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# Morphological and Molecular Characterization of *Apanteles mohandasi* Sumodan & Narendran (Hymenoptera: Braconidae), a Solitary Endoparasitoid of *Pammene critica* Meyrick (Lepidoptera: Tortricidae), with Notes on Biology from India

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# MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *APANTELES MOHANDASI* SUMODAN & NARENDRAN (HYMENOPTERA: BRACONIDAE), A SOLITARY ENDOPARASITOID OF *PAMMENE CRITICA* MEYRICK (LEPIDOPTERA: TORTRICIDAE), WITH NOTES ON BIOLOGY FROM INDIA[1]

**Ankita Gupta[2], Amalendu Ghosh[3], Nesil Liz Baby,[2] and S. K. Jalali[2]**

ABSTRACT: *Apanteles mohandasi* Sumodan & Narendran (Hymenoptera: Braconidae) is a specific solitary endo-parasitoid of *Pammene critica* Meyrick (1905) earlier known as *Grapholita* (*Cydia*) *critica* (Lepidoptera: Tortricidae), a major pest on *Cajanus cajan* (L.). This study provides biological details, a brief morphological diagnosis with illustrations, and molecular characterization of the parasitoid. The taxonomic studies confirm the correct placement of *A. mohandasi* Sumodan & Narendran 1990 **stat. rev.** in place of *Dolichogenidea mohandasi*. *Apanteles mohandasi* is also compared with the closely allied species, *A. taragamae* Viereck and *A. sauros* Nixon, and DNA barcode data obtained for *A. mohandasi* and *A. taragamae*. The size of amplified CO1 gene was 494bp for the two species. The alignment score of 75% was obtained between the two species on pair-wise alignment by Clustal W tool using Megalign, DNASTAR Inc. for the sequences of *A. mohandasi* (GenBank Acc.No: JX083404) and *A. taragamae* (GenBank Acc.No: JX0830405*)* based on the sequence data using universally approved species identification marker CO1. The mean duration of *A. mohandasi* from egg laying to pupation, and pupation to adult emergence was 15.16 and 5.75 days respectively. The mean total duration from egg to adult emergence was 23.61±0.5 days and the adult mean longevity was 2.7 days in the laboratory. The hyperparasitoid, *Elasmus anticles* Walker (Hymenoptera: Eulophidae) was also bred in the laboratory from field collected cocoons of *A. mohandasi*.

KEYWORDS: *Apanteles mohandasi*, larval parasitoid, *Pammene critica*, diagnosis, biology, hyperparasitoid, India, PCR, primer, DNA barcode

## INTRODUCTION

Pigeon pea, *Cajanus cajan* (L.), is an important legume crop grown in the tropics and subtropics, mostly in Asia, Africa, Latin America and the Caribbean region occupying 6.5 percent of the world's total pulse area and contributing 5.7 percent to the total pulse production. In Asia, pigeonpea is grown on 4.1 million ha and India alone accounts for 86 percent of Asia's total pigeonpea area and contributes 82 percent to the total production followed by Myanmar and is the single largest producer of pigeonpea in the world (ICRISAT 2012). In India, pulses are grown on about 23 million ha annually, with a production of 14 million tons. However, the demand is around 17 million tons (ICRISAT, 2011).

More than 250 insect pests are reported to attack pigeonpea (Gopali et al., 2010). The pod borer complex including *Pammene critica* Meyrick (1905) is the major limiting factor in its production (Singh and Cheema 2006). *Pammene crit-*

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Mailed on January 24, 2013

*ica* is the new name for *Grapholita* (*Cydia*) *critica* (Lepidoptera: Tortricidae) (Beccaloni et al., 2003). The caterpillar of *P. critica* feeds on foliage, buds, flowers and sometimes even on pods. In the vegetative stage, larvae web the leaves together and feed inside the web. Although damage by the larva is greater during the vegetative stage, sometimes its attack extends up to the reproductive stage injuring the flowers and pods. Several hymenopteran parasitoids and hyperparasitoids reported are *A. sauros* Nixon (Braconidae), *Temelucha minuta* (Morley) (Ichneumonidae), three Eulophidae species *viz.*, *Elasmus albopictus* Crawford, *E. anticles* Walker and *Pediobius cydiae* Khan, and one Eurytomid species *Eurytoma* sp. (Yu, 2012). From India *A. taragamae* Viereck and *Protapanteles obliquae* (Wilkinson) (Braconidae), *Goniozus* sp. (Bethylidae), *Pristomerus microdon* Cushman (Ichneumonidae), and *Copidosoma* (*Paralitomastix*) sp. (Encyrtidae), are reported from different areas attacking *P.* (*Grapholita*) *critica* (Pramanik and Basu 1968, Sachan 1986, Lateef and Reddy 1984).

