Discovery of a New Species of *Telenomus* (Hymenoptera: Scelionidae) Parasitic on Eggs of *Bombyx mandarina* and *Bombyx mori* (Lepidoptera: Bombycidae) in Japan and Taiwan

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Received 8 May 2018; Editorial decision 29 June 2018

Abstract

We reared a *Telenomus* species from eggs of *Bombyx mandarina* (Moore) (Lepidoptera: Bombycidae) and *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae) in Japan, and from eggs of *B. mandarina* in Taiwan. Morphological examination revealed that this *Telenomus* species is new to science. In this article, we describe it as *Telenomus moricolus* Matsuo et Hirose, sp. nov. Because *B. mori* is considered to be an ancestor of *B. mandarina*, a domestic insect, it is reasonable to assume that *B. mandarina* is an original host of *T. moricolor*. This is the second discovery of an egg parasitoid attacking wild and domesticated silkworms, following the first discovery of *T. theophilae*, a Chinese species. The significance of the discovery of *T. moricolor* is discussed in relation to examining the effects of host-insect domestication on egg parasitism.

Key words: egg parasitoid, host domestication, *Telenomus moricolus*

The genus *Bombyx* Linnaeus (Lepidoptera: Bombycidae) contains six species, *B. huttoni* Westwood, *B. horsfieldii* (Moore), *B. incomposita* van Eecke, *B. lemeepauli* Lemée, *B. mandarina* (Moore), and *B. mori* (Linnaeus) (Wang et al. 2015). These six species are all distributed in Asian countries, except for *B. mori*, a domesticated species which is cultured around the world for sericulture, insect physiology, and genetics (Yokoyama 1963, Horie and Watanabe 1980, Mita et al. 2004, Goldsmith et al. 2005, Srivastava 2011, Nation 2015, Wang et al. 2015).

The genus *Telenomus* Haliday (Hymenoptera: Scelionidae) contains at least 570 species worldwide (Johnson 1992). They are egg parasitoids attacking a wide variety of insects belonging to the Lepidoptera, Hemiptera, Diptera, and Neuroptera (Johnson 1984). Among them, two *Telenomus* species, i.e., *T. tetratomus* (Thomson) and *T. theophilae* Wu and Chen, are known as egg parasitoids of *Bombyx* species. Fahringer (1922) demonstrated that *T. bombycis* Mayr (presently *T. tetratomus*) can utilize *B. mori* as a host insect under laboratory conditions. *T. theophilae*, a Chinese species, is an egg parasitoid of *B. mandarina* and *B. mori* under wild and laboratory conditions, respectively (Wu and Chen 1980, Chen et al. 1997). Although these two species share the same host insect, they have different life history strategies. *T. tetratomus* has a trans-Palaearctic distribution and a wider host range, attacking various lepidopteran eggs (Kozlov 1988, Moraal 1990, Yao et al. 2005). In contrast, *T. theophilae* is known only from China, and can parasitize only *B. mandarina* and *B. mori* (Chen et al. 1997).

In Japan, Nawa (1936) reported the occurrence of unidentified egg parasitoid(s) of the wild silkworm, *B. mandarina*. According to Ômura (1950), rates of egg parasitism of this silkworm ranged from 77 to 100% in Hino, Tokyo in August 1942. In spite of such high rates of parasitism, the egg parasitoid(s) were not identified.

In 2008, Y.N., one of the authors, reared adults of a *Telenomus* species from eggs of *B. mandarina* that were laid on *Morus australis* Poiret (Moraceae) in Taiwan. In 2010–2011, Ms. Haru Hiyama and T.Y. reared adults of a *Telenomus* species similar to the Taiwanese species from *B. mori* egg traps attached to *Morus alba* Linnaeus in Fuchu, Tokyo, Japan. In 2011, T.Y. also reared the *Telenomus* from eggs of *B. mandarina* on *M. alba* in the same locality of Tokyo.
The aim of this study was to identify the Taiwanese and Japanese species of *Telenomus*. In identifying these, we paid attention to *T. theophrasti* and *T. tetratomus* for morphological comparison, because these two species share the same host insects, and have a geographic distribution similar to both the Taiwanese and Japanese *Telenomus*. In addition, we used molecular sequencing data to strengthen the results of morphological study.

**Materials and Methods**

**Field Survey and Laboratory Rearing**

For our taxonomic study, eggs of *B. mandarina* were collected from Japan and Taiwan in 2011 and 2008–2012, respectively. Egg masses of *B. mori*, which were laid on filter papers, were attached to branches of *M. alba* as egg traps from May to November 2010–2011 in Fuchu, Tokyo to obtain adults of *Telenomus* species. The silk-worm eggs were provided by Tokyo University of Agriculture and Technology and Kyushu University, with partial support from the National Bio Resource Project of the MEXT, Japan. Each egg trap was deployed for 7 d in the field. Field-collected eggs of *B. mandarina* and egg traps of *B. mori* were kept at room temperature (15–25°C) until the emergence of adult wasps. Adults that emerged from eggs of *B. mandarina* and *B. mori* were preserved in 99% ethanol for morphological observation and DNA analysis.

