Trichogramma papilionis* Nagarkatti**
(Figs. 15-17)

***Trichogramma papilionis* Nagarkatti** 1974: 391; Oatman, Pinto and Platner 1982: 9;
Sorokina 1993: 44

Distribution: Japan, Hawaii (Sorokina 1993)

Hosts: *Papilio xuthus* Linnaeus, *Papilio memnon thunbergi* Siebold (Lepidoptera:
Papilionidae) (Sorokina 1993).

Distinguishing Characters of Genital Capsule: The SEM photos represent a few distinctive features of *Trichogramma papilionis*: rectangular and truncate volsellae clearly separate from the parameres, an obviously robust intervolsellar process with adnate and prominent ventral processes and a dorsal lamina with slightly developed shoulders and a sublinguiform posterior extension.

Fig. 1. Dorsal view of the male genital capsule of *T. cultella* n. sp. (736X).

Fig. 2. Ventral view of the male genital capsule of *T. cultella* n. sp. (656X).

Fig. 3. Ventral view of the apical distance of *T. cultella* n. sp. (1662X).

Fig. 4. *T. cultella* n. sp. 4a, Dorsal and lateral view of the antennae. 4b, Dorsal and ventral view of the male genital capsule. 4c, Forewing. 4d, Hind wing. Scale line equals 0.05 mm (courtesy of Laurel V. Taylor).

Fig. 5. Dorsal view of the male genital capsule of *T. uvera* n. sp. (637X).

Fig. 6. Ventral view of the male genital capsule of *T. umeria* n. sp. (603X).

Fig. 7. Ventral view of the apical distance of *T. umera* n. sp. (2789X).

Fig. 8. *T. umeria* n. sp. 8a, Antennae. 8b, Dorsal and ventral view of the male genital capsule. 8c, Forewing. 8d, Hind wing. Scale line equals 0.05 mm (courtesy of Laurel V. Taylor).

Fig. 9. Dorsal view of the male genital capsule of *T. chilonis* (654X).

Fig. 10. Ventral view of the male genital capsule of *T. chilonis* (568X).

Fig. 11. Ventral view of the apical distance of *T. chilonis* (2906X).

Fig. 12. Dorsal view of the male genital capsule of *T. dendrolimi* (506X).

Fig. 13. Ventral view of the male genital capsule of *T. dendrolimi* (690X).

Fig. 14. Ventral view of the apical distance of *T. dendrolimi* (2259X).

Fig. 15. Dorsal view of the male genital capsule of *T. papilionis* (416X).

Fig. 16. Ventral view of the male genital capsule of *T. papilionis* (503X).

Fig. 17. Ventral view of the apical distance of *T. papilionis* (3000X).

DISCUSSION

Trichogramma cultella n. sp. and *T. umera* n. sp. represent a determined attempt to circumvent the typological approach that has pervaded *Trichogramma* taxonomy. The two new species in this paper are supported by molecular evidence (J. Honda unpublished data); scanning electron microscope photographs which can capture morphological characteristics far superior any compound microscopes; and diagnoses from nearly identical congeners based on major anatomical differences.

As with the case with many *Trichogramma* species (*i.e.*, *T. suorangelica* Pinto 1998) descriptions of *T. cultella* and *T. umera* are based on specimens from a single locality (Iriomote Island, Okinawa and Ishigaki Island, Okinawa, respectively). As recommended by Pinto *et al.* (1989) these *Trichogramma* collected from limited sample sizes are described because major anatomical differences were found. The diagnoses accompanying the *T. cultella* and *T. umera* species descriptions expound on these anatomical differences as they are compared to several almost identical congeners. Morphological data for the new species were reported as a range or with the mean $\pm$ 1 standard deviation to establish estimates of intraspecific variation and to account for phenotypic plasticity. Unreliable taxonomic characters such as "simple characters" discussed by Pinto *et al.* (1989) were avoided as diagnostic criterion for species differentiation. Simple characters (those measured and not defined by a ratio) include: flagellum length, longest flagellar setae, longest fringe setae on the forewing, forewing width, hind tibial length, genital capsule length, genital capsule width, aedeagus length, female antennal club length, and ovipositor length (Pinto *et al.* 1989). Those characters

utilizing ratios are less sensitive to phenotypic plasticity (Pinto *et al.* 1989). With most reliable characters being the genital width to genital length ratio (GW/GL), aedeagus length to hind tibial length (AL/HTL), apical distance to genital length (AD/GL), and ovipositor length to hind tibial length (OL/HTL) (Pinto *et al.* 1989).

