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Discovery of a New Endoparasitoid Wasp (Hymenoptera: Braconidae: Microgastrinae) on *Bombyx* Larvae (Lepidoptera: Bombycidae) from Japan

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Abstract. A new species of *Protapanteles* Ashmead, 1898 (Hymenoptera, Braconidae, Microgastrinae), *P. bombycis* Tokuhira, Fernández-Triana & Maeto, **sp. nov.**, is described from Honshu Island, Japan, including its bionomics and DNA barcodes. The new species is a solitary koinobiont endoparasioid of the larvae of *Bombyx mandarina* (Moore, 1872) and *B. mori* (L., 1758) (Lepidoptera, Bombycidae). This study is considered to present the first reliable host record of microgastrine wasps parasitizing *Bombyx* (and the second record for the entire family Bombycidae). Besides its unique host association, *P. bombycis* **sp. nov.** is recognizable because of its distinctly developed curved spinule in the female protarsus and a white basal spot on the gena.

Key words: *Bombyx mandarina*, *Bombyx mori*, *Cotesia*, koinobiont, *Morus*, *Protapanteles bombycis*.

Introduction

The subfamily Microgastrinae (Hymenoptera: Braconidae) is a species-rich taxon of wasps that represents the single most important group of parasitoids of Lepidoptera in the world, in both economic terms and species richness (Mason 1981; Whitfield 1997). These wasps are all koinobiont endoparasitoids and have been recorded across almost the entire taxonomic and biological spectrum of Lepidoptera, with the probable exception of the four basal superfamilies (Shaw & Huddleston 1991; Quicke 2015; Whitfield *et al*. 2018; Fernández-Triana *et al*. 2020).

The moth family Bombycidae (Lepidoptera) consists of more than 200 species within 27 genera (Kitching *et al*. 2018), including economically important species such as silk moths. There are a few records of microgastrine wasps parasitizing Bombycidae (data compiled in Shenefelt 1972, Yu *et al*. 2016), but the majority are likely to be incorrect. The only verified record we are aware of is that of *Glyptapanteles trilochae* Gupta, 2013 which was reared from the Greenish silk moth *Trilocha varians* (Walker, 1855) in southern India (Gupta 2013).

According to Shenefelt (1972) and Yu *et al*. (2016), three microgastrine species, i.e. *Cotesia glomerata* (L., 1758), *C. tibialis* (Curtis, 1830), and *Sathon belippae* (Rohwer, 1918), are regarded as parasitoids of the domesticated silk moth *Bombyx mori* (L., 1758) (Lepidoptera, Bombycidae) (e.g., Dalla Torre 1898; Telenga 1955; Chatterji & Sarup 1962; Sathe & Jadhav 2001), but these records are most likely to be incorrect (see further discussion about that in the section "Parasitism of Bombycidae by Microgastrinae"). Similarly, no braconid parasitoids have been reported for its closest relative, the wild silk moth *B. mandarina* (Moore, 1872), which is widely distributed in eastern Asia (Nakamura *et al*. 1999).

However, the second and fifth authors recently reared an undescribed microgastrine species from *B. mandarina* in the field and later also from *B. mori* in the laboratory. In this study, we describe the wasp as a new species in the genus *Protapanteles* Ashmead, 1898 and report

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it as the first reliable case of microgastrine wasps parasitizing the genus *Bombyx* L., 1758.

Materials and methods

Field surveys were conducted at a plantation of *Morus alba* L. (Moraceae) (35.681873°N, 139.484946°E, altitude ca. 60m) located on the campus of the Tokyo University of Agriculture and Technology, Saiwaicho, Fuchu City, Tokyo Prefecture, Honshu Island, Japan (Yokoyama 2019). This plantation is small (0.3 ha) and isolated within an urban area, but is situated only ~3.5 km far from the riparian forests of the Tamagawa River, in which wild mulberries are abundant.

Wasp cocoons and larvae of *B. mandarina* were collected from the leaves of *M. alba* in the plantation and reared in the laboratory in 2019, 1920, and 2021. Emerged adult wasps were provided with early larvae of *B. mandarina* or *B. mori* for oviposition testing. The moth larvae were reared on leaves of *M. alba* at 25 °C under 16L: 8D illumination until pupation or emergence of the adult wasps. Adult wasps obtained from the wild cocoons and parasitized larvae were examined for species identification and description.

Type series are deposited in the Insect Museum, National Agriculture and Food Research Organization, Tsukuba, Japan (NARO, previously referred to as NIAES) and the Canadian National Collection of Insects, Ottawa, Canada (CNC).

