JHR 98: 69–84 (2025)
doi: 10.3897/jhr.98.131742
https://jhr.pensoft.net

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

A new record of the genus *Parabioxys* (Hymenoptera, Braconidae, Aphidiinae) and a redescription of *Bioxys japonicus* from South Korea

Sangjin Kim[1*], JuHyeong Sohn[1*], Yerim Lee[1], Yeonghyeok Yu[1], Hyojoong Kim[1]

1 *Animal Systematics Laboratory, Department of Biology, Kunsan National University, Gunsan 54150, Republic of Korea*

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

Academic editor: Jovana M. Jasso-Martínez | Received 10 July 2024 | Accepted 23 December 2024 | Published 3 February 2025

https://zoobank.org/4BF03FC8-FAB8-4C68-9CC9-2F86BCA62D2B

Citation: Kim S, Sohn J, Lee Y, Yu Y, Kim H (2025) A new record of the genus *Parabioxys* (Hymenoptera, Braconidae, Aphidiinae) and a redescription of *Bioxys japonicus* from South Korea. Journal of Hymenoptera Research 98: 69–84. https://doi.org/10.3897/jhr.98.131742

Abstract

The genera *Bioxys* Starý & Schlinger, 1967 and *Parabioxys* Shi & Chen, 2001 (Hymenoptera: Braconidae: Aphidiinae) are each known to contain only one species worldwide. *Bioxys japonicus* Starý & Schlinger, 1967 was recorded in South Korea in 1983, but this record should be considered doubtful. *Parabioxys songbaiensis* Shi & Chen, 2001 is now reported for the first time in South Korea. In this study, we provide detailed descriptions and photographic documentation of these two little-known species, *B. japonicus* and *P. songbaiensis*. Additionally, we present mitochondrial cytochrome c oxidase subunit I (COI) barcode region data and conduct a Bayesian tree analysis of closely related taxa.

Keywords

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

* These authors contributed equally.

Introduction

The genus *Bioxys* Starý & Schlinger, 1967, *Parabioxys* Shi & Chen, 2001, and *Sergeyoxys* Davidian, 2016 (Hymenoptera: Braconidae: Aphidiinae) are each known to contain only one species worldwide (Starý and Schlinger 1967; Chen and Shi 2001; Davidian 2016). The two genera, *Bioxys* and *Parabioxys*, have been recorded in South and North Korea, respectively (Starý et al. 2010; NIBR 2023), while the genus *Sergeyoxys* has been recorded in Far East Russia (Davidian 2016). Starý and Schlinger (1967) established *Bioxys* as a new genus, characterized by the fusion of the original paired prongs into a single, unique median prong. However, Takada (1968) described *Trioxys machilaphidis* Takada, 1968 and later concluded it was synonymous with *B. japonicus* Starý & Schlinger, 1967, suggesting that *Bioxys* should be considered a subgenus of the *Trioxys* Haliday, 1833 rather than a genus. Starý (1975) later maintained *Bioxys* as a distinct genus, arguing that the unique median prong could develop independently of the paired prongs known in *Trioxys*, thus highlighting the distinctiveness of *Bioxys*.

The genus *Parabioxys* shares a similar fused prong with *Bioxys* but is distinguished by a deep incision on the apical metasomal sternite (Starý 2010), a feature also present in *Sergeyoxys*. Shi and Chen (2001) established *Parabioxys* as a new genus, describing its fused prong and secondary tubercles on the petiole. However, the figure of the prong was not provided, and the figure of the petiole appears questionable. Starý (2010) redescribed *Parabioxys* using North Korean specimens, emphasizing the morphology of the prong and its deep incision at the apex. Davidian (2016) established the new genus *Sergeyoxys* based on specimens from Far East Russia, comparing them to the figures in Shi and Chen (2001), without taking the previous description by Starý (2010) into consideration.