No experiments addressing reproductive compatibility were conducted in this study. Complete incompatibility is not a prerequisite for *Trichogramma* species separation (Pinto and Stouthamer 1994). Species such as *T. platneri* Nagarkatti (1975) were defined reproductively and such definitions should be avoided (Pinto 1998). Reproductive incompatibility should rarely, if at all, be the only character for species discrimination (Pinto *et al.* 1991). When a relational trait such as reproductive compatibility defines a species relative to another species the species under examination lacks characters of their own (Pinto 1998). The species definition used in this study is, the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (Pinto 1998, Nixon and Wheeler 1990).

Trichogramma cultella and *T. umera* have been placed in the nominate subgenus *Trichogramma*, primarily defined by the male antennae with all flagellar segments (except the anellus 1) fused into a single claval segment (Pinto 1998). The other subgenera include *Trichogrammanza* Carver characterized by a two-segmented funicle and one-segmented clavus in males (Pinto and Stouthamer 1994) and *Vanlisus* Pinto where the flagellum consists of two distinct funicular and three claval segments (Pinto 1998).

Trichogramma cultella is positioned in the Thalense section of the nominate subgenus characterized by: a relatively narrow genital capsule ($GW/GL > 0.30$), a narrow posterior extension of the dorsal lamina and the immediately to slightly anterior position of the ventral processes relative to the ventral ridge (Pinto 1998). Arguably, *T. cultella* can be categorized in the Parkeri section, considered the most primitive of the subgenus *Trichogramma* (Pinto 1998). This argument is substantiated by: the narrow genital capsule ($GW/GL > 0.30$), a narrow posterior extension of the dorsal lamina and short intervalsellar process indicative of both the Parkeri section (Pinto 1998) and *T. cultella*. Final placement within the Thalense section is based on additional characters defining the Parkeri section: a distinct subapical constriction, arcuate parameres and lateral placement of the ventral processes with respect to the ventral ridge (Pinto 1998) not represented in *T. cultella*.

Trichogramma umera is placed in the Exiguum section, the largest section within *Trichogramma*. This section is defined by: a posteriorly narrowed genital capsule with a slight constriction at the base of the parameres, unmodified and relatively straight parameres, usually linguiform posterior extension of the dorsal lamina, elongate intervalsellar processes with ventral processes basal to the intervalsellar process and immediately lateral to the ventral ridge (Pinto 1998). *Trichogramma umera* clearly embodies the characteristics constituting this section. The Exiguum section comprises of many minimally differentiated and difficult to identify species (Pinto 1998). Many *Trichogramma* utilized in biological control worldwide belong here (Pinto 1998).

SEM photographs of *T. dendrolimi* and *T. chilonis* are consistent with diagrams accompanying redescrptions of the species from Taiwan published recently by Chan and Chou (2000). However, when compared to their diagram of *T. dendrolimi* the SEM photo reveals a dorsal lamina with smaller lobes and a shorter more linguiform posterior extension. This documents the phenotypic plasticity inherent with the dorsal lamina (Pinto *et al.* 1989). This variation is seen in *T. pretiosum*, where the dorsal lamina is typically moderately lobed when reared at 18 and 24°C and only slightly lobed or not lobed at 29°C (Pinto *et al.* 1989). Upon evaluation of the SEM photo of *T. chilonis* with diagrams of the same species by Chan and Chou (2000) variation was noticed with the intervolsellar process. Chan and Chou (2000) depict the intervolsellar process nearly triangular and attaining a lesser portion of the apical distance when compared to the SEM photo of the Japanese *T. chilonis*. Disparities with the intervolsellar process have been documented with *T. minutum*, depending on form (A or B) the structure varies from relatively narrow, spine like and elongate to narrowly subtriangular and moderately elongate (Pinto 1998). The preceding intraspecific variation should be considered if describing any new species morphologically similar to *T. dendrolimi* or *T. chilonis*, especially if those variations shown with the dorsal lamina and intervolsellar process as demonstrated in the SEM photographs are observed. The SEM photographs of *T. papilionis* revealed prominent ventral processes adnate to the intervolsellar process. These were not depicted in diagrams in the original new species description and subsequent publications (Nagarkatti 1974, Oatman *et al.* 1982). This is significant because ventral processes adnate to the intervolsellar process were used to differentiate *T.*