Adult specimens were observed under a stereomicroscope (SNZ645, Nikon, Tokyo, Japan). Photographs of the specimens were taken using a digital camera (ILCE-7RM2, Sony, Tokyo, Japan), with either a microscope objective (M Plan APO 10X 378-803-3, Mitutoyo, Kanagawa, Japan) with a 210 mm focal length close-up lens (Raynox CM-2000 1.5x, Yoshida Industry, Tokyo, Japan) as a tube lens, or a macro lens (Laowa 25 mm f/2.8 2.5-5x Ultra Macro, Venus Optics, Anhui, China). Specimens were illuminated with two defused flashes (TT600, GODOX Photo Equipment, Shenzhen, China). The camera system was attached to a WeMacro focus stacking rail and a vertical stand (Ultramacro, Wickhambrook, UK). Multiple images were captured through different object planes and then stacked using the software Zerene Stacker v.1.04 (Zerene Systems LLC, Washington, USA) to generate a single, fully focused image. Images were processed using the software RawTherapee v.5.5 (Horváth and RawTherapee Development Team, https://www.rawtherapee.com) and GIMP ver. 2.10.8 (The GIMP Development Team, https://www.gimp.org).

Morphological terms and measurements follow those of Mason (1981), Sharkey & Wharton (1997), Karlsson & Ronquist (2012), and Fernández-Triana et al. (2014). The following abbreviations are used for descriptions: T1, T2, T3, T4, and T5 – metasomal tergites 1, 2, 3, 4, and 5; F1, F2, F15, and F16 – antennal flagellomeres 1, 2, 15, and 16; POL – posterior ocellar line (minimum distance between posterior ocelli); OOL – ocular ocellar line (minimum distance between posterior ocellus and eye); OD – maximum diameter of posterior ocellus; H – height; L – length; and W – width.

DNA was extracted from whole bodies of two paratypes stored in 99.9% ethanol. Partial fragments of the mitochondrial cytochrome c oxidase 1 (CO1, widely known as a barcoding gene for many invertebrate taxa) were amplified using a pair of primers, CO1 lco hym (5' – CAA ATC ATA AAG ATA TTG G – 3') and CO1 hco extB (5' – CCT ATT GAW ARA ACA TAR TGA AAA TG – 3') (Schulmeister 2003). The laboratory protocols followed those of Shimizu et al. (2020) and Fujie *et al*. (2021).

Results and Discussion

Taxonomy

Protapanteles Ashmead, 1898

Protapanteles Ashmead, 1898: 166. Gender: masculine. Type species: (*Protapanteles ephyrae* Ashmead, 1898) = *Apanteles paleacritae* Riley, 1881, by subsequent designation (Viereck 1914: 123).

Remarks. This is a small genus which hitherto contained 26 valid species from the Holarctic and Oriental regions (Fernández-Triana *et al*. 2020; Abdoli *et al*. 2021). Seven species have been recorded in Japan (Fujie & Maeto 2020).

Generic delimitation between *Protapanteles* and its related common genus *Cotesia* Cameron, 1891 has long been questionable (Mason 1981; Fernández-Triana *et al*. 2020). See details in "Generic position of the new species".

Protapanteles bombycis
Tokuhira, Fernández-Triana & Maeto, **sp. nov.**
[Japanese name: Kuwako-samurai-komayubachi]
(Figs 1–16)
ZooBank taxon LSID: zoobank.org:act:D81A5893-6B31-4909-98F2-F625073BD1D1

Type series. Holotype: ♀ (NARO): "Saiwaicho, Fuchu City, Tokyo Prefecture, JAPAN; 35.681873°N, 139.484946°E; Altitude ca. 60m; S. Ono leg.", "Offspring of wasps em. from larvae of *Bombyx mandarina* co11. on 10.VII.2020 from leaves of *Morus alba*; Adult em. on 1.VIII.2020", "Host larva: *Bombyx mandarina* in the laboratory". **Paratypes:** Same locality and collector as holotype. 1♀ (NARO), cocoon coll. on 14.V.2020 from a leaf of *M. alba*, adult em. on 15.V.2020; 1♂ (NARO), offspring of a wasp em. from a cocoon co11. on 14.V.2020 from a leaf of *M. alba*, adult em. on 14.VI.2020, host larva: *B. mandarina* in the laboratory; 2♂ [DNA extracted] (NARO), host larva coll. on 5.X.2020 from a leaf of *M. alba*, adult em. on 19.X.2020, host larva: *B. mandarina* on *M. alba*; 2♀3♂ (NARO), 1♀1♂ (CNC), offspring of wasps em. from larvae of *B. mandarina* coll. in IV–V.2021 from leaves of *M. alba*, adult em. on 17.VI.2021, host larva: *B. mori* in the laboratory.