The genus *Bioxys* has been reported as a parasitoid of *Machilaphis machili* (Takahashi, 1928) (Hemiptera: Aphididae: Phyllaphidinae) in Japan and Taiwan (Takada 1968; Chou 1999). It has also been reported as a parasitoid of callipterine aphids on *Ficus* sp. (Starý and Schlinger 1967), with Calaphidinae Oestlund, 1919, now recognized as a distinct subfamily within Aphididae (Quedneau and Remaudière 1994). Chang and Youn (1983) reported *Bioxys* in South Korea, but their description differs in several key aspects from other records: the antenna 11-segmented, the pterostigma length/width ratio is 2.5, the petiole shape of figure is different, and the host is *Aphis gossypii* Glover, 1877, while, in *Bioxys* indicate the antenna 12-segmented, the pterostigma length/width ratio more than 3.0, and it is a parasitoid of *M. machili* and calaphidine aphids (Starý and Schlinger 1967; Takada 1968; Starý et al. 2010). Given these discrepancies, the record of *Bioxys* from South Korea should be considered doubtful. In contrast, the genus *Parabioxys* is known as a parasitoid of *Greenidea kuwanai* (Pergande, 1906) on *Quercus dentata* (Thunberg, 1784) in North Korea (Starý et al. 2010), which was also collected from pines and cypresses in Shennongjia, Hubei, China (Shi and Chen 2001).

In this study, we provide detailed descriptions of two known species, *Bioxys japonicus* and *Parabioxys songbaiensis*. Additionally, we present *COI* barcode data for both species.

Materials and methods

Field and taxonomic works

Bioxys japonicus samples were collected by locating *Machilaphis machili* mummies on *Machilus thunbergii* Siebold & Zucc, 1876. Leaves hosting these mummified aphids were collected and placed in clean insect breeding dishes (SPL Life Sciences, Korea). To ensure parasitoids emerge, the dishes were maintained at room temperature in the laboratory. Emerging parasitoid wasps were monitored daily and collected using an insect aspirator. Subsequently, the collected wasps were preserved in 80% ethanol at -19 °C. The deposited dried specimens of *Parabioxys songbaiensis* were obtained from the Kunsan National University Insect collection (Gunsan-si, Republic of Korea).

Morphological identification was conducted using references from Starý and Schlinger (1967), Takada (1968), Chen and Shi (2001), Starý et al. (2010) and Starý collection from IECA, České Budějovice (Institute of Entomology Czech Academy of Sciences). We sorted out specimens with similar phenotypes using a stereomicroscope (OLYMPUS SZX16, Leica M205C). After this initial sorting, we labeled the samples and proceeded with DNA extraction.

Following morphological identification, we measured previously unrecorded species. For photography and characterization, we used a LEICA DMC2900 digital camera mounted on a LEICA M205 C microscope (Leica Geosystems AG). In case of Starý collection, we utilized a Canon EOS 60D digital camera mounted on a WeMacro rail (Shanghai Macro Photoelectric CO., China). Multiple images were taken at various focal heights using Mosaic V2.3 (Tucsen Software), and the image stacking process was carried out using HeliconFocus 7 (Helicon Soft). After stacking, illustrations were generated using Adobe Photoshop CS6. To determine the precise shape of the specimens, we employed Mosaic V2.3 (Tucsen Software) (Berkovitch et al. 2009).

Molecular analysis

Total genomic DNA was extracted using a LaboPass Tissue Kit (COSMOgenetech, Korea) following the manufacturer's protocol with slight modifications. To preserve the morphological integrity of the specimens, we adapted the "freezing method" described by Yaakop et al. (2013). Our modification involved extending the incubation period from 30 minutes to 2 hours at 56 °C with 200 µl of TL buffer and 20 µl of proteinase K. This adjustment allowed for effective DNA extraction while minimizing damage to the specimen's structure. Each sample underwent individual genomic DNA extraction to ensure sample-specific results.

For molecular identification, we targeted a 658-bp fragment from the front partial region of mitochondrial COI gene. This region was amplified using the primers, LCO1490 (forward) 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 (reverse) 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994), with AccuPower PCR PreMix (Bioneer Corp., Daejeon, Korea). The polymerase chain

reaction (PCR) was performed in a 20 μl reaction mixture consisting of 3 μl of DNA extract, 2 μl of primer, and 15 μl of ddH_2O. The PCR protocol was carried out as follows: initial denaturation for 5 min at 95 °C, followed by 35 cycles of 60 s at 94 °C, 60 s at 54 °C, and 90 s at 72 °C, with a final extension at 72 °C for 7 min. PCR products were visualized on agarose gel electrophoresis. Visible bands were sent to Macrogen (Daejeon, Korea) for purification and sequencing analysis.