Parasitization of *P. critica* (Lepidoptera: Tortricidae) by *Apanteles mohandasi* (Hymenoptera: Braconidae), a solitary endo-parasitoid, is reported from Kanpur, Uttar Pradesh. *A. mohandasi* was first described by Sumodan and Narendran in 1990 from Kerala, India. There has been a report of parasitization of *P. critica* by *Dolichogenidea molgandasi* from Orissa (Mohapatra and Sahu, 2003). The present research paper deals with the identification of the parasitoid and its comparison with the closely allied species *A. taragamae* based on morphological and molecular characterization besides studying the life cycle of the parasitoid. In the recent past much importance is given to DNA barcode sequences of braconid parasitoids that provide additional information to clarify the limits of some species (Fernandez-Triana, 2010). The iterative process of combining morphological analysis along with DNA barcoding results in a much more fine-scaled understanding of parasitoid diversity and host specificity (Smith et al., 2008). Thus, this study was aimed to initiate efforts to study braconid wasps by combining traditional taxonomic skills along with molecular analysis.

## METHODS

### *Sample collection*

The incidence of *P. critica* and its parasitoids was monitored in pigeonpea (Variety UPAS 120) at New Research Farm (26˚31'09.63"N, 80˚15'00.58"E, elev. 125 m) of the Indian Institute of Pulses Research (IIPR), Kanpur, Uttar Pradesh, India, during 2011. Cocoons of the parasitoid were collected from the field and observed under laboratory conditions. First and second instar larvae of *P. critica* were field-collected and reared in the laboratory on apical twigs of pigeonpea as a natural diet, to get next generation unparasitized larvae. Cocoons of the parasitoid were kept in insect rearing cages (30 $cm^3$) for emergence and 50% honey (fortified with brewer's yeast and ascorbic acid) was provided as adult food. Water-saturated filter paper was kept within the cage. The wasps after emergence were collected and released in a 1 liter glass jar containing unparasitized 1st/2nd

instar larvae of *P. critica*. In each jar 10 pairs of *A. mohandasi* and 20 larvae of *P. critica* were released and covered with a muslin cloth. A honey-water impregnated cotton swab was provided in the jar. After 48 hours, the larvae were collected and placed in a separate jar for parasitoid development; subsequently larval period, pupal period and adult longevity were recorded. The experiment was replicated 4 times for each generation. Two generations of parasitoids were observed from August to November, 2011. The entire laboratory observation was conducted at 27±2ºC and 70±10% relative humidity.

The taxonomic studies were conducted by the first author in the Biosystematics Laboratory of National Bureau of Agriculturally Important Insects, Bangalore. The slide-mounted specimens were prepared following normal dehydration process keeping 15 minutes each in 50%, 70%, 90%, and 100% alcohol. Later, specimens were transferred in 100% alcohol+ Terpineol in 50:50 ratio for 15 minutes and finally mounted with Canada balsam. The remaining specimens were dry mounted on cards.

The wasp images were taken using an Olympus SZX 16 stereo zoom microscope with DP 25 inbuilt camera and images were later stacked using Combine ZP software. The slide-mounted specimen was photographed using Leica DFC 425 camera attached to Leica DMLB compound microscope. The images were further processed using Adobe Photoshop Elements 8.

**Specimens examined.** *Apanteles mohandasi*– 3 females (Two dry mounted on card and one dissected on slide) and 4 males on card, Kanpur, Uttar Pradesh, India, 26˚31'09.63"N, 80˚15'00.58"E, elev. 125 m, ex. cocoon associated with larva of *Pammene critica* on *Cajanus cajan* (L.), 05.09.2011, leg. Amalendu, No. Br. 511 (NBAII COLL.).