Description of holotype. Female (Figs 1, 2). Body L 2.6 mm; fore wing L 2.7 mm; antenna L 2.8 mm.

Head (Figs 3, 5–7). Head behind eyes rounded in dorsal view; W of head in dorsal view 1.7× its median L; head and eyes with dense setae; clypeal W 3.5 × its H; face, clypeus, temples and genae weakly punctuate; OD: OOL: POL = 1.0: 1.8: 1.8; ocelli in low triangle, H of the triangle about as long as OOL; antennae slightly longer than body; L/W ratio of F1: 3.1, F2: 3.1, F15: 1.9, and F16: 2.6.

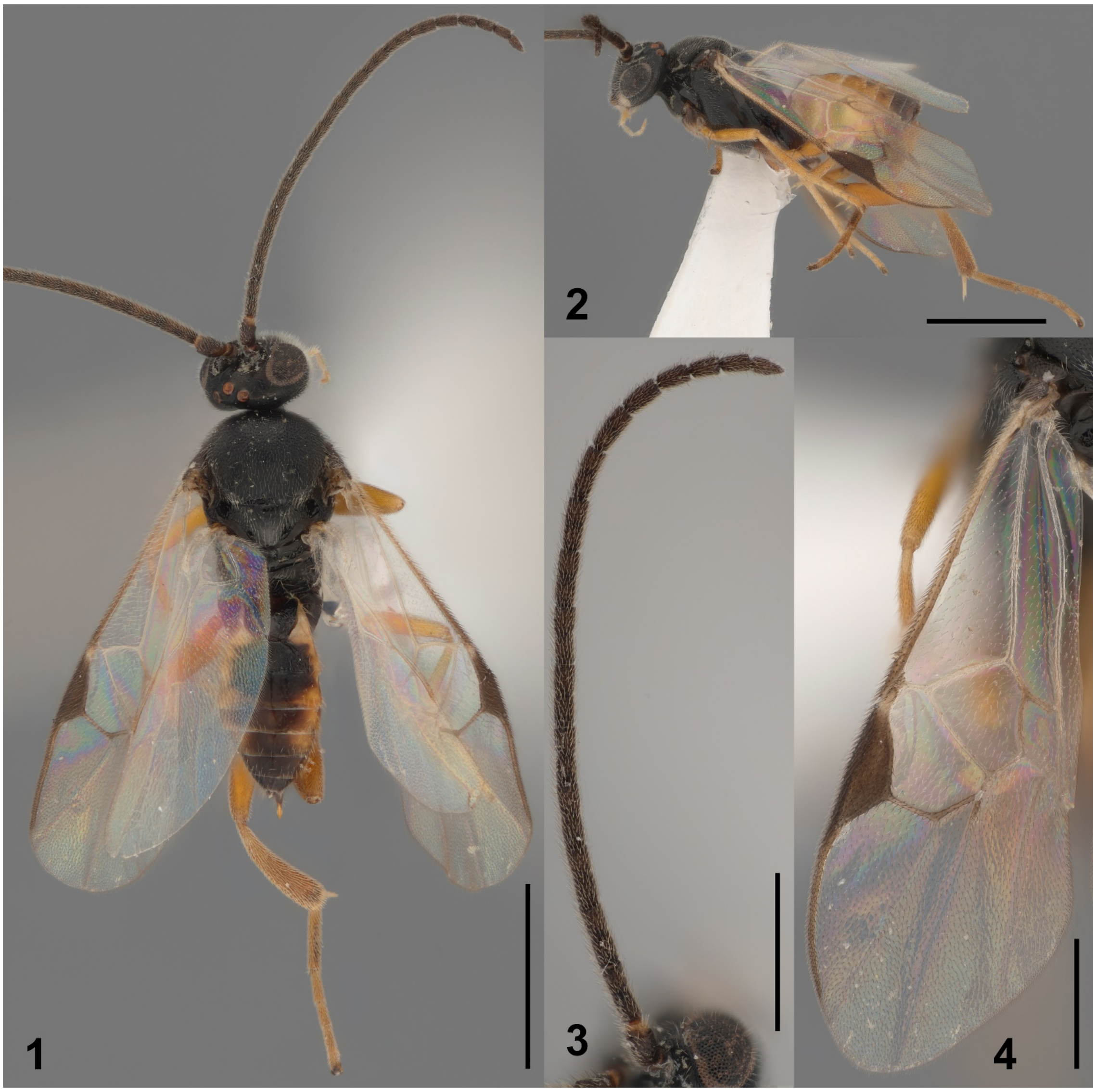
Mesosoma (Fig. 8). L of mesosoma 1.4 × its maximum H; pronotum weakly rugose, weakly punctuate to smooth; propleuron weakly punctuate and setose; anteromesoscutum weakly and densely punctate, shiny; notauli absent; scutoscutellar sulcus relatively narrow, crenulae smaller and shallower laterally; scutellar disc weakly and sparsely punctate, shiny; setae on scutellar disc somewhat longer than those on anteromesoscutum; phragma of scutellum exposed laterally; mesopleuron smooth and shiny, while punctate and setose on anterior 0.3; propodeum rugulose, weakly rugose medially, smoother anteriorly, sparsely setose, without a median longitudinal carina, lateral parts almost smooth with disperse punctuations (cf. Fig. 13).