Sequence alignment was performed using MAFFT version 7 (Katoh et al. 2019) with default settings, and frameshifts were checked to exclude pseudogenes. Sequences divergence was calculated using the 'p-distance' model with 1,000 bootstrap replicates and pairwise deletion for data gaps in MEGA version 7.0 (Kumar et al. 2016).

A gene tree was inferred using the Bayesian method for tree reconstruction in BEAST2 (Bouckaert et al. 2014), with Markov chain Monte Carlo (MCMC) analysis conducted for 100 million generations to ensure robust parameter estimation. To achieve accurate gene tree reconstruction, we performed best-fit substitution model testing in IQ-TREE (Trifinopoulos et al. 2016) under the GTR model, using the Bayesian Information Criterion (BIC). In BEAUti, we applied a strict clock model (Ferreira and Suchard 2008), with site options based on IQ-TREE results. MCMC analysis was conducted and verified using Tracer (Rambaut et al. 2018) and DensiTree (Bouckaert 2010). Subsequently, we generated a consensus tree using TreeAnnotator with a posterior probability limit set to 1.0.

A 658 bp of the COI fragment was sequenced for *B. japonicus.* For comparative analysis, we retrieved 28 sequences representing 16 species, including the outgroup, from GenBank (Table 1).

Results

Systematic accounts

Genus *Parabioxys* Shi & Chen, 2001

Parabioxys Shi & Chen, 2001: 122–123.

Type species. *Parabioxys songbaiensis* Shi & Chen, 2001.

Parabioxys songbaiensis Shi & Chen, 2001

Fig. 1A–K

Parabioxys songbaiensis Shi & Chen, 2001.

Redescription. Female. Length of body about 2.3–2.4 mm (Fig. 1A). Length of forewing 1.8 mm (Fig. 1K).

Table 1. GenBank accession numbers of retrieved (1–10, 13–28) and newly generated molecular data (11–12).

No	Species	NCBI accession number	Host for this specimen	General host range
1	*Binodoxys acalephae* Marshall, 1896	MT946064	*Myzus persicae* (Sulzer, 1776)	All known host is Aphidinae
2	*B. acalephae*	MT946065	*M. persicae*	
3	*B. angelicae* Haliday, 1833	MG443441	–	All known host is Aphidinae
4	*B. angelicae*	JF730315	–	
5	*B. brevicornis* Haliday, 1833	MK080162	*Hyadaphis foeniculi* (Passerini, 1860)	All known host is Aphidinae
6	*B. centaurae* Haliday, 1833	JN620611	–	All known host is Aphidinae
7	*B. centaurae*	JN620612	–	
8	*B. communis* Gahan, 1926	MF850294	*Aphis gossypii* Glover, 1877	All known host is Aphidinae
9	*B. communis*	MK070445	–	
10	*B. heraclei* Haliday, 1833	MF287648	*Cavariella aegopodii* (Scopoli, 1763)	1 known genus in Chaitophorinae 6 known genera in Aphidinae
11	*Bioxys japonicus* Starý & Schlinger, 1967	PQ483234	*Machilaphis machili* (Takahashi, 1928)	*M. machili*
12	*B. japonicus*	PQ483235	*M. machili*	
13	*Trioxys auctus* Haliday, 1833	KY887993	–	All known host is Aphidinae
14	*T. auctus*	MK080163	*Rhopalosiphum nymphaeae* (Linnaeus, 1761)	
15	*T. complanatus* Quilis, 1931	KJ848479	–	2 known genera in Calaphidinae 3 known genera in Aphidinae
16	*T. liui* Chou & Chou, 1993	MT324249	*Takecallis* sp.	1 known genus in Phyllaphidinae 1 known genus in Calaphidinae
17	*T. liui*	PP373116	*T. taiwana* (Takahashi, 1926)	
18	*T. liui*	PP373117	*T. taiwana*	
19	*T. pallidus* Haliday, 1833	KM973342	*Chromaphis juglandicola* (Kaltenbach, 1843)	12 known genera in Callaphidinae 1 known genus in Thelaxinae
20	*T. pallidus*	KM973234	*C. juglandicola*	1 known genus in Aphidinae
21	*T. parauctus* Starý, 1960	MK080164	*Hyadaphis molluginis* Börner, 1939	3 known genera in Aphidinae 1 known genus in Lachninae
22	*T. remaudierei* Starý & Rakhshani, 2017	PP373118	*T. arundinariae* (Essig, 1917)	*T. arundinariae*
23	*T. remaudierei*	PP373119	*T. arundinariae*	
24	*T. sunnysidensis* Fulbright & Pike, 2007	KR789186	*R. padi* (Lineeaeus, 1758)	*Rhopalosiphum padi*
25	*T. sunnysidensis*	MG438589	*R. padi*	
26	*T. ulmi* Čkrkić & Tomanović, 2021	MT873046	*Tinocallis takachihoensis* Higuchi, 1972	*T. takachihoensis*
27	*Aphidius uzbekistanicus* Luzhetzki,1960	ON827045		
28	*A. uzbekistanicus*	ON759206		

