

# Three new species of the genus *Aphidius* (Hymenoptera, Braconidae, Aphidiinae) from South Korea

Sangjin Kim<sup>1\*</sup>, Željko Tomanović<sup>2\*</sup>, Yeonghyeok Yu<sup>1</sup>, JuHyeong Sohn<sup>1</sup>,  
Yunjong Han<sup>1</sup>, Gyeonghyeon Lee<sup>1</sup>, Hyojoong Kim<sup>1</sup>

**1** Animal Systematics Laboratory, Department of Biology, Kunsan National University, Gunsan 54150, Republic of Korea **2** University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg 16, 11000 Belgrade, Serbia

Corresponding author: Hyojoong Kim ([hkim@kunsan.ac.kr](mailto:hkim@kunsan.ac.kr))

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## Abstract

Several species of the genus *Aphidius* are well known as commercial biocontrol agents of pest aphids, and more than 130 species of the genus have been recorded worldwide. To date, only 15 *Aphidius* species have been recorded in South Korea. Using the DNA barcode region (ca. 658 bp) of mitochondrial cytochrome c oxidase subunit I (COI), we amplified sequences of 15 *Aphidius* Korean species, aligned them in combination with 23 reference species retrieved from GenBank for comparison and identification, and then reconstructed a barcode phylogeny by the neighbour-joining method. As a result, three *Aphidius* species were found to be new to Science. Descriptions and illustrations of the three species new to Science – *Aphidius longicarpus* **sp. nov.**, *A. longistigmus* **sp. nov.**, and *A. asiaticus* **sp. nov.** – are provided, together with their phylogenetic position within the genus *Aphidius*. In addition, a redescription of *A. areolatus*, a parasitoid of maple aphids (*Peryphillus* spp.), is also given.

## Keywords

DNA barcoding, natural enemy, parasitoid wasps, systematics, taxonomy

\* These authors contributed equally to this work.

## Introduction

The genus *Aphidius* consists of more than 130 species around the world and belongs to the subfamily Aphidiinae, which includes approximately 63 genera and 650 species (Yu et al. 2016). All *Aphidius* species are solitary koinobiont endoparasitoids of aphids, and many of them (e.g. *A. colemani*, *A. gifuensis*) are commercially used and produced as biocontrol agents against pest aphids worldwide (Hågvar and Hofsvang 1991; Blackman and Eastop 2000). As a species-rich genus with a huge diversity of host aphids, many new species have been discovered and described in recent studies (Tomanović et al. 2007; Davidian and Gavrilyuk 2010; Petrović et al. 2020).

Members of the genus *Aphidius* are medium-sized wasps, only 15 species of which have been recorded in South Korea (Starý and Choi 2000; Yu et al. 2016; Hwang et al. 2018; NIBR 2019; Kim et al. 2020). Since *Aphidius* species such as *A. colemani* and *A. ervi* are used as imported biocontrol agents against pest aphids worldwide, especially in agriculture and horticulture (Sequeira and Mackauer 1992; Henter and Via 1995; Fernandez and Nentwig 1997; Takada 1998), it is important to explore indigenous *Aphidius* species and to confirm their diversity in South Korea and surrounding regions.

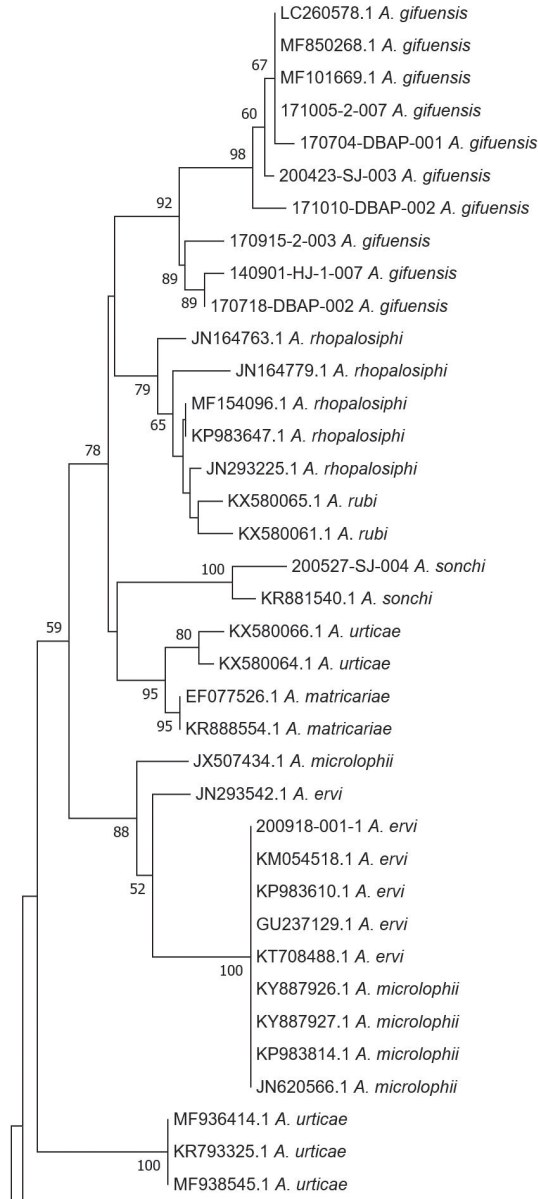
In this study, we describe and diagnose three new *Aphidius* species and present their phylogenetic relationships with other congeners. We also confirm the presence of *A. areolatus*, which was poorly known and often misidentified, in South Korea and redescribe it.