Wings (Figs 1, 4). Fore wing slightly longer than body, evenly setose; areolet open (vein r-m absent); L of pterostigma 2.9 × its W; L of discal cell equal to its W; vein 1CUb 1.4 × as long as vein 1CUa; vein R1 slightly shorter than pterostigma; vein R1 4.5 × as long as distance of vein R1 to vein 3RSb; vein r longer than maximum W of pterostigma; vein r and vein 2SR meeting each other angularly; vein r slightly longer than vein 2SR. Hind wing setose, vein cu-a slightly curved; vannal lobe slightly convex, hind margin without setae subapically (cf. Fig. 14).

Legs (Fig. 2). Protibial spur about as long as first segment of protarsus; fifth segment of protarsus modified with a strongly curved spinule on outer side and with a large emargination opposite to it (cf. Figs 11, 12); mesotibial inner spur slightly longer than first segment of mesotarsus; metacoxa in lateral view coriaceous with sparse and shallow punctures, setose; metacoxa L 1.5 × its maximum W; metafemur L 3.3 × its maximum W; metatarsus L 1.2 × metatibia L; inner metatibial spur slightly longer than outer one, L of inner spur 0.6 × L of first segment of metatarsus (cf. Fig. 12).

Metasoma (Figs 1, 9, 10). T1 subparallel sided, gradually and slightly narrowing toward posterior end; posterior sides of T1 converging, rounded apically; anterior half of T1 smooth and depressed; posterior half of T1 deeply punctuate and rugose except for a median smooth part, with some setae; T1 L 1.3 × its maximum W; T2 transverse, W 3.1 × its median L; median field of T2 laterally marked by divergent grooves, subtriangular to trapezoidal, weakly rugose laterally and smooth medially; the grooves fainting on posterior half; anterior margin of T3 weakly crenulated medially; T3 1.2 × as long as T2; T3 and remaining tergites smooth and shiny; hypopygium short, blunt, sclerotized and setose; ovipositor sheath short, about 0.2 × as long as metatibia; ovipositor sheath sparsely setose subapically, without modified setae.