Head. Head wider than mesosoma (head and mesoscutum width ratio = 1.2) with sparse long setae. Eyes oval, sparsely setose. Face with sparse long setose, width/height ratio 1.3 (Fig. 1D). Tentorial index 0.3 (Fig. 1D). Clypeus oval with 6 long setae. Malar space 0.2 times as long as longitudinal eye diameter. Antenna 11-segmented (Fig. 1B). F1 subequal to F2 (length F1/F2 = 1.0–1.1) (Fig. 1C). F1 and F2 3.2–3.3 and 2.8–3.2 times as long as their width at the middle, respectively. F1 and F2 with one or two and two or three longitudinal placodes respectively. Maxillary palp with four palpomeres, labial palp with two palpomeres.

Mesosoma. Mesoscutum with notaulices on anterolateral margin, effaced dorsally; dorsal surface smooth, with two rows of 5–6 long setae along the dorsolateral part of mesoscutum (Fig. 1E). Scutellum nearly triangular, bearing 3–4 long setae on each side. Propodeum with pentagonal central areolated, length/width of areola = 0.9–1.1 (Fig. 1F). Pterostigma 3.0 times as long as width. Pterostigma longer than vein R1 (=metacarpus) 0.5 times. Vein r & RS sclerotized.

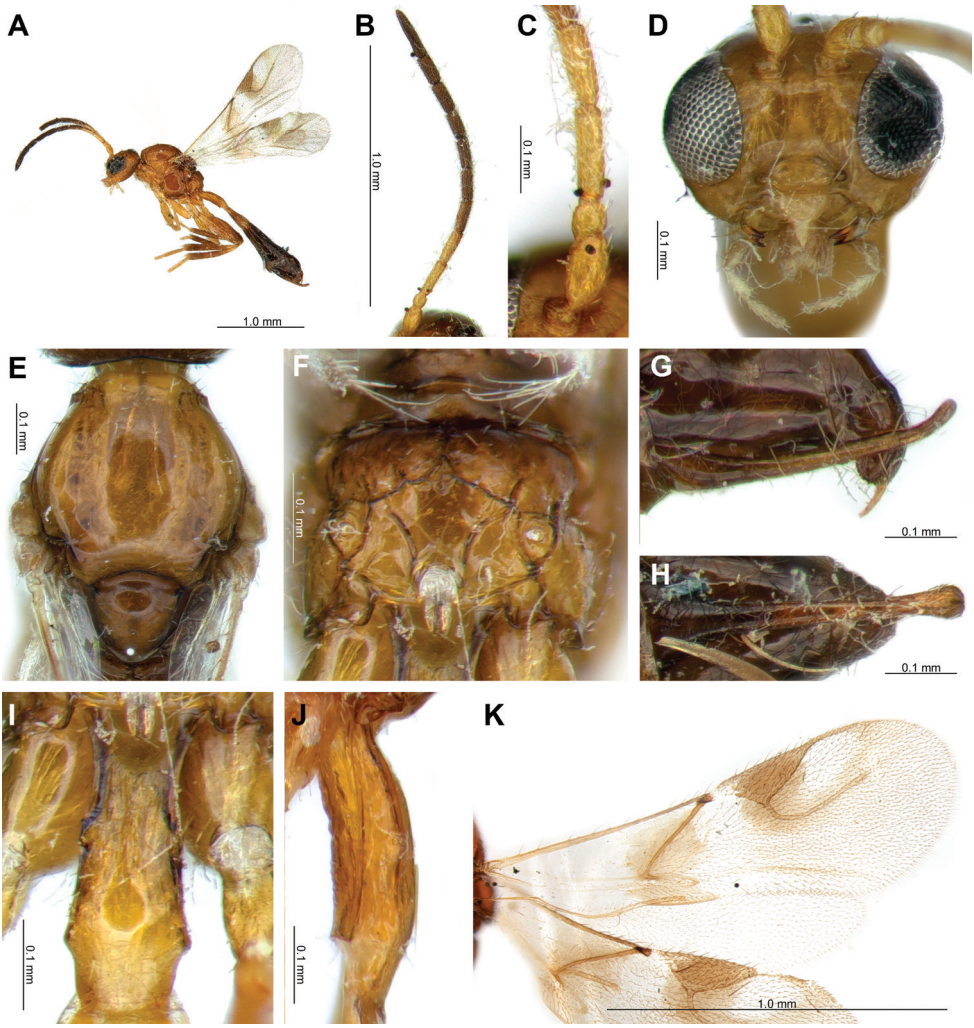

Figure 1. *Parabioxys songbaiensis* female **A** habitus, lateral view **B** antenna **C** scape, pedicel, F1 and F2 **D** head **E** mesonotum **F** propodeum **G** ovipositor **H** basal view of prong **I** dorsal view of petiole **J** lateral view of petiole **K** forewing.

Metasoma. Petiole long and slender, 3.3 times as long as wide at level of spiracles and 2.4 times as long as wide at level of secondary tubercles. Distance between spiracular and secondary tubercles is 1.2 times as long as width at level of spiracular tubercles. Distance between secondary tubercles and the apex of petiole is 0.6 times the width at level of secondary tubercles. (Fig. 1I, J). Ovipositor sheath semi-circular, with 5–7 short and dense setae positioned at basal apex as in genus *Diaeretus* Förster,1863. Prong long and straight, with the end curved upward, present deep incision on the apical metasomal sternite, four setae at dorsal and two setae at ventral part (Fig. 1G), having three setae laterally on each side in dorsal view (Fig. 1H).