## Materials and methods

Samples were borrowed from the Korean National Arboretum (Pocheon, South Korea). All of them were obtained with Malaise traps in South Korea. They are stored in 95% ethyl alcohol at -19 °C.

Specimen morphological identification was based on Shaw and Huddleston 1991; Wharton et al. 1997; Yu et al. 2016; and Rakhshani et al. 2019. We first performed morphological sorting of similar phenotypes and labeling of these samples using a dissecting microscope (OLYMPUS SZX16, Leica M205C, NIKON SMZ 1500), after which DNA extraction was performed. Total genetic DNA extraction was performed using a LaboPass Tissue Kit (COSMOgenetech, Korea) following the manufacturer's protocol. For DNA extraction, samples consisted of single or several individuals from the same colony.

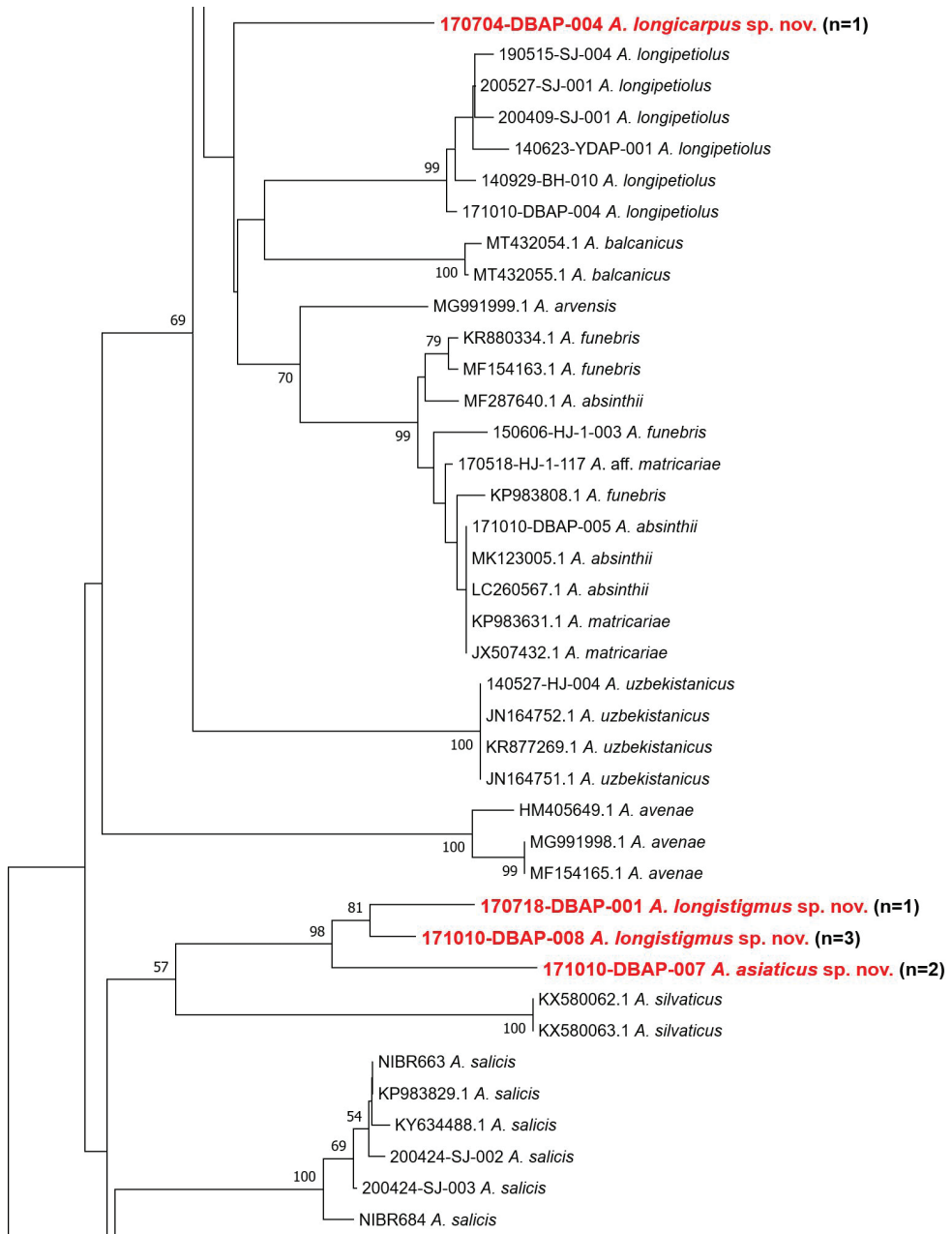
The target site for molecular identification was the front partial region of mitochondrial COI, viz., a 658-bp fragment, amplified using primers LCO1490 (forward) 5'-GGTCAACAAATCATAAAGATATTTGG-3' and HCO2198 (reverse) 5'-TAAACTTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994) and AccuPower PCR PreMix (Bioneer Corp., Daejeon, Korea). Polymerase chain reaction (PCR) amplification was conducted with 20 µl of a reaction mixture consisting of 3 µl of DNA extract, 2 µl of primer, and 15 µl of H<sub>2</sub>O. It was carried out as follows: denaturation for 5 min at 95 °C; 38 cycles of 20 s at 95 °C, 30 s at 45 °C, and 40 s



**Figure 1.** Neighbour-joining tree of 28 *Aphidius* spp. from South Korea based on their COI DNA barcode. *Diaeretiella rapae* was used for an outgroup.