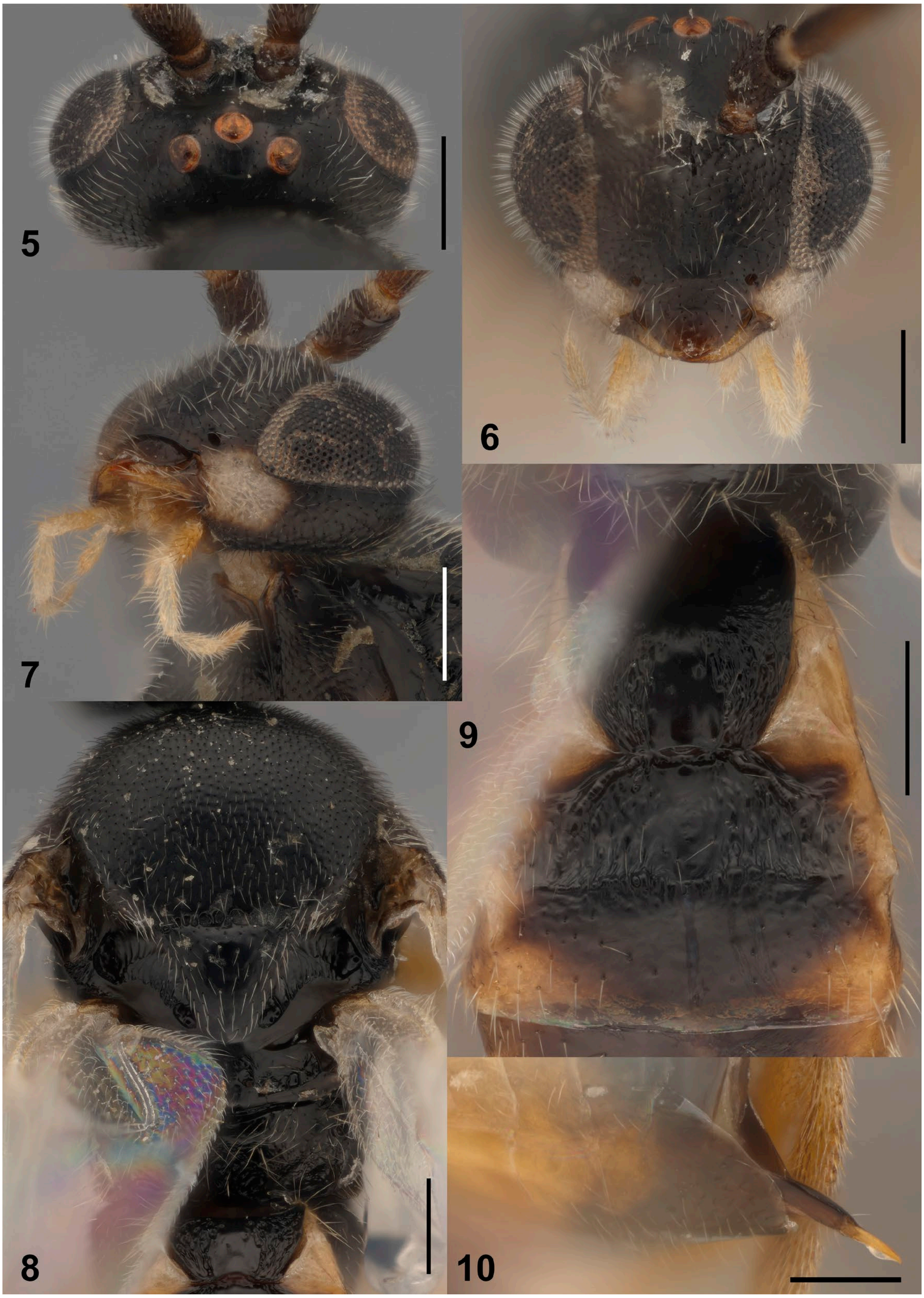
Coloration (Figs 1–10). Body black; antenna dark brown, somewhat lighter ventrally; mouthparts and palpi whitish yellow; mandible brown, yellowish apically; gena with a distinct white spot ventrally; tegula black; humeral



FIGURES 1–4. *Protapanteles bombycis* **sp. nov.**: female holotype. 1, habitus, dorsal view; 2, habitus, lateral view; 3, antenna, dorsal view; 4, fore wing. Scales: 1.0 mm (1, 2), 0.5 mm (3, 4).

complex dark brown; procoxa dark brown, yellowish ventrally; mesocoxa dark brown; metacoxa black, partially dark brown; apical 0.2 of metatibia and metatarsus brown, except lighter metatibial spurs; remaining legs yellow; wings hyaline; pterostigma brown (basal white spot absent); wing venation mostly transparent, R1, r, 2RS, 2M, 1CUa, 1CUb, 1cu-a (fore wing) and R1 (hind wing) somewhat brown; setae on wing membrane transparent, except for brown ones on distal 0.4 of fore wing; T1 and T2 black; T3 black, lightened to yellowish posterolaterally; T4 and T5 dark brown, lightened to yellowish laterally; remaining tergites black; laterotergites yellow, darkened toward apex of metasoma; hypopygium and ovipositor sheath dark brown.

Variations in females. Body L 2.5–3.1 mm; fore wing L 2.7–2.9 mm (but fore wing of the largest individual was shrunk and thus not measured); W of head in dorsal view 1.7–1.9 × its median L; OD: OOL: POL = 1.0:1.6–1.8:1.6–1.8; L/W ratio of F1: 3.1–3.4, F2: 3.1–3.5, F15: 1.6–1.9, and F16: 2.4–2.6; L of pterostigma 2.8–2.9 × its W; L of metafemur 3.3–3.5 × its W; metatarsus L 1.2–1.3 × metatibia L; L of metatibial inner spur 0.56–0.65 × L of first segment of metatarsus; T1 L 1.25–1.30 × its maximum W; T2 W 2.7‑3.1 × its median L; T3 1.15–1.23 × as long as T2; metacoxa and T3– T5 dark brown to entirely black.