***Colour*.** Antenna brown; scape, pedicel, F1 and F2 yellowish brown, with F2 sometimes partly dark brown. Head, face and clypeus with mouthparts light brown. 1M and base of r&RS vein of forewing with brown spot. Mesosoma light brown and metasoma dark brown; petiole and sternite 2 light brown. Legs light brown with dark apices.

Note. The maxillary palps are broken in the specimen that was collected in 1998. In original description, there is no setae in the surface of prong, and the same is observed in our dried specimens. However, when examined in alcohol, some pores at the apex of a prong are visible.

Remarks. Davidian (2016) defined *Parabioxys* as having an immovable prong without a preapical spur (= deep incision at the apex) of the prong, while *Sergeyoxys* possesses an independent structure separated from the base of the last sternite, with a deep incision at the apex of the prong. In Shi and Chen (2001), there was neither a mention of deep incision at the apex of the prong nor any figure of the genitalia, even although it was first noted in Starý (2010). Upon examining the specimens used in the referenced papers, we confirmed, as mentioned in Davidian (2016), that the prong is an independent structure, movably connected. After comparing the traits commonly mentioned in the three references, we considered that the samples from Starý (2010), Davidian (2016), and our samples could potentially belong to the same species (Fig. 2). However, while *Sergeyoxys* may be a synonym of *Parabioxys*, we cannot confirm this with certainty without examining the holotype (personal communication with Dr. Davidian, Table 2). In addition, the samples from Starý (2010) and this study bear a "two-segmented" labial palp and "no setiferous pore" at the base of the prong, while *Sergeyoxys* in Davidian (2016) has "three-segmented" labial palp and "a distinct setiferous pore". Since it is uncertain whether this trait falls within the range of variation, further specimens and molecular experiments are needed to reach a definitive conclusion in the future study.