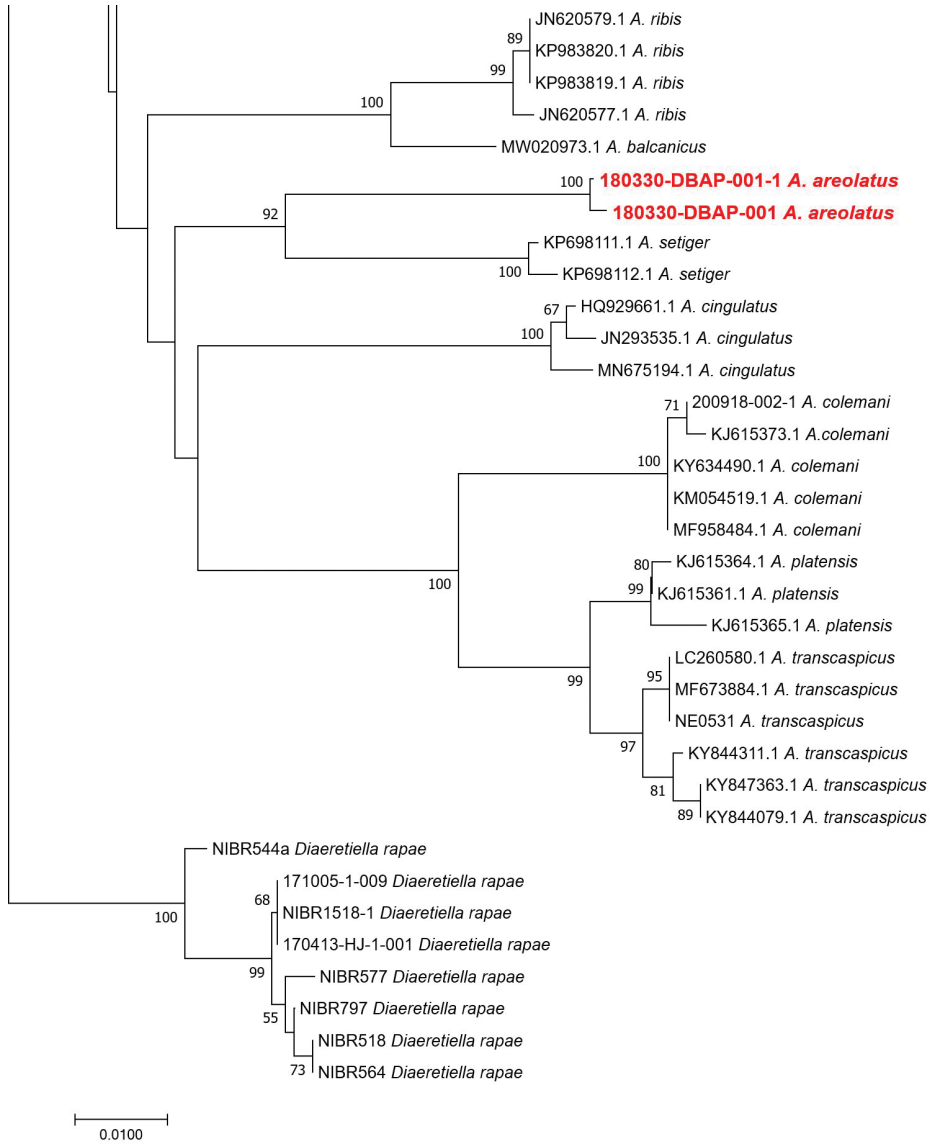
at 72 °C; and final extension at 72 °C for 5 min. PCR products were tested by electrophoresis on agar gel and if a band existed, we commissioned Bionics (Korea) for analysis and purification.

Sequences were aligned using Clustal W default setting and their frame-shifts were checked to avoid pseudogenes. Alignments were translated to amino acids using



**Figure 1.** Continued.

MEGA, version 7.0. We calculated sequence divergences using the ‘*p*-distance’ model commonly employed to analyze *COI* barcoding data. A phylogeny tree was constructed using the neighbor-joining method with 1,000 bootstrapping replications and complete deletion in data gaps.



**Figure 1.** Continued.

After morphological and molecular identification, measurements of the new species were carried out. A LEICA DMC2900 digital camera and a LEICA M205 C microscope (Leica Geosystems AG) were used for photography and characterization, several pictures being taken for each height using multifocusing technology. LAS V4.11 (Leica Geosystems AG) and HeliconFocus 7 (Helicon Soft) software were used for stacking work. After stacking work, illustrations were created using Adobe Photoshop CS6. LAS V4.11 (Leica Geosystems AG) was used to ascertain the shape of specimens (Berkovitch et al. 2009; Arias-Penna et al. 2013).