FIGURES 5–10. *Protapanteles bombycis* **sp. nov.**: female holotype. 5, head, dorsal view; 6, head, frontal view; 7, head, anterolateroventral view; 8, mesosoma, dorsal view; 9, metasoma, dorsal view; 10, apical portion of metasoma, posterolateral view. Scales: 0.2 mm.

Males. As females (Fig. 15), but fifth segment of protarsus normal without a curved spinule and its opposite emargination. Body L 2.0–2.6 mm; fore wing L 2.0–2.7 mm; antennae distinctly longer than body; OOL and POL slightly longer (OOL 1.7–1.9 × OD, POL 1.7–2.0 × OD); propodeum less rugulose, smooth and shiny laterally.

Cocoons. Single, white or light yellow; spindle-shaped with an apical cap for adult emergence; L 3.3–4.3 mm; W 1.2–1.7 mm; L/W 2.4–2.6 (Fig. 16).

DNA barcodes. We obtained DNA sequences from two male paratypes (with Process ID: JPMIC001-22 and JPMIC002-22 in BOLD). They were 1,029 bp in length each and were unique among the 5,000+ sequences available in BOLD as of March 2022 for Holarctic species of the genera *Protapanteles*, *Cotesia*, *Glyptapanteles* Ashmead, 1904, *Lathrapanteles* Williams, 1985 and *Sathon* Mason, 1981. Those sequences represent 380 different putative species or BINs (Barcode Index Number, which is a system that uses DNA barcodes to indicate possible species limits; for more details on the BIN concept see Ratnasingham and Hebert 2013) and *P. bombycis* **sp. nov.** is distinctive among all Holarctic species of those genera with known DNA barcodes (Supplementary Figure 1).

Distribution. Japan (Honshu Island: Tokyo Pref.).

Hosts. *Bombyx mandarina* (Moore, 1872) and *B. mori* (L., 1758) (Lepidoptera, Bombycidae).

Etymology. Named after the genus name of host Lepidoptera, *Bombyx*.

Differential diagnosis. *Protapanteles bombycis* **sp. nov.** resembles *P. albigena* Abdoli, Fernandez-Triana & Talebi, 2021 from Iran and *P. enephes* (Nixon, 1965) widely distributed within the Palearctic region. The three species have a white, basal spot on the gena, a markedly modified fifth segment of the female protarsus with a strongly curved spinule and an emargination opposite to it, and a rugulose propodeum except for smooth anterior and lateral portions (Nixon 1976; Papp 1984; Abdoli *et al.* 2021). However, *P. bombycis* has metatibial inner spur shorter (0.56–0.65 × as long as first segment of metatarsus), pterostigma relatively wider (L / W = 2.8–2.9), and OOL shorter (OOL 1.6–1.8 × OD), whereas *P. albigena* has metatibial inner spur much longer (0.8 × as long as first segment of metatarsus), pterostigma much narrower (L / W = 3.1), and OOL longer (OOL 2.0 × OD). And also, *P. bombycis* has head behind eyes rounded in dorsal view, T2 mostly smooth (with weak longitudinal striae restricted to margins), T3 to T5 laterally yellow and centrally reddish-brown, and tegula black, whereas *P. enephes* has head behind eyes linearly constricted in dorsal view, T2 mostly sculptured (with strong longitudinal striae covering most of tergite's surface except for small central area which is smooth), all tergites dark brown to black, and tegula yellow. Additionally, *P. bombycis* is parasitic on the Bombycidae (*Bombyx*), while *P. enephes* is known to parasitize Geometridae (*Ennomos* and *Erannis*) (Nixon 1965).