Specimens examined. North Korea • 3 ♀, Mt. Taesong, Phyongyan-si 22.VI.1987. reared from *Greenidea kuwanai* (Pergande, 1906) on *Quercus dentata* Thunberg ex Murray, 1874. leg. J. Havelka. 1 dried specimen; 2 slide mounted specimen (original numbers: 617a, 617c). All specimens from North Korea are deposited in IECA, České Budějovice. South Korea • 1 ♀, Bibong-myeon, Hwaseong-si, Gyeonggi-do, 01.VI.1994. leg. DS. Ku; • 1 ♀, 57, Hoegi-ro, Dongdaemun-gu, Seoul, 20.V.1998. leg. SH. Kang.

Table 2. Comparative morphological characters from literature.

	Parabioxys **(Holotype)**	***Parabioxys*** **(Starý, 2010)**	***Sergeyoxys*** **(Davidian, 2016)**	**Our samples**
F1 l/w	4.0	3.5–4.0	3.5	3.2–3.3
Pterostigma l/w	3.5	2.2–2.3	2.3	3.0
Length of Pterostigma : vein R1	2:1	1.65–1.7 : 1	1.5:1	2 : 1
Petiole l/w at spiracular tubacles	4.0	2.90–2.95	3.0	3.3
Petiole l/w at secondary tubercles	–	2.40–2.45	2.5	2.4
Forewing l/w	–	2.7	3.0	2.9
Segment of maxillary palp	–	4	4	4
Segment of Libial palp	–	2	3	2
Setiferous pore in base of prong	–	absent	present	absent
Deep incision at the apex (= preapical spur) of prong	–	present	present	present

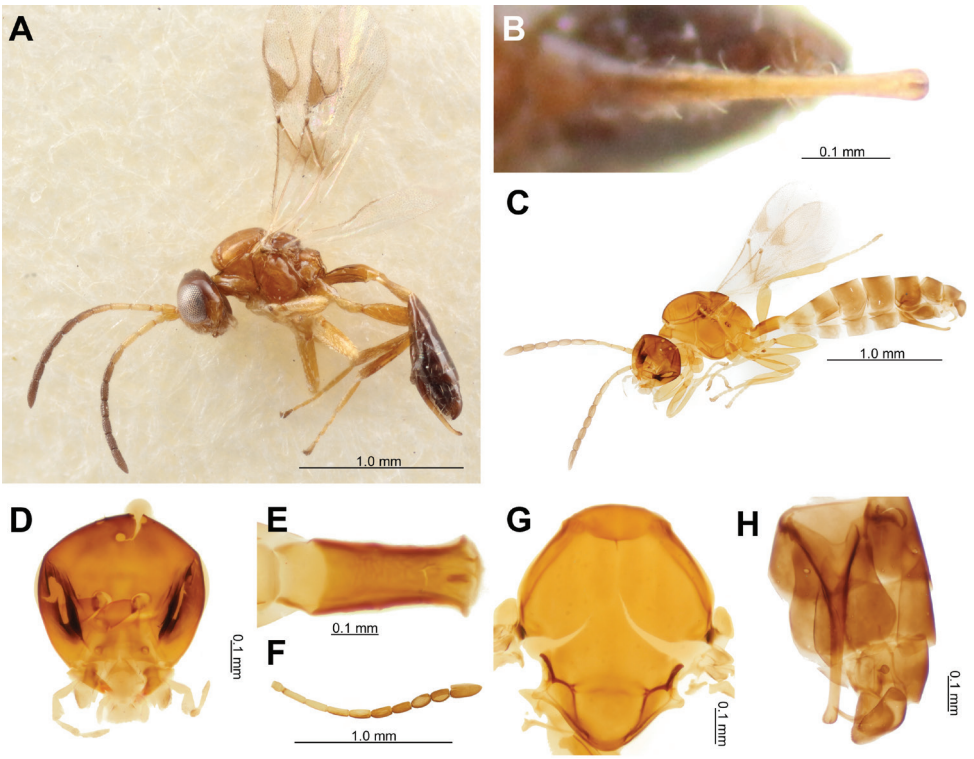

Figure 2. *Parabioxys songbaiensis* from North Korea deposited in IECA, České Budějovice **A** habitus, lateral view **B** basal view of Prong **C** habitus, in slide, 617c **D** head, 617a **E** petiole, 617a **F** antennae, 617a **G** mesonotum, 617a **H** ovipositor sheath with prong, 617a.

Genus *Bioxys* Starý & Schlinger, 1967

Bioxys Starý & Schlinger, 1967: 32–33.