## Results

A total of 110 COI MOTUs ( $\geq 525$ ) of 28 species, including the outgroup, were used to establish the phylogenetic tree. Fourteen species recorded in South Korea (but not *A. pleotricophori* because of a lack of sequence data) were used in phylogenetic analysis. Altogether, sequences of 28 species containing 72 reference sequences of 22 *Aphidius* species from GenBank (Suppl. material 1: Table S1), were used to explore the phylogenetic position of the three newly described species. *Diaeretiella rapae* was used as an outgroup.

*Aphidius longistigmus* sp. nov. contains two MOTUs (molecular operational taxonomic units), 171010-DBAP-008 in three specimens and 170718-DBAP-001 in one specimen. *Aphidius asiaticus* sp. nov. is clustered with *Aphidius longistigmus* sp. nov. and is present with two specimens (171010-DBAP-007). Genetic divergence between *Aphidius asiaticus* sp. nov. and *Aphidius longistigmus* sp. nov. is in the range of 3.1–3.9%, as compared with the two MOTUs in *Aphidius longistigmus* sp. nov., whose average genetic divergence is 1.6%.

## Systematic accounts (description on the basis of females)

### *Aphidius longicarpus* Kim & Tomanović, sp. nov.

<http://zoobank.org/D82A1BC5-37CE-44FB-A78C-F6D6CAA643A4>

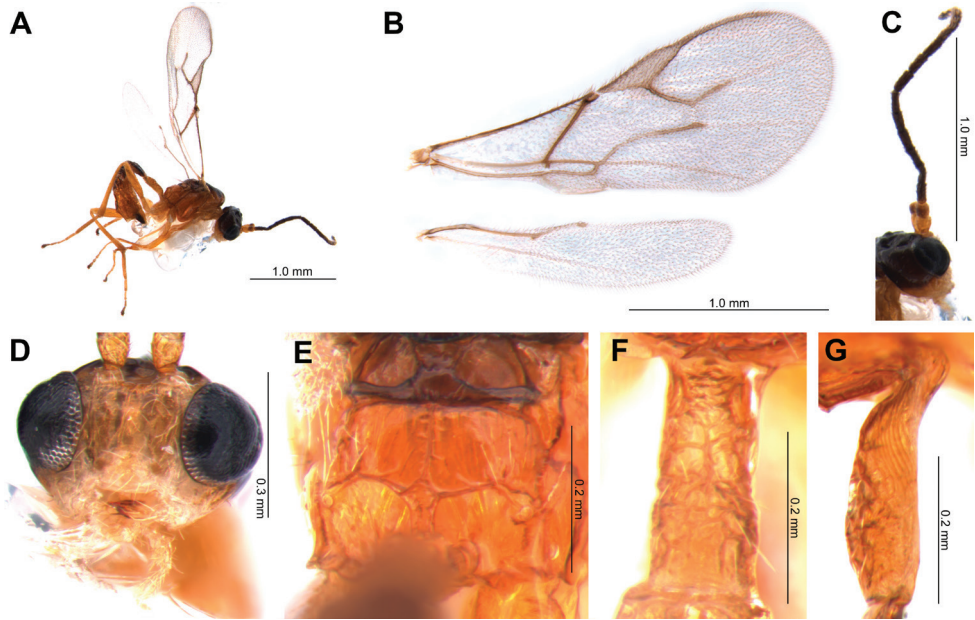
Fig. 2A–G

**Diagnosis.** In some morphological characters (shape of the first flagellomere and antennae, number of antennal segments, shape of the pterostigma, and number of maxillary and labial palps), *Aphidius longicarpus* sp. nov. is similar to *A. funebris* and *A. balcanicus*. However, it clearly differs from *A. balcanicus* in having a shorter R1 vein (the R1 vein is subequal to pterostigma length in *Aphidius longicarpus* sp. nov., while in *A. balcanicus* the ratio of pterostigma length to R1 vein length is 1.4–2.0). It differs from *A. funebris* in possessing a more elongate pterostigma (the pterostigma length/width ratio is 4.06 in *Aphidius longicarpus* sp. nov., vs. 3.0–3.5 in *A. funebris*) and a more elongate petiole (the petiole length/width ratio at the spiracle level is about 3.14 in *Aphidius longicarpus* sp. nov., vs. 2.5–2.9 in *A. funebris*). The new species is distinguished from all other congeneric *Aphidius* species by a combination of the following characters: 16-segmented antennae, short first flagellomere (length/width ratio of about 2.6), and subequal length of the R1 vein and pterostigma.

**Description. Female.** Length of body about 2.26 mm (Fig. 2A). Length of forewing 2.06 mm (Fig. 2B).

**Head.** Tentorial index 0.53 (Fig. 2D). Malar space  $0.29 \times$  longitudinal eye diameter. Antenna 16-segmented (Fig. 2C). F1 slightly shorter than F2. F1 and F2 2.61 and 3.35 times as long as their width at the middle, respectively. F1 and F2 with three and four longitudinal placodes, respectively. Maxillary palp with four palpomeres, labial palp with three palpomeres. Ratio of eye to temple in dorsal view 1.40. Face width/height ratio 1.25 (Fig. 2D).