**Morphological Observation and Depository of Specimens**

For the microscopic study, the ethanol-stored specimens were dried using the method described in Matsuo and Yukawa (2009). Specimens were observed under a binocular microscope (LEICA S8APO). Several specimens were gold-coated for microphotography with a JEOL JSM-5600LV scanning electronic microscope. Antennae and male genitalia were mounted on slides in Canada balsam, using ethanol and clove oil. Drawings were made with the aid of a drawing tube. Adult morphological terminology follows usage described in Mikó et al. (2007) for the head and mesosoma, Johnson (1984) for the metasoma, and Polaszek and Kimani (1990) for the male genitalia.

Holotype, paratypes, and other specimens used in this study are deposited in the collection of the Biosystematics Laboratory, Faculty of Social and Cultural Studies, Kyushu University, Japan (BLKU) and the National Museum of Natural Science, Taichung, Taiwan (NMNST).

**DNA Analysis**

For every individual, total DNA was extracted from the whole body with the Qiagen DNeasy Blood and Tissue Kit following the manufacturer’s instructions. A region of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA (mtDNA) was amplified by the following procedures: denaturation step at 94°C for 35 s, annealing at 47°C for 50 s and extension at 72°C for 60 s, with 35 cycles being performed. The primers used in this study and methods of DNA data analysis are described in Matsuo et al. (2014). In constructing a neighbor-joining tree, *Telenomus chrysopae* Ashmead and *Trissolcus plautiae* (Watanabe) (Hymenoptera: Scelionidae) were used as outgroup taxa. The sequence data was deposited in the DNA Databank of Japan (DDBJ) with accession numbers (Table 1).

**Nomenclature**

This article and the nomenclatural act it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:C9C9C189-3415-4F9E-9786-FEB7981A3FFB.

**Results**

**Species Identification**

Based on the morphological comparison, *Telenomus* reared from eggs of *B. mandarina* in Japan and Taiwan were found to be the same species, as were the *Telenomus* reared from *B. mori* egg traps in Japan. Moreover, the *Telenomus* reared in our study was found to have different morphological characteristics from other congeners. We named the *Telenomus* found in our study *T. moricolus* (see below). We found color variation between *T. moricolus* collected from Japan and Taiwan. The antennal segments A1–A5 were darker in the Japanese population, and paler in the Taiwanese population.

**Taxonomy**

*Telenomus moricolus* Matsuo et Hirose, sp. nov.

LSID: urn:lsid:zoobank.org:act:8E04A12B-CBD6-452E-B4E8-35A48926DFFA

**Etymology**

The specific name, *moricolus*, is Latin meaning an inhabitant of *Morus*.

**Material Examined**

Holotype: one female, emerged on 25–26 April 2011 from an egg of *B. mandarina* on *M. alba* collected by T. Yokoyama on 11 April 2011 from Saiwai, Fuchu, Tokyo, Japan (BLKU). Paratypes: four females and five males, same data as the holotype (BLKU); two males, emerged on 16 March 2012 from eggs of *B. mandarina* on *M. australis* collected by K.M. on 6 March 2012 from Daxi, Taoyuan, Taiwan (NMNST); one female and two males, emerged on 17 March 2012.