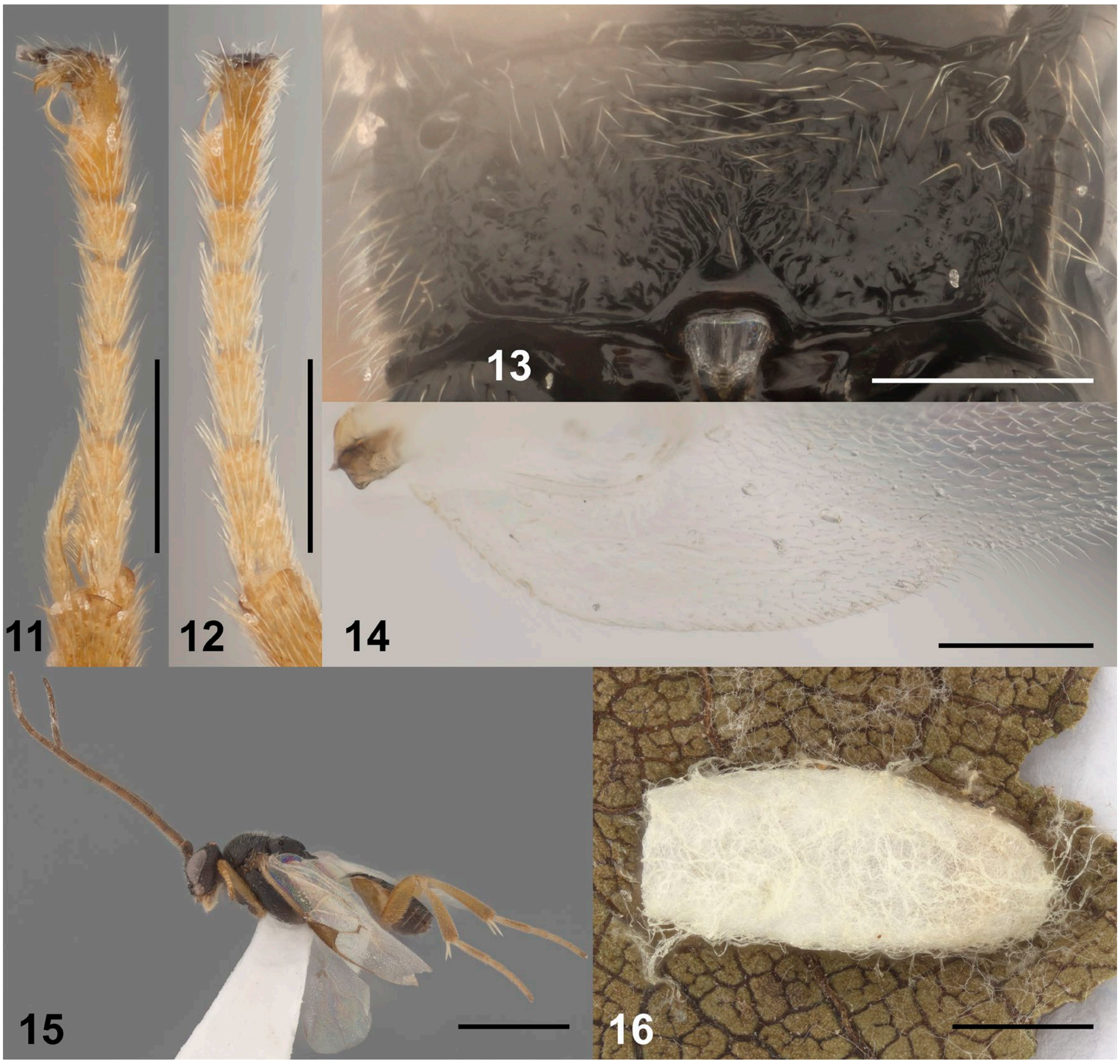
Generic position of the new species

Generic delimitation between *Protapanteles* and *Cotesia* is somewhat uncertain (Fernández-Triana et al. 2020). The new species is here assigned to *Protapanteles* because of the propodeum lacking carinae, the modified female protarsus, and the apical rounding of the first metasomal tergite (Mason 1981). However, this species is rather aberrant in having a distinctly sculptured propodeum and second metasomal tergite, similar to some *Cotesia* species (Fernández-Triana et al. 2020). Therefore, the present generic assignment may be revised in future.

Notable morphological characters

In most *Protapanteles* and some *Cotesia* species, the fifth segment of the female protarsus is modified, with a curved spinule, to various degrees (Nixon 1965, 1976; Mason 1981; Papp 1984; Fernández-Triana *et al.* 2020), occasionally showing a ring-like structure as in *P. bombycis* (Figs 11, 12). This modification is observed only in females and thus probably has some unknown function in oviposition or host manipulation.

Another peculiar characteristic of *P. bombycis* and some congeners is the white spotted gena of both sexes (Figs 6, 7, 15). However, the gena with a pale spot is not unique to *Protapanteles* as is present in all known species of the genus *Alphomelon* Mason, 1981 and a few species of several unrelated genera (Fernández-Triana *et al.* 2020), though its function is unknown.



FIGURES 11–16. *Protapanteles bombycis* **sp. nov.**: female paratypes (11–14), male paratype (15), and the cocoon of holotype (16). 11, protarsus, lateral view; 12, protarsus, dorsal view; 13, propodeum, dorsal view; 14, vannal lobe of hind wing; 15, habitus, lateral view. Scales: 0.2 mm (11–14), 1.0 mm (15, 16).