**Mesosoma.** Propodeum areolated, areola length/width ratio 2.00 (Fig. 2E). Pterostigma 4.06 times as long as wide. Ratio of pterostigma length to R1 vein (=metacarpus) length 1.14 (Fig. 2B).



**Figure 2.** *Aphidius longicarpus* Kim & Tomanović, sp. nov., female **A** body **B** wing **C** antennae **D** head **E** propodeum **F** dorsal view of petiole **G** lateral view of petiole.

**Metasoma.** Petiole 3.14 times as long as wide at spiracles (Fig. 2F, G), with eight curved costulae in the anterolateral area (Fig. 2G). Petiole rugose on dorsal side, with prominent dorsal carina (Fig. 2F).

**Colour.** Antenna black; scape yellowish-brown; pedicel brown to black from base to apex. Head black. Face brown, clypeus with mouthparts yellowish-brown. Dorsal side of mesoscutum and metasoma dark brown, except yellowish brown propodeum and petiole. Legs yellowish-brown with dark apices.

**Etymology.** The name of the new species refers to the very long fore wing R1 vein (=metacarpus).

**Specimen examined. Holotype:** Korea, 1 ♀; DMZ Botanical Garden, Mandae-ri, Haean-myeon, Yanggu-gun, Gangwon-do, collected by Malaise trap: 20.VI.–04.VII.2017, leg. H.T. Shin, S.J. Kim. Holotype deposited in the Korean National Arboretum, Gyeonggi-do, Republic of Korea.

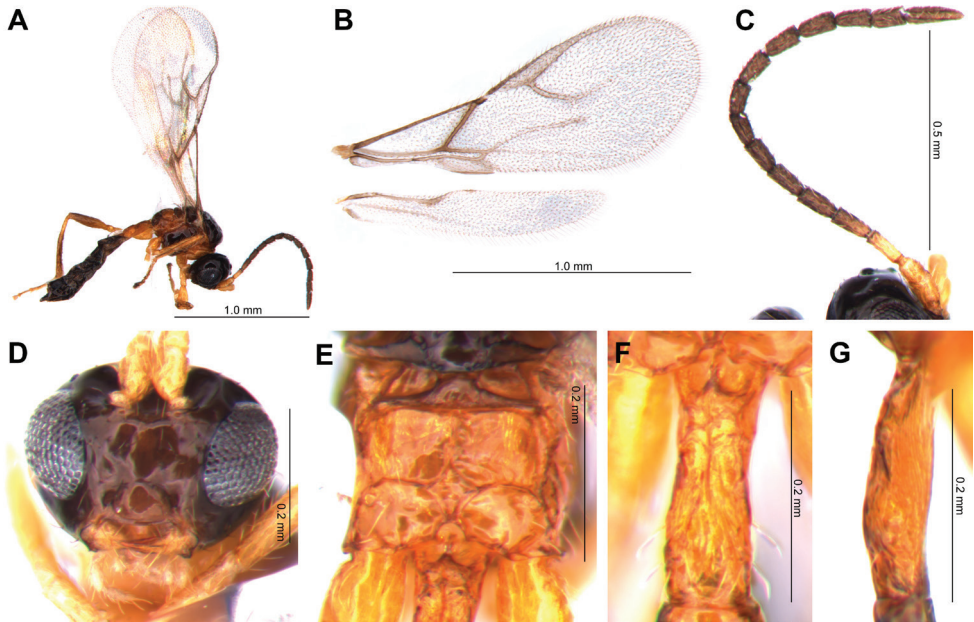
***Aphidius longistigmus* Kim & Tomanović, sp. nov.**

<http://zoobank.org/720E0253-F2D9-4F99-AFBF-6635FBB2DBA8>

Fig. 3A–G

**Diagnosis.** On the basis of the number of maxillary (three) and labial (two) palpomeres, number of antennal segments, and possession of an elongate pterostigma, the new species is morphologically related to *A. matricariae*. However, it differs clearly from *A. matricariae* in having a very elongate fore wing pterostigma (fore wing pterostigma





**Figure 3.** *Aphidius longistigmus* Kim & Tomanović, sp. nov., female **A** body **B** wing **C** antennae **D** head **E** propodeum **F** dorsal view of petiole **G** lateral view of petiole.

length/width ratio of 4.96–5.46 in *Aphidius longistigmus* sp. nov., vs. 3.50–4.00 in *A. matricariae*) and shorter flagellomere 1 (F1 length/width ratio of 2.11–2.52 in *Aphidius longistigmus* sp. nov., vs. 2.50–3.00 in *A. matricariae*).

**Description. Female.** Length of body about 1.85 mm (Fig. 3A). Length of fore wing 1.45 mm (Fig. 3B).

**Head.** Tentorial index 0.39–0.50 (Fig. 3D). Malar space 0.26–0.30 times longitudinal eye diameter. Antenna 13–15-segmented (Fig. 3C). F1 slightly shorter than F2. F1 and F2 2.11–2.52 and 1.93–2.28 times as long as their width at the middle, respectively. F1 with 2–3 and F2 with 3–4 longitudinal placodes. Maxillary palp with three palpomeres, labial palp with two palpomeres. Ratio of eye to temple in dorsal view 0.98–1.13. Face width/height ratio 1.43–1.51 (Fig. 3D).