**Table 1. Telenomus moricolus and outgroup taxa used for DNA analysis**

| Parasitoid       | Host insect   | Associated plant | Collecting site                      | No. specimen | Accession No. |
|------------------|---------------|------------------|-------------------------------------|--------------|--------------|
| *T. moricolus*   | *B. mandarina*| *M. alba*        | Saiwai, Fuchu, Tokyo, Japan          | 11 females   | LC363957-67  |
| *T. moricolus*   | *B. mori*     | *M. alba*        | Saiwai, Fuchu, Tokyo, Japan          | 5 females    | LC363951-55  |
| *T. moricolus*   | *B. mandarina*| *M. australis*    | Fushiing, Taoyuan, Taiwan           | 5 females    | LC363941-45  |
| *T. moricolus*   | *B. mandarina*| *M. australis*    | Daxi, Taoyuan, Taiwan               | 3 females    | LC363948-50  |
| *T. moricolus*   | *B. mandarina*| *M. australis*    | Sihkanshuei, Taipei, Taiwan         | 2 females    | LC363946-47  |
| Outgroup         | Unidentified chrysopid | *Quercus glauca* | Hikarigouka, Chikushino, Fukuoka, Japan | 1 female    | LC363956   |
| *T. chrysopae*   | *Plautia stali*| *M. alba*        | Hakozaiki, Fukuoka, Fukuoka, Japan  | 1 female    | LC363968   |
| *Trissolcus plautiae* | *Plautia stali* | *M. alba*        | Hakozaiki, Fukuoka, Fukuoka, Japan  | 1 female    | LC363968   |
from eggs of *B. mandarina* on *M. australis* collected by K.M. on 6 March 2012 from Daxi, Taoyuan, Taiwan (BLKU); two females, emerged on 8 March 2012 from eggs of *B. mandarina* on *M. australis* collected by K.M. on 5 March 2012 from Sihkanshuei, Taipei, Taiwan (BLKU); two females, emerged on 8 March 2012 from eggs of *B. mandarina* on *M. australis* collected by K.M. on 5 March 2012 from Sihkanshuei, Taipei, Taiwan (NMNST); one female and four males, emerged in May 2010 from eggs of *B. mori* deployed on *M. alba*, and collected by T.Y. from Saiwai, Fuchu, Tokyo, Japan (BLKU). Other specimens: the following specimens were excluded from type series because their body color had faded. Two females and two males, emerged on 7 March 2008 from eggs of *B. mandarina* on *M. australis* collected by Y.N. from Fuhsing, Taoyuan, Taiwan (BLKU); two females, emerged on 10 March 2008 from eggs of *B. mandarina* on *M. australis* collected by Y.N. from Shihtzutou, Nantou, Taiwan (BLKU).

**Female**

Body length 0.9–1.1 mm. Head, mesosoma, and metasoma black. Antenna black; A1–A5 brownish yellow in Taiwanese specimens. Fore wing hyaline. All coxae and femora black; all tibiae brownish medially, paler in basal and apical tips; first to fourth tarsomeres of all legs black.

Head in dorsal view (Fig. 1) 1.9–2.0 times as wide as long, weakly converged; vertex coriaceous; hyperoccipital carina absent; occipital sculptured as vertex, smoother around occipital carina; occipital carina complete medially, simple. Head in frontal view (Fig. 2) 1.2–1.3 times as wide as high; malar space 0.5–0.6 times length of eye; orbital band absent; preocellar pit absent; frons coriaceous; frontal depression weakly developed; malar space coriaceous. Antenna (Fig. 6) 10-segmented; clava five-segmented; length of A2 as long as A3; A5 quadrate.

Mesosoma (Fig. 3) 1.1–1.2 times as long as wide; mesoscutum evenly coriaceous; parapsidal line absent; notauli absent; scutellum smooth, setose; posterior mesoscutellar sulcus smaller than punctures on metascutellum; pleural pit distinct (Fig. 4); acetabular carina simple; sternaulus absent; postacetabular patch setose, entering femoral depression, not reaching intercoxal space; intercoxal space 1.5–1.7 times as long as fore coxa; femoral depression well developed; mesopleural carina absent; mesepimeral sulcus present, shallow; posterior mesepimeral area smooth; metapleural pit distinct; metapleuron with a furrow arising from metapleural pit continuing to posterior margin; metapleural carina present. Fore wing 2.5–2.6

**Figs. 1–5.** *Telenomus moricolus.* (1) female head, dorsal view; (2) female head, frontal view; (3) female mesosoma, dorsal view; (4) female mesosoma, lateral view; (5) female metasoma, dorsal view.
times as long as wide. Width of hind wing 2.0–2.3 times as long as length of marginal fringe.

Metasoma (Fig. 5) slightly as long as mesosoma; T1 with three to four pairs of lateral setae and one pair of sublateral setae; costae on T1 extending over 2/3 length of sclerite; greatest length of basal costae on T2 short, extending 1/5 length of sclerite.

Male

Differs from the female as follows: Antenna (Fig. 7) 12-segmented, slender than that of female; A2 shorter than A3. Genitalia (Fig. 8); basal ring 0.3–0.4 times as long as aedeago-volsellar shaft; aedeago-volsellar shaft constricted medially; end of laminae volsellars strongly incised; digitus with three digital teeth; aedeagal lobe entirely truncate.

Host Information

This species was reared from field-collected eggs of *B. mandarina* that were laid on branches of *M. alba* and *M. australis*. In addition, it was frequently reared from *B. mori* egg traps attached to *M. alba*, and females that emerged were allowed to oviposit *B. mori* eggs in the laboratory (Fig. 9).
Distribution
Japan and Taiwan.

DNA Analysis
A total of 26 individuals of *T. moricolus* were used for DNA analysis (Table 1). The length of the amplified mtDNA COI gene fragment was 424 bp. We recognized three haplotypes among individuals collected from Japan and Taiwan (Fig. 10). The sequence divergence was 1.65% between the Japanese and Taiwanese populations of *T. moricolus*. All individuals that were reared from *B. mandarina* and *B. mori* in Japan shared the same haplotype.