Type species. *Bioxys japonicus* Starý & Schlinger, 1967.

Bioxys japonicus Starý & Schlinger, 1967

Fig. 3A–K

Trioxys machilaphidis Takada, 1968: 113.
Trioxys staryi Mackauer, 1968: 73.

Redescription. Female. Length of body about 2.4 mm (Fig. 3A). Length of forewing 1.7–1.9 mm (Fig. 3K).

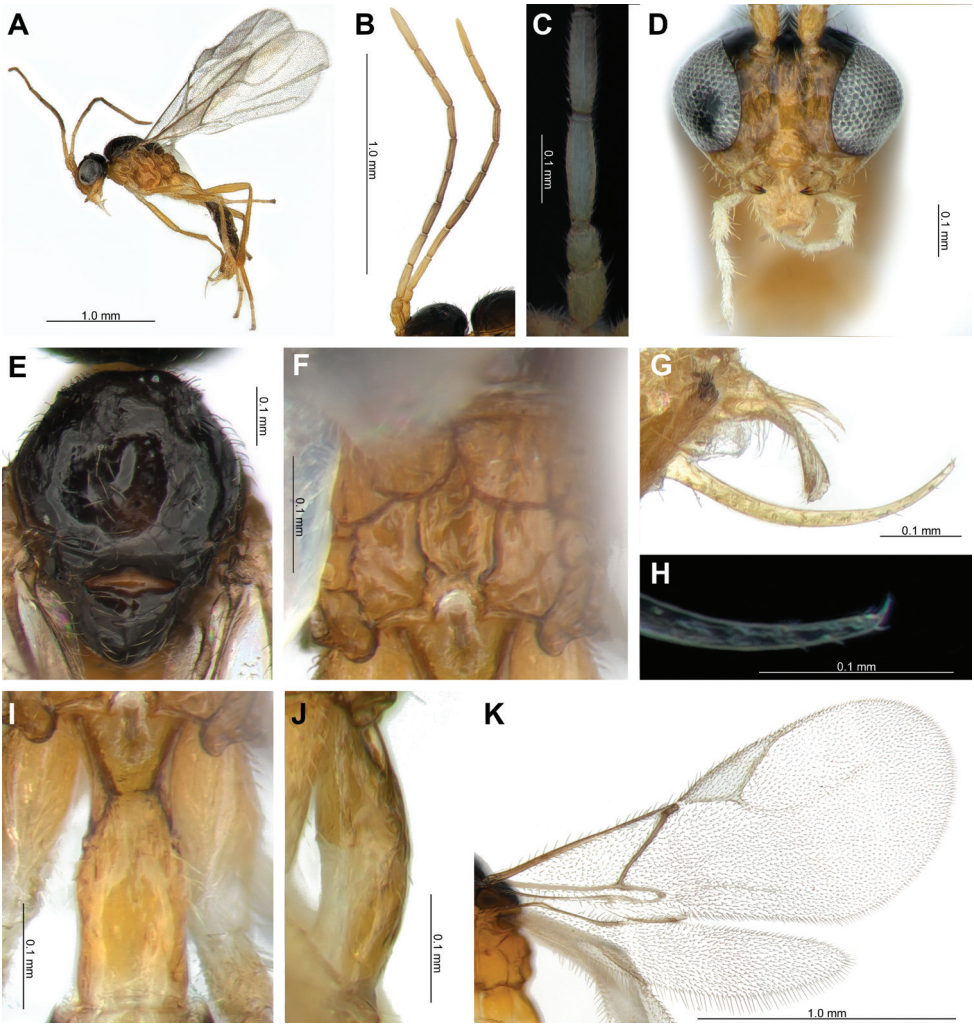

Figure 3. *Bioxys japonicus* female **A** habitus, lateral view **B** antenna **C** sacpe, Pedicel, F1 and F2 **D** head **E** mesonotum **F** propodeum **G** ovipositor and prong **H** apex of prong **I** dorsal view of petiole **J** lateral view of petiole **K** forewing.