*Elasmus anticles*– 2 females on card, Kanpur, Uttar Pradesh, India, 26˚31' 09.63"N, 80˚15'00.58"E, elev. 125 m, ex. cocoon associated with larva of *Pammene critica* on *Cajanus cajan* (L.), 05.09.2011, leg. Amalendu, No. Elas. 511 (NBAII COLL.).

*Apanteles taragamae*– 4 females on card, GKVK, Bangalore, India, 12°58'N, 77°35'E, elev. 930 m, ex. cocoons on *Cajanus cajan* (L.), 16.10.2010, leg. Ankita Gupta, No. Br. 510 (NBAII COLL.).

Voucher specimens of *Apanteles mohandasi* (Vr. No. Br. 511) and *A. taragamae* (Vr. No. Br. 510) used in molecular studies are deposited (NBAII COLL.).

### *DNA extraction, PCR amplification and Sequencing*

DNA extraction and PCR amplification was done in the Molecular Entomology Laboratory of National Bureau of Agriculturally Important Insects, Bangalore. DNA was extracted using DNeasy Blood and tissue Kit (Qiagen) and stored at -80°C until used following the manufacturer's protocols. Amplification, sequencing and sequence analysis closely followed standard methods. PCR amplification of the CO1 gene was done by using the Universal primer. PCR was performed with a total reaction mixture of 50 µl consisting of 10x Taq Buffer

(Complete Buffer with $MgCl_2$), 10 mM dNTP mix (Genei), Universal primer HCO1–2198 (20pm/µl), LCO1–1490(20 pm/ µl) (Folmer et al., 1994), template DNA (50ng/ µl), Taq DNA polymerase (Genei 1U/µl) and sterile water. The DNA extracted was amplified under the following conditions: Initial denaturation at 94°C for 5 min. followed by 30 cycles of denaturation (94° C for 1 min), annealing (45°C for 1 min), extension (72°C for 1 min) and a final extension step at 72°C for 10 min. To detect any possible contamination a negative control was kept in the PCR amplification. The PCR amplification was performed in a thermal cycler (Bio-Rad, C1000 Thermal Cycler). The amplified genes by PCR were visualized on 1.8% gel with a low range ladder (Fermentas Mass Ruler 1000bp).

The PCR products from which the partial sequences of CO1 region were obtained were sequenced. The PCR products sequenced were edited by BioEdit and aligned using BLAST2 and verified by BLASTn for species homology. Nucleotide sequences were submitted to GenBank. The sequences were aligned for studying the similarity between the *A. mohandasi* and *A. taragamae* using Megalign, DNASTAR Inc. (Lin and Wood, 2002).

The GenBank accession numbers are as follows:

| S. No. | **Species name** | GenBankAccession No. |
|---|---|---|
| 1. | *Apanteles mohandasi* Sumodan & Narendran | JX083405 |
| 2. | *Apanteles taragamae* Viereck | JX083404 |

## RESULTS AND DISCUSSION

**General Biology.** Incidence of *P. critica* commenced at the vegetative stage of pigeonpea in the field during the 1st half of August with its peak population during the 2nd half of September. Thereafter, it declined at the initiation of pod formation. The peak activity of *A. mohandasi* coincided with the peak activity of the pest. *A. mohandasi* parasitized larvae are noticed in the field from the 2nd half of August and they gradually increase around mid-September. It was observed to be a solitary endo-parasitoid. The adults mated immediately after emergence. Females have an ovipositor through which eggs are injected inside the early instar larvae of *P. critica*. The parasitized larva of *P. critica* first turns slightly sluggish, ceases feeding, and gradually becomes immobile thus transforming itself into a dull black colored and shrunken sized larva. Twelve days after oviposition the mature larva of *A. mohandasi* comes out of the host larva and spins a white fuzzy cocoon. From one parasitized host larva only one cocoon was associated indicating and confirming its solitary behavior. The host larva, as a black cadaver, was sometimes found loosely attached with the wasp cocoon. After 6 days the adult wasp of *A. mohandasi* emerges out of the cocoon by making a short hole at one end. In the field, the parasitoid cocoons were found associated with parasitized larvae as well as in the web.