**Mesosoma.** Propodeum with clearly defined central areola, areola length/width ratio 1.00 (Fig. 3E). Pterostigma 4.96–5.46 times as long as wide. Ratio of pterostigma length to R1 vein (=metacarpus) length 1.00–1.34 (Fig. 3B).

**Metasoma.** Petiole 3.09–3.29 times as long as wide at spiracles (Fig. 3F, G), with about 10 curved costulae in anterolateral area (Fig. 3G). Petiole with prominent dorsal carina (Fig. 3F).

**Colour.** Antenna dark-brown; scape, pedicel, and flagellomere 1 yellowish–brown, partly dark-brown. Head black. Face with clypeus dark-brown, mouth parts yellowish-brown. Dorsal side of mesoscutum and metasoma dark–brown, except for propodeum with petiole. Legs yellowish-brown with dark apices.

**Etymology.** The name of the new species refers to the very long fore wing pterostigma.



**Specimens examined. *Holotype*:** Korea, 1 ♀; DMZ Botanical Garden, Mandae-ri, Hae-an-myeon, Yanggu-gun, Gangwon-do, collected by Malaise trap: 05.IX.–19.IX.2017, leg. H.T. Shin, S.J. Kim. Holotype deposited in the Korean National Arboretum, Gyeonggi-do, Republic of Korea.

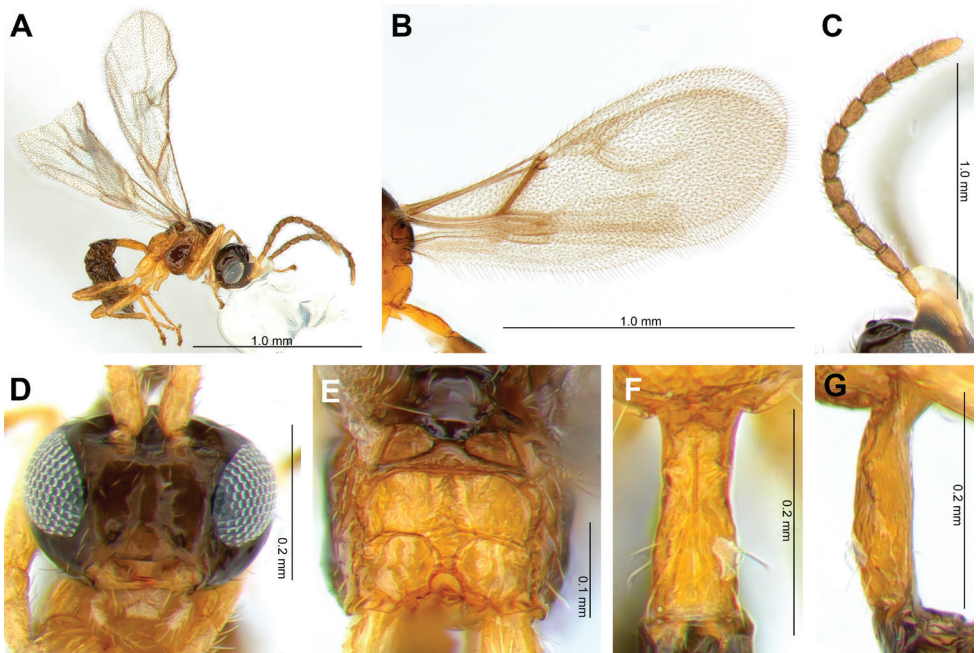
***Paratypes*:** Korea, 3 ♀; 1 ♀, DMZ Botanical Garden, Mandae-ri, Hae-an-myeon, Yanggu-gun, Gangwon-do, collected by Malaise trap: 04.VII.–18.VII.2017, leg. H.T. Shin, S.J. Kim. 2 ♀, same locality, collected by Malaise trap: 19.IX.–10.X.2017, leg. H.T. Shin, S.J. Kim. Paratype specimens deposited as dry and immersion-mounted in the Korean National Arboretum, Gyeonggi-do, Republic of Korea.

***Aphidius asiaticus* Kim & Tomanović, sp. nov.**

<http://zoobank.org/D381A6F3-D58F-4799-A2AE-F9C58A1610E4>

Fig. 4A–G

**Diagnosis.** On the basis of the number of antennal segments and wing venation pattern, the new species is morphologically related to *A. matricariae* and *Aphidius longistigmus* sp. nov.. However, it differs clearly from *A. matricariae* in having a more elongate fore wing pterostigma (the fore wing pterostigma length/width ratio is 4.62–4.79 in *Aphidius asiaticus* sp. nov., vs. 3.50–4.00 in *A. matricariae*) and a shorter flagellomere 1 (The F1 length/width ratio is 2.23–2.49 in *Aphidius asiaticus* sp. nov., vs. 2.50–3.00 in *A. matricariae*). *Aphidius asiaticus* sp. nov. differs from *Aphidius longistigmus* sp. nov.