Discussion

Taxonomic Remarks
We found color variation between Japanese and Taiwanese populations of *T. moricolus*. The antennal segments A1–A5 were darker in the Japanese population and paler in the Taiwanese population. In addition, 1.65% sequence divergence was detected between Japanese and Taiwanese populations of *T. moricolus*. We treated these differences as intraspecific variations because a difference in coloration is not enough to justify that they are different species, and sequence divergence was too subtle. For example, extremely similar species, *Trissolcus plautiae* and *T. japonicus* (Ashmead) that can be discriminated by presence or absence of sublateral setae on the T1, have approximately 13% sequence divergence in mtDNA COI region, even though they share the same host insects, and are sympatrically distributed (Matsuo et al. 2014, Mita et al. 2015).

Although *T. moricolus* and *T. tetratomus* share the same host insect, their morphological characteristics were quite different from each other. Females of *T. moricolus* have 10-segmented antennae, whereas those of *T. tetratomus* have 11-segmented antennae. Moreover, the head of *T. moricolus* is weakly converged in the dorsal view, whereas it is strongly converged in *T. tetratomus* (see Fig. 236-17 of Kozlov 1988).
Telenomus moricolus and T. theophilae have similar morphological characteristics: vertex coriaceous; female antenna 10-segmented; female club five-segmented; scutellum smooth. In the female, T. theophilae has a strongly transverse head, 3.5 times as wide as long. In the male genitalia, T. theophilae has a parallel-sided aedeago-volsellar shaft, weakly incised laminae volsellar, and a pointed aedeagal lobe (Wu and Chen 1980). Telenomus moricolus can be distinguished from T. theophilae by the following characteristics: head in dorsal view 1.9–2.0 times as wide as long; aedeago-volsellar shaft constricted medially; ends of laminae volsellar strongly incised; aedeagal lobe entirely truncate.

Telenomus moricolus is quite similar to T. sudoensis Ryu, a Korean species, because of the following shared features: female antenna 10-segmented; female club five-segmented; scutellum smooth; T1 with three to four pairs of lateral setae and a pair of sublateral setae. Telenomus sudoensis has a transverse head, 2.8 times as wide as long, basal costae which extends 1/3 length of the sclerite, yellowish femora of all legs (Ryu and Hirashima 1985). Telenomus moricolus can be distinguished from T. sudoensis by the following characteristics: head in dorsal view 1.9–2.0 times as wide as long; greatest length of basal costae on T2 short, extending 1/5 length of sclerite; all femora black.

Host Association

Bombyx mori completely depends on humans for survival and reproduction. Its nearest ancestor is thought to be B. mandarina, because of the similarity of their morphological and developmental characteristics (Sasaki 1899, Yoshitake 1968, Chikushi 1972) and genetic information (Li et al. 2010). Therefore, it is reasonable to assume that B. mandarina is an original host insect of T. moricolus. Nawa (1936) and Ômura (1950) reported the occurrence of unidentified egg parasitoid(s) of B. mandarina in Japan. We suggest that most of egg parasitoid(s) reported by Nawa (1936) and Ômura (1950) might be T. moricolus, because no other egg parasitoids were reared from eggs of B. mandarina during the course of this study. Ômura (1950) examined 274 egg capsules of B. mandarina from which unidentified egg parasitoid(s) had emerged. Among them, 270 egg capsules had a single emergence hole. Two egg capsules had two emergence holes, and the remaining two had a larger emergence hole. A total of 272 eggs, including the former two, may have been parasitized by T. moricolus. Existence of two emergence holes on a single egg capsule may have been the result of superparasitism by T. moricolus. A larger emergence hole may have been opened by a minor egg parasitoid species that was not collected in this study.

Significance of the Discovery of T. moricolus

Telenomus moricolus and T. theophilae parasitize both wild and domesticated silkworms. We consider that these Telenomus species are good materials for examining the effect of host-insect domestication on egg parasitism. Previous studies have focused on effects of host-plant domestication on guild members, including herbivores and their parasitoids (Benrey et al. 1998, Chen and Welter 2003, Macfadyen and Bohan 2010). In these studies, parasitoids received indirect effects of host-plant domestication through their host insects. However, direct effects of host-insect domestication on primary parasitism have never been examined. Future research on the direct effects of host-insect domestication on egg parasitism by T. moricolus and T. theophilae is needed.

Acknowledgments

We thank Dr. M. Maruyama and Ms. Y. Matsuo for their support in analyzing mtDNA of Telenomus species. We also thank the Material Management Center at Kyushu University for substantial support to implement appropriate procedures to obtain parasitized specimens. This study was partly supported by the Global COE Program (Center of Excellence for Asian Conservation Ecology as a Basis of Human-Nature Mutualism), Ministry of Education, Culture, Sports, Science and Technology, Japan to K.M.

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