The development mean times of *A. mohandasi* recorded from egg laying to pupation, and pupation to adult emergence were 15.16±0.5 and 5.75±0.5 days, respectively. Total life cycle was completed in 23.61±0.5 days and the adult longevity recorded was 2.7 days. In the present observation, it is noted that *A. mohandasi* is responsible for high parasitism of *P. critica* in pigeonpea and therefore plays an important role in controlling leaf roller population.

**Distribution.** ***Apanteles mohandasi*** **Sumodan & Narendran–**India: Uttar Pradesh (New Record) and Kerala. There has also been a report of parasitisation of *P. critica* by *Dolichogenidea molgandasi* from Orissa (Mohapatra and Sahu 2003).

***Apanteles taragamae*–**India: Cosmopolitan in nature and often reported from Karnataka, West Bengal, Kerala, Tamil Nadu and Orissa.

**Diagnosis of *A. mohandasi* Sumodan & Narendran (Plate I & II)**

**Female.** 2.0-2.3 mm long; predominantly black in color. Antennae dark brown to black; ocelli light brown; scape, all coxae, and trochanters blackish brown. Head, antennae, mesosoma, and metasoma black. Apex of fore femora, apical half of mid femora, fore and mid tarsi, and basal half of hind femora with yellow coloration. Apical half of fore and hind tibia with brown infuscation. Hind tibial spur pale white. Hind metatarsus (basitarsus) yellow on basal 1/4th; wing veins colorless; forewings with stigma pallid.

**Head.** Antennae shorter than body; head transverse and densely setose with short setae; vertex rugose; frons, face and malar space with fine sculpture and minute punctuations.

**Mesosoma.** Longer than broad. Mesoscutum distinctly punctate and densely setose; punctations more distinct and setae more dense near the scuto-scutellar margin. Scuto-scutellar margin with thirteen shallow crenulated grooves. Scutellum smooth and nitid without any punctuations, both the lateral edges with long pale white setae. Mesopleuron with fine sculpture and long setae on anterior half. Propodeum with distinct areola and costulae; setose on the apical half on either sides of areola; spiracles round. Forewings longer than body. Hind tibial spur distinctly shorter than first tarsal segment.

**Metasoma.** Shorter than combined length of head and mesosoma. First dorsal tergite of metasoma with coarse sculpture and distinct longitudinal striations; slightly wider at apex than extreme base. Second tergite with fine sculpture. Rest of the tergites smooth and shiny. Ovipositor sheath very stout and uniformly curved apically (in *A. taragamae* ovipositor sheaths more slender and longer; PLATE-III); with long white setae throughout its length; shorter than metasoma (0.6-0.7x metasoma); as long as hind tibia.

This species can be easily confused with the closely allied species, *A. taragamae*, and the latter can be easily distinguished by the following characters: Ovipositor sheaths not unusually thick and as long as metasoma, distinctly little shorter than hind tibia; forewings with stigma pale and colorless but with distinct

dark brown border. First tergite parallel sided and narrow at apex; one and a half times as long as wide at apex.

It can also be distinguished from another associated parasitoid of *P. critica*, *Apanteles sauros* (Braconidae), which has segments 2-4 of hind tarsus dirty whitish yellow as is also the base of basitarsus. However in *A. mohandasi,* segments 2-4 of hind tarsus are brownish black and basal half of basitarsus is yellowish white.

**Taxonomic remarks. *A. mohandasi* Sumodan & Narendran, 1990 stat. rev. is the confirmed identity of *Dolichogenidea mohandasi* (Sumodan & Narendran).** The genus *Dolichogenidea* differs from *Apanteles* in having convex and evenly fringed vannal lobe of hindwing and in having distinct punctures posteriorly on the mesonotum (Whitfield et al., 2009). In the species *A. mohandasi* vannal lobe of hindwing is distinctly concave and flattened with fringe of setae absent over the flattened part.

### *Molecular Characterization and DNA Barcode*

The CO1 gene has proved to be suitable for species identification in a large range of animal taxa, including butterflies and moths (Hebert et al., 2004) and wasps (Smith et al., 2008). The sequences were submitted to the GenBank and accession numbers *A. mohandasi* (GenBank Acc.no: JX083404 ) and *A. taragamae* (GenBank Acc. no: JX0830405) were obtained. The sequences of the amplified product on pair-wise alignment gave an alignment score of 75% using Megalign, DNASTAR Inc. (Fig. 2). Sequences were submitted to Barcode of Life datatabase (BOLD) (www.boldsystems.org) and DNA barcodes were obtained (Fig.3). The barcode developed is a diagnostic tool for identification of these parasitoids (Armstrong et al., 2005).