ostrinae from a nearly morphologically identical *T. pretiosum* (Pinto 1998). The discovery of this character in *T. papilionis* may aid in distinguishing this species from other similar congeners.

Inevitably, more *Trichogramma* species will be discovered; with technologies such as DNA sequencing and scanning electron microscopes become more prevalent and accessible to taxonomists it is the expectation of this author that these tools complement traditional taxonomic investigations when feasible. Although, it may be impossible to require taxonomists to use these tools, these tools should certainly be recruited to solidify ambiguous and ill-defined species barriers. Clearly, DNA sequencing and scanning electron microscopy may alleviate some of the nomenclatural inaccuracies and instabilities typical in this genus.

Finally, it is always the responsibility of the taxonomist to adopt rigorous species criterion (based on significant anatomical differences). It is perhaps the combination of taxonomists adhering to those rigorous species criterion while embracing new technologies (as mentioned previously) that will bring stability and order to *Trichogramma* taxonomy.

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APPENDIX A

Table 1. Hosts of *Trichogramma japonicum* Ashmead

Host	Order	Family	Source
<i>Chilo suppressalis</i> (Walker)	Lepidoptera	Pyralidae	Sorokina 1993
<i>Scirpophaga incertulas</i> (Walker)	Lepidoptera	Pyralidae	Sorokina 1993
<i>Trichoplusia ni</i> (Hübner)	Lepidoptera	Noctuidae	Sorokina 1993
<i>Sesamia exigua</i> (Butler)	Lepidoptera	Noctuidae	Sorokina 1993
<i>Naranga aenescens</i> Moore	Lepidoptera	Noctuidae	Sorokina 1993
<i>Leucania</i> sp.	Lepidoptera	Noctuidae	Sorokina 1993
<i>Lampides boeticus</i> (Linnaeus)	Lepidoptera	Lycaenidae	Nagarkatii & Nagaraja 1971
<i>Parnara guttata</i> (Bremer and Grey)	Lepidoptera	Hesperiidae	Nagarkatii & Nagaraja 1971
<i>Sepedon sauteri</i> Hendel	Diptera	Sciomyzidae	Nagarkatii & Nagaraja 1971