**Figure 4.** *Aphidius asiaticus* Kim & Tomanović, sp. nov., female **A** body **B** wing **C** antennae **D** head **E** propodeum **F** dorsal view of petiole **G** lateral view of petiole.

in having a less elongate pterostigma (the pterostigma length/width ratio is 4.62–4.79 in *A. asiaticus* sp. nov., vs. 4.96–5.46 in *Aphidius longistigmus* sp. nov.). Additionally, *A. asiaticus* sp. nov. has four maxillary palpomeres (or three when the last one is very long and undivided), while *A. longistigmus* sp. nov. has three maxillary palpomeres.

**Description. Female.** Length of body about 1.85 mm (Fig. 4A). Length of fore wing 1.45 mm (Fig. 4B).

**Head.** Tentorial index 0.47–0.50 (Fig. 4D). Malar space 0.28–0.31 times longitudinal eye diameter. Antenna 13–15-segmented (Fig. 4C), thickened at apex. F1 slightly shorter than F2. F1 and F2 2.23–2.49 and 2.11–2.14 times as long as their width at the middle, respectively. F1 with 0–3 and F2 with 1–3 longitudinal placodes. Maxillary palps with four or three palpomeres (in the latter case, the last palpomere is long and undivided), labial palp with two palpomeres. Ratio of eye to temple in dorsal view ca. 1.00. Face width/ height ratio 1.44–1.54 (Fig. 4D).

**Mesosoma.** Propodeum with clearly defined central areola, areola length/ width ratio 1.18–1.20 (Fig. 4E). Pterostigma 4.62–4.79 times as long as wide. Ratio of pterostigma length to R1 vein (metacarpus) length 1.26–1.32 (Fig. 4B).

**Metasoma.** Petiole 3.08–3.12 times as long as wide at spiracles (Fig. 4F, G), with about 10 curved costulae in anterolateral area (Fig. 4G). Mediodorsal carina of petiole well developed (Fig. 4F).

**Colour.** Antennal scape, pedicel, and flagellomere 1 yellow, flagellomere 2 yellow at the base, remaining parts of antennae yellowish to light-brown. Head black. Face with clypeus dark-brown, mouth parts yellow. Dorsal side of mesoscutum and metasoma dark-brown except for the yellow to light-brown propodeum (propodeum sometimes dark-brown) with yellow petiole. Legs yellow with dark apices.

**Etymology.** The name of the new species is derived from its known geographic distribution.

**Specimens examined. Holotype:** Korea, 1 ♀; DMZ Botanical Garden, Mandae-ri, Haean-myeon, Yanggu-gun, Gangwon-do, collected by Malaise trap: 19.IX.–10.X.2017, leg. H.T. Shin, S.J. Kim. Holotype deposited in the Korean National Arboretum, Gyeonggi-do, Republic of Korea.

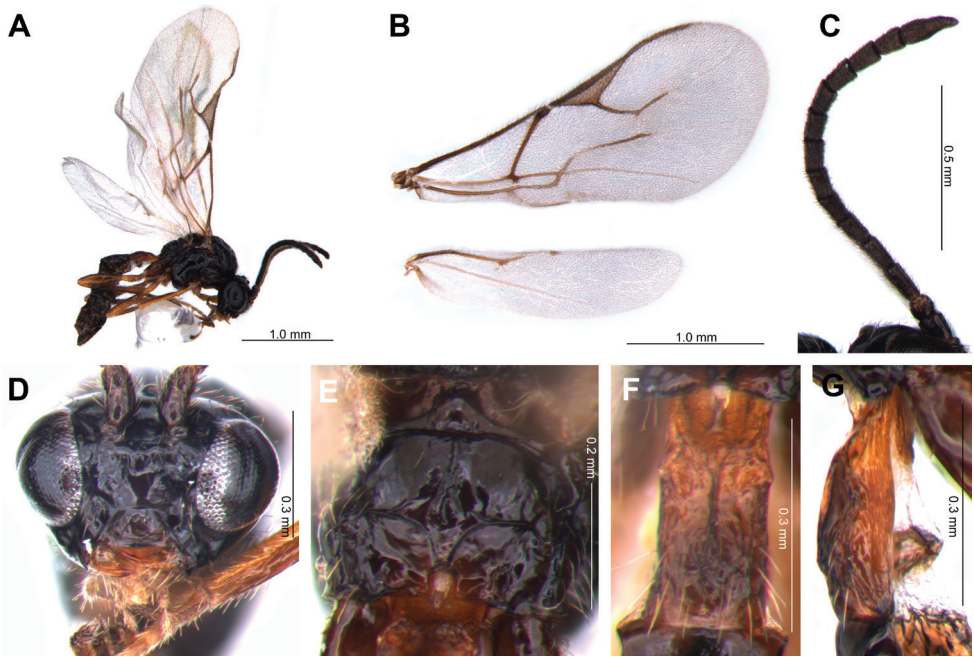
**Paratypes:** Korea, 1 ♀; DMZ Botanical Garden, Mandae-ri, Haean-myeon, Yanggu-gun, Gangwon-do, 15.VII.2014, leg. H.T. Shin. Paratype specimen deposited as fluid-mounted in the Korean National Arboretum, Gyeonggi-do, Republic of Korea.

### *Aphidius areolatus* Ashmead, 1906

Fig. 5A–G

*Aphidius areolatus* Ashmead, 1906; Starý and Schlinger 1967; Chang and Youn 1983; Starý and Choi 2000.