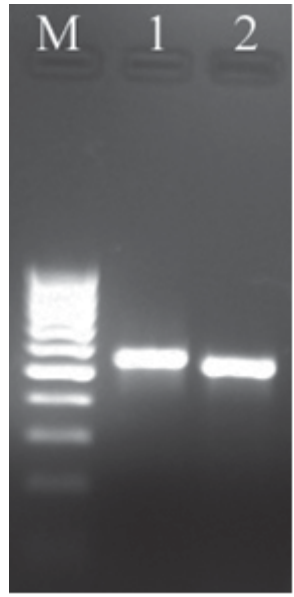


Fig. 1. The amplified genes by PCR on 1.8% gel. (Ladder 1- *A. mohandasi*; Ladder 2- *A. taragamae*)

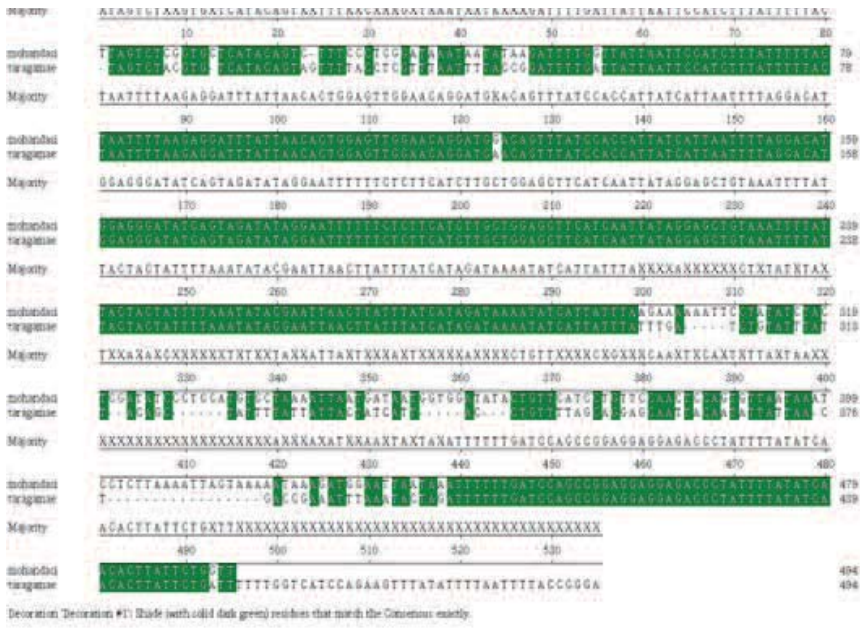

Fig. 2. Pairwise alignment between *Apanteles* spp. using Megalign, DNASTAR Inc. (The shaded bases show the misaligned to differentiate from aligned bases.)

*Apanteles mohandasi* Sumodan & Narendran


*Apanteles taragamae* Viereck

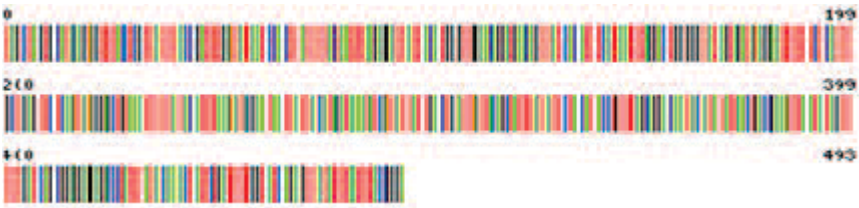


Fig. 3. DNA Barcodes of *Apanteles* spp.

Our main aim was to clarify morphological and molecular distinctions between *A. taragamae* and *A. mohandasi* as these two closely allied species are commonly associated with pests of *Cajanus cajan*. The taxonomy, systematics, distribution, and biology of *A. mohandasi* are reviewed. Hence this work provides integrated assessment on identification along with notes on biology of the parasitoid from India.

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Plate I – 1. Larva of *Pammene critica* Meyrick, 2. Solitary cocoon of *A. mohandasi*, 3. Adult wasp of *A. mohandasi*, 4. Adult wasp of *A. taragamae*.