Table 2. Hosts of *Trichogramma chilonis* Ishii

Host	Order	Family	Source
<i>Chilo simplex</i> Butler	Lepidoptera	Pyalidae	Sorokina 1993
<i>Chilo infuscatellus</i> Snellen	Lepidoptera	Pyalidae	Sorokina 1993
<i>Chilo suppressalis</i> (Walker)	Lepidoptera	Pyalidae	Sorokina 1993
<i>Chilo sacchariphagus</i> (Bojer)	Lepidoptera	Pyalidae	Sorokina 1993
<i>Ostrinia nubilalis</i> (Hübner)	Lepidoptera	Pyalidae	Sorokina 1993
<i>Tryporysa incertulas</i> Walker	Lepidoptera	Pyalidae	Sorokina 1993
<i>Heliothis armigera</i> (Hübner)	Lepidoptera	Noctuidae	Sorokina 1993
<i>Spodoptera litura</i> Fabricius	Lepidoptera	Noctuidae	Sorokina 1993
<i>Trichoplusia ni</i> (Hübner)	Lepidoptera	Noctuidae	Chan & Chou 2000
<i>Plutella xylostella</i> (Linnaeus)	Lepidoptera	Plutellidae	Chan & Chou 2000
<i>Ochyrotica yanoi</i> Arenberger	Lepidoptera	Pterophoridae	Chan & Chou 2000
<i>Orgyia postica</i> (Walker)	Lepidoptera	Lymantriidae	Chan & Chou 2000
<i>Agrius convolvuli</i> (Linnaeus)	Lepidoptera	Sphingidae	Sorokina 1993
<i>Macroglossum pyrrhostictum</i> (Butler)	Lepidoptera	Sphingidae	Chan & Chou 2000
<i>Parnara guttata</i> (Bremer and Grey)	Lepidoptera	Hesperiidae	Sorokina 1993
<i>Pelopidas mathias</i> Fabricius	Lepidoptera	Hesperiidae	Sorokina 1993
<i>Udaspes folus</i> (Cramer)	Lepidoptera	Hesperiidae	Chan & Chou 2000
<i>Lampides boeticus</i> (Linnaeus)	Lepidoptera	Lycaenidae	Chan & Chou 2000
<i>Jamides bochus formosanus</i> Fruhstorfer	Lepidoptera	Lycaenidae	Chan & Chou 2000
<i>Princeps demoleus libanius</i> Fruhstorfer	Lepidoptera	Papilionidae	Chan & Chou 2000
<i>Acraea issoria</i> (Hübner)	Lepidoptera	Nymphalidae	Chan & Chou 2000
<i>Danaus chrysippus</i> (Linnaeus)	Lepidoptera	Danaidae	Chan & Chou 2000

Table 3. Hosts of *Trichogramma dendrolimi* Matsumura

Host	Order	Family	Source
<i>Dendrolimus sibiricus</i> Tschetverkov	Lepidoptera	Lasiocampidae	Sorokina 1993
<i>Dendrolimus pini</i> Linnaeus	Lepidoptera	Lasiocampidae	Sorokina 1993
<i>Gastropacha quercifolia</i> Linnaeus	Lepidoptera	Lasiocampidae	Sorokina 1993
<i>Orgyia antiqua</i> Linnaeus	Lepidoptera	Lymantriidae	Sorokina 1993
<i>Pygaera curtula</i> Linnaeus	Lepidoptera	Notodontidae	Sorokina 1993
<i>Pheosia tremula</i> Clerck	Lepidoptera	Notodontidae	Sorokina 1993
<i>Panolis flammea</i> Denis and Schifferrmüller	Lepidoptera	Noctuidae	Sorokina 1993
<i>Mamestra brassicae</i> (Linnaeus)	Lepidoptera	Noctuidae	Sorokina 1993
<i>Spodoptera litura</i> (Fabricius)	Lepidoptera	Noctuidae	Chan & Chou 2000
<i>Hyphantria cunea</i> Drury	Lepidoptera	Arctiidae	Sorokina 1993
<i>Ostrinia nubilalis</i> (Hübner)	Lepidoptera	Pyalidae	Sorokina 1993
<i>Archips rosana</i> (Linnaeus)	Lepidoptera	Tortricidae	Sorokina 1993
<i>Laspeyresia pomonella</i> (Linnaeus)	Lepidoptera	Tortricidae	Sorokina 1993
<i>Homona magnanima</i> Diakonoff	Lepidoptera	Tortricidae	Chan & Chou 2000
<i>Princeps demoleus libanius</i> Fruhstorfer	Lepidoptera	Papilionidae	Chan & Chou 2000
<i>Papilio memnon heronus</i> Fruhstorfer	Lepidoptera	Papilionidae	Chan & Chou 2000
<i>Elymnias hypermnestra</i> <i>hainana</i> Moore	Lepidoptera	Nymphalidae	Chan & Chou 2000
<i>Pristiphora pallipes</i> Leppel.	Hymenoptera	Tenthredinidae	Sorokina 1993
<i>Caliroa cenasi</i> (Linnaeus)	Hymenoptera	Tenthredinidae	Sorokina 1993