Bionomics

This species is a solitary koinobiont endoparasitoid of the early larvae of *B. mandarina* in the field (Fig. 17) and also of *B. mori* in the laboratory. Cocoons and parasitized host larvae were collected from April to October (see Type series). In the laboratory at 25 °C under 16L: 8D illumination, the period from oviposition into the first instar host larvae to mature larval emergence of wasps from the host larvae in their second molt (the late second or early third instar larvae) was 10—16 days on *B. mandarina* (n = 31) and 8—12 days on *B. mori* (n = 124); the period from cocoon formation to adult emergence was 5 — 7 days on *B. mandarina* (n = 6) and 6 days on *B. mori* (n = 23). Therefore, *P. bombycis* is no doubt multivoltine, although its overwintering stage is currently unknown.

We did not find any evidence of *P. bombycis* parasitizing hosts other than *B. mandarina* in the field. Indeed, *Glyphodes pyloalis* Walker, 1859 (Lepidoptera, Crambidae) cohabited with *B. mandarina* in the same mulberry plantation, but *P. bombycis* was never reared from *G. pyloalis* larvae (S. Ono unpublished data). Larvae of *B. mandarina* continuously occur throughout the year in Japan, except for winter (Kawamoto &

Tomita 2019); therefore, *P. bombycis* may be able to last for generations on only *B. mandarina*.

Parasitism of Bombycidae by Microgastrinae

Shenefelt (1972) recorded *Cotesia glomerata* [as *Apanteles glomeratus*] and *C. tibialis* [as *A. congestus* (Nees, 1834)] as parasitoids of *B. mori* based on the Hymenoptera catalogue by Dalla Torre (1898). However, Dalla Torre wrongly cited the description by Girard (1870), in which these two species were noticed as examples of natural enemies of other caterpillars, but not of *B. mori* (Girard 1870: 369). Telenga (1955) also recorded these two *Cotesia* species as parasitoids of *B. mori* but did not provide any information on the host data he was using (most likely based on Dalla Torre 1898). Much later, Sathe & Jadhav (2001) reported the searching behavior of *C. glomerata* (as *C. glomeratus*) on larvae of *B. mori* in a laboratory in India but provided no evidence of morphological identification and host-parasitoid association with *B. mori*.

Sathon belippae [as *Apanteles belippae*], originally reared from Limacodidae in Java (Rohwer 1918), was once recorded from *B. mori* in India (Chatterji & Sarup 1962), but no evidence of identification was given and no additional host record was subsequently provided (Austin & Dangerfield 1992); thus it is also likely to be incorrect.

From Chatterji & Sarup (1962) and Sathe & Jadhav (2001), it seems that there could be some microgastrine parasitoids of *B. mori* in India. However, there is not convincing, published data indicating this, and the latest review on the diversity of Lepidoptera hosts of Microgastrinae in India (Gupta & Fernández-Triana 2014) did not mention any parasitoids on *Bombyx*.

Based on the evidence discussed above we consider our paper to present the first reliable host record of microgastrine wasps on the genus *Bombyx* and the second verified host record of Microgastrinae parasitizing Bombycidae (after that of Gupta 2013).

Previously known species of *Protapanteles* are mostly solitary (seldom gregarious) parasitoids, mainly attacking exposed larvae of Geometridae, Noctuidae and Erebidae (e.g., Nixon 1965, 1976, as the *popularis* group of *Apanteles*; see also a general compilation of host data in Yu *et al*. 2016). Geometridae and Noctuidae are two of the top ten lepidopteran families that are parasitized by microgastrines (Fernández-Triana *et al*. 2020). Based on what is currently known, parasitism of Bombycidae by Microgastrinae seems to be rather limited, i.e. only by two microgastrine species, *Glyptapanteles trilochae* (Gupta 2013) and *Protapanteles bombycis* (this study), both belonging to closely related genera within the *Cotesia* group (sensu Fernandez-Triana *et al*. 2020).

Much genomic information has been accumulated on *B. mori*, as a promising model organism as well as an industrial insect (Yang *et al*. 2021). Physiological interactions between lepidopteran hosts and koinobiont wasp parasitoids may be more deeply investigated using this newly discovered host–parasitoid system.

FIGURE 17. Mature larva (left) and cocoon (right) of *Protapanteles bombycis* **sp. nov.** emerged from a larva of *Bombyx mandarina* on 12 May 2019 (T. Yokoyama).

Acknowledgments

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Supporting information

Supplementary Figure 1. Neighbour-joining tree (Kimura 2 parameter) of all BINs available in BOLD as of March 2022 for Holarctic species in the genera *Protapanteles*, *Cotesia*, *Glyptapanteles*, *Lathrapanteles* and *Sathon*. The new species, *P. bombycis*, is highlighted inside a red box.
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