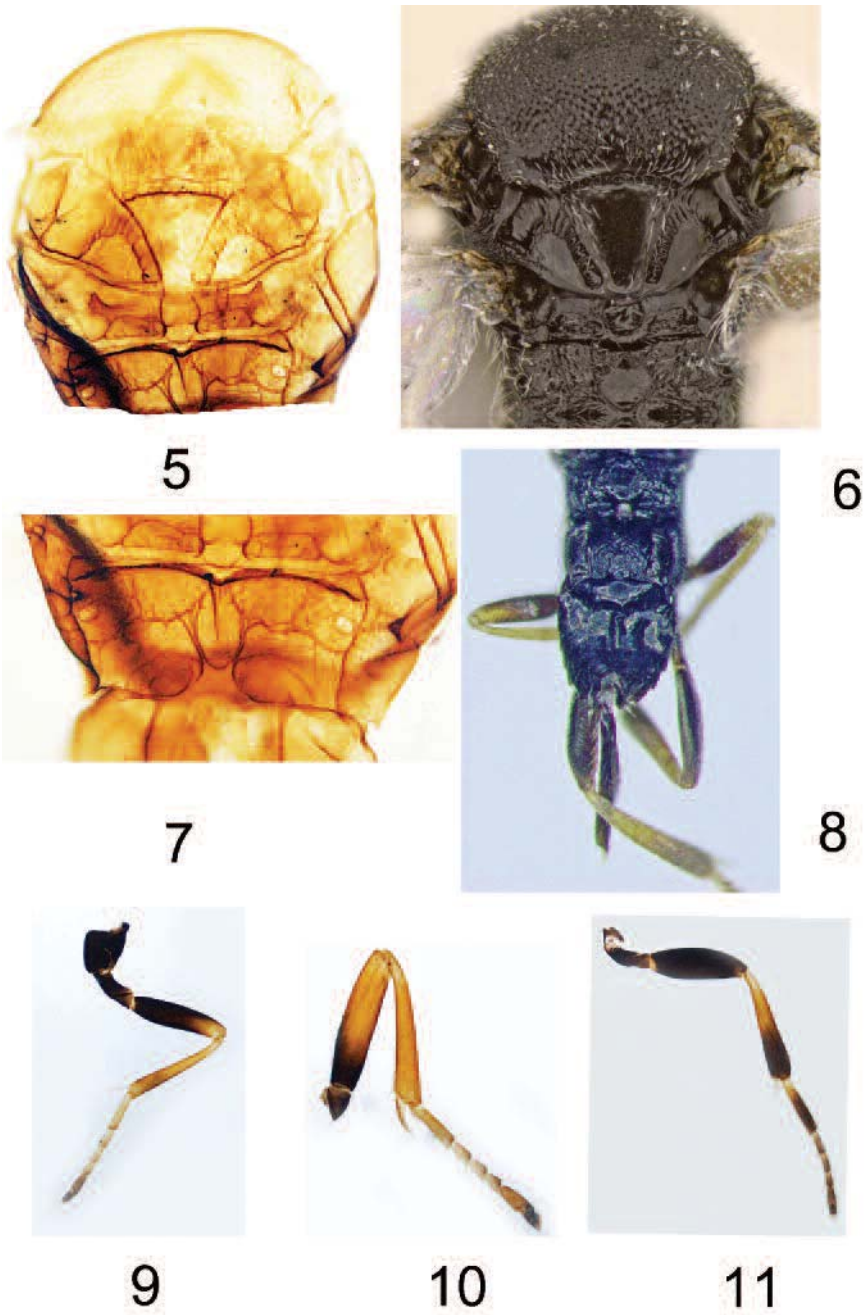


Plate II – *Apanteles mohandasi* 5. Mesoscutum (slide mounted), 6. Mesoscutum (card mounted), 7. Propodeum (slide mounted), 8. Metasoma, 9. Fore leg, 10. Mid leg, 11. Hind leg.

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13

14

Plate III – 12. *Apanteles taragamae* Viereck in dorsal view, 13. Mesoscutum along with propodeum (card mounted), 14. Propodeum with metasomal tergites (1st, 2nd, and part of 3rd).