Table 4. Biological Control Uses¹ of *Trichogramma japonicum*², *T. dendrolimi* and *T. chilonis*

Species	Crop	Pest	Source
<i>T. chilonis</i>	Sugarcane	<i>Chilo infuscatellus</i> Snellen	Rachappa & Naik 2000
<i>T. chilonis</i>	Pigeonpea	<i>Helicoverpa armigera</i> (Hübner)	Romeis <i>et al.</i> 1999
<i>T. chilonis</i>	Sorghum	<i>Helicoverpa armigera</i> (Hübner)	Romeis <i>et al.</i> 1999
<i>T. chilonis</i>	Sugarcane	<i>Tryporyza nivella</i> (Fabricius)	Ashraf <i>et al.</i> 1993
<i>T. chilonis</i>	Sugarcane	<i>Emmalocera depressella</i> (Swinh.)	Ashraf <i>et al.</i> 1993
<i>T. chilonis</i>	Sugarcane	<i>Chilo auriclius</i> Dudgeon	Varma <i>et al.</i> 1991
<i>T. chilonis</i>	Maize	<i>Ostrinia furnacalis</i> (Guenée)	Nafus & Schreiner 1986
<i>T. chilonis</i>	Sweetpotato	<i>Agrius convolvuli</i> Linnaeus	Nafus & Schreiner 1986
<i>T. chilonis</i>	Sugarcane	<i>Argyroploce schistaceana</i> Snellen	Wen 1986
<i>T. chilonis</i>	Sugarcane	<i>Proceras venosatus</i> Walker	Wen 1986

¹ Biological control uses including *Trichogramma japonicum*, *T. dendrolimi* and *T. chilonis* have been reviewed extensively by: Cock 1985, Hassan 1993, Li 1994, Smith 1996 and Greenberg *et al.* 1998. References shown were not included in those reviews or were published after 1998.

² Although no references are made here, reviews utilizing this parasitoid are included in: Cock 1985, Hassan 1993, Li 1994, Smith 1996 and Greenberg *et al.* 1998.

APPENDIX B

Key to the Species of *Trichogramma* Westwood of Japan (Males)

1. IVP short, poorly developed and occupying no more than 0.20 the AD (Figs. 2, 3); GC relatively narrow, 0.3 as wide as long (Figs. 1, 2).....2
- 1'. IVP prominent, well developed and occupying greater than 0.33 the AD (Figs. 6, 7, 10, 11, 13, 14, 16, 17); GC greater than 0.3 as wide as long (Figs. 5, 6, 9, 10, 12, 13, 15, 16).....3

- 2(1). DLA broad and rounded with a linguiform posterior extension; parameres elongate and straight; AD 0.34 the GL (Pinto 1998).....*T. japonicum*
- 2'. DLA narrow with a sharply tapering posterior extension (Fig. 1); parameres slightly convergent at apex (Figs. 2,3); AD 0.19 the GL (Fig. 2)....*T. cultella* n. sp.

- 3(1'). GC relatively broad, 0.4-0.5 as wide as long (Figs. 9, 10, 12, 13).....4
- 3'. GC not as broad, only 0.32-0.34 as wide as long (Figs. 5, 6, 15, 16).....5

- 4(3). Lobes of DLA reaching edge of GC (Fig. 12); apex of IVP equal in length to volsellae (Figs. 13, 14).....*T. dendrolimi*
- 4'. Lobes of DLA do not reach edge of GC (Fig. 9); apex of IVP below volsellae apex (Figs. 10, 11).....*T. chilonis*

- 5(3'). VP prominent and papilliform (Figs. 16, 17); volsellae rectangular and truncate clearly separate from parameres (Figs. 16, 17); IVP robust with slightly curved edges (Figs. 16, 17).....*T. papilionis*
- 5'. VP not obviously protuberant (Figs. 6, 7); volsellae relatively straight and appear "attached" to parameres (Figs. 6, 7); IVP elongate with straight edges (Figs. 6, 7).....*T. umera* n. sp.