**Redescription. Female.** Length of body 2.93 mm (Fig. 5A). Length of fore wing 2.63 mm (Fig. 5B).



**Figure 5.** *Aphidius areolatus* Ashmead, female **A** body **B** wing **C** antennae **D** head **E** propodeum **F** dorsal view of petiole **G** lateral view of petiole.

**Head.** Tentorial index 0.35–0.39 (Fig. 5D). Malar space 0.27–0.30 times longitudinal eye diameter. Antenna 14-segmented, thickened at apex with short antennal segments (Fig. 5C). F1 slightly longer than F2. F1 and F2 2.12–2.14 and 1.63–1.76 times as long as their width at the middle, respectively. Maxillary palp with four palpomeres, labial palp with three palpomeres. Ratio of eye to temple in dorsal view 1.42. Face width/height ratio 1.46–1.57 (Fig. 5D).

**Mesosoma.** Propodeum areolated, areola length/width ratio ca. 1.53 (Fig. 5E). Pterostigma 3.61 times as long as wide. Ratio of pterostigma length to R1 vein length ca. 1.14 (Fig. 5B).

**Metasoma.** Petiole 2.17 times as long as wide at spiracles (Fig. 5F, G), with about 10 straight costulae in anterolateral area (Fig. 5G).

**Colour.** Antenna black. Head black. Face with clypeus black, mouth parts light-brown. Mesoscutum and metasoma black, except for brown petiole. Legs brown with dark apices.

**Male.** Antenna 19–20-segmented. Maxillary palp with four palpomeres, labial palp with three palpomeres.

**Specimens examined.** Korea, 2 ♀, 6 ♂; Korean National Arboretum, Gwangneung Forest, Soheul-eup, Pocheon-si, Gyeonggi-do, collected by Malaise trap: 19.III.–30.III.2018, leg. M.H. Kim, M.C. Kim, S.W. Jo, O. Ki.

**Remarks.** Morphologically, the examined specimens exhibited more variability than was known before (Starý and Schlinger 1967). The pterostigma is more

triangular and the petiole more quadrate than in the original description. Chang and Youn (1983) reported *A. areolatus* from South Korea (one male specimen), but their record of this endemic Japanese species is doubtful. Due to misidentification (Chang and Youn 1983), *Aphidius areolatus* should be deleted from the previously known Korean parasitoid fauna (personal communication with Prof. Paik). It follows that *A. areolatus* is recorded from South Korea for the first time in the present study.

## Discussion

It is well known that parasitoids, as a hyperdiverse and understudied group of organisms, generally are characterized by cryptic speciation (Derocles et al. 2012). Important biocontrol agents, aphidiine parasitoids in particular remain a largely unexplored group. In the last decades, through an integrative approach including DNA barcoding, there has been a rapid increase of knowledge about aphidiine diversity and cryptic species (Tomanović et al. 2018; Črkrić et al. 2019; Kocić et al. 2020).

Here, we use DNA barcoding to describe three new *Aphidius* species from the Korean Peninsula, an area with a poorly known aphidiine fauna that includes only 58 known species (Starý and Choi 2000; Starý et al. 2001; Yu et al. 2016; Choi et al. 2017; Choi and Kim 2018; Hwang et al. 2018; Kim et al. 2020), which is not consistent with their economic and ecological importance. *Aphidius longicarpus* sp. nov. is a sister species to the *A. absinthii* – *A. funebris* – *A. longipetiolus* clade, with a long and narrow pterostigma as a clear apomorphic character, along with a short flagellomere 1 and long R1 vein as clear plesiomorphies. *Aphidius longistigmus* sp. nov. and *Aphidius asiaticus* sp. nov. are clustered with *A. silvaticus*, a member of the *A. urticae* group (Jamhour et al. 2016). Both species share a long and narrow pterostigma, along with three maxillary and two labial palpomeres in *A. asiaticus* sp. nov. as apomorphies and a short flagellomere 1 as a plesiomorphic character. In addition, we here redescribe *A. areolatus*, an interesting species related to *Periphyllus* aphids as hosts in the Far East (Starý and Schlinger 1967). This species is morphologically similar to the European and Central Asian *A. setiger*, replacing it in the forest type of habitats in the Far East, where it parasitizes *Periphyllus* aphids on *Acer* spp. Correspondingly, in our phylogeny, *A. areolatus* is clustered with *A. setiger* and *A. cingulatus*, both parasitoids of aphids on *Acer* spp. (*Periphyllus* spp. and *Pterocomma* spp., respectively).

We presume that the Korean parasitoid fauna is extremely rich due to habitat and plant diversity (NIE 2017a, 2017b; NIBR 2019). This group is very important because most of its members are already being used or are tentatively applicable as biological control agents. Further research should therefore be conducted to explore this rich parasitoid biodiversity and detect tritrophic (host plant-aphid-parasitoid) interactions.

## Acknowledgements

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## Supplementary material I

### Table S1

Authors: Sangjin Kim, Željko Tomanović, Yeonghyeok Yu, JuHyeong Sohn, Yunjong Han, Gyeonghyeon Lee, Hyojoong Kim

Data type: table

Explanation note: List of 72 reference sequences of 22 *Aphidius* spp. retrieved from GenBank \* nine *Aphidius* spp. are not recorded in South Korea but included for molecular identification.

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