Head. Head weakly wider than metasoma (head and mesoscutum width ratio = 1.1) with sparse long setae. Eyes oval, sparsely setose. Face with densely long setae, width/height ratio 1.2–1.4 (Fig. 3D). Tentorial index 0.4–0.5 (Fig. 3D). Clypeus oval with 12 long setae. Malar space 0.2 times longitudinal eye diameter (Fig. 3D). Antenna 11-segmented (Fig. 3B). F1 equal to F2 (Fig. 3C). F1 and F2 3.4–4.4 (average 3.9) and 3.4–4.2 times (average 3.8) as long as their width at the middle, respectively. F1 with two longitudinal placodes and F2 with three longitudinal placodes. Maxillary palp with four palpomeres, labial palp with two palpomeres.

Mesosoma. Mesoscutum with notaulices on anterolateral margin, effaced dorsally; Dorsal surface smooth, with four rows of long setae (5–8 setae per row) arranged along the dorsolateral parts of the mesoscutum, with two rows on each side (Fig. 3E). Scutellum nearly triangular, bearing 4 long setae on each side of dorsolateral. Propodeum areolated, areola length/width ratio is 1.5–1.8 (average 1.7) (Fig. 3F). Pterostigma 3.0 times as long as width. Pterostigma longer than vein R1 (=metacarpus) 1.7–2.0 (average 1.8) times. Vein r and RS extended.

Metasoma. Petiole long and slender, 3.2–3.4 (average 3.3) times as long as wide at level of spiracles and 3.1–3.3 (average 3.2) times as long as wide at level of secondary tubercles. Distance between spiracular and secondary tubercles is 2.6–2.9 (average 2.8) times as long as width at level of spiracular tubercles. Distance between secondary tubercles and apex of petiole is 1.2–1.3 (average 1.2) times width at level of secondary tubercles. (Fig. 3I, J). Dorsolateral part of anterior spiracles is concave on each side (Fig. 3I). Ovipositor sheath slender and long, curved downwards. Ratio of ovipositor sheath width/length is 2.2 at the base (Fig. 3G). Prong is long, quite curved upwards, having one claw like bristle with 2 short setae and 9 setae at dorsal side (Fig. 3G, H).

Colour. Antenna partly brown; scape, pedicel, F1, 2 (at least partly yellowish brown at F2) and F7–9 yellowish brown, F3–6 brown. Head black, face brown, clypeus with mouthparts yellowish brown. Mesosoma yellowish brown and metasoma brown; Mesonotum black; Petiole yellowish brown; Sternite 2–4 and part of sternite 5 is brown; part of sternite 5 and others, and ovipositor sheath with prong are yellowish brown. Legs light brown with dark apices.

Description. Male (Fig. 4). Length of body about 1.8–2.3 mm (Fig. 4A). Length of forewing 1.8 mm (Fig. 4J).

Head. Face width/height ratio 1.6 (Fig. 4D). Tentorial index 0.4–0.6 (average 0.5) (Fig. 4D). Clypeus oval with 8 long setae. F1 and F2 2.9–3.2 (average 3.1) and 2.8–3.2 times (average 3.0) as long as their width at the middle, respectively. F1 with three or four longitudinal placodes and F2 with four or five longitudinal placodes (Fig. 4B, C).

Mesosoma. Scutellum nearly triangular, bearing 4–5 long setae on each side of dorsolateral. Propodeum areolated (Fig. 4E). Pterostigma 3.2–3.5 (average 3.3) times as long as width. Pterostigma longer than vein R1 (=metacarpus) 2.2–2.3 (average 2.2) times.

Metasoma. Petiole long and slender, 3.4 times as long as wide at spiracles (Fig. 4H, I). Male genitalia as in Fig. 4G.

Colour. Antenna brown; scape, pedicel, F1, part of F2 yellowish brown. Head black, face with clypeus dark brown, mouthparts yellowish brown. Mesosoma and metasoma dark brown; Mesonotum black; Petiole yellowish brown; Legs light brown with dark apices.

Specimens examined. South Korea • 1 ♀, Mangeung-dong, Yeosu-si, Jeollanam-do, 22.VI.2014, reared from *Machilaphis machili* on *Machilus thunbergii*, leg. Yerim Lee.; • 7 ♀ 3 ♂, 413, Gamsan-ri, Andeok-myeon, Seogwipo-si, Jeju-do, 33°15'21.5"N, 126°21'14.1"E, 30.IV. 2024, reared from *M. machili* on *M. thunbergii*, leg. Sangjin Kim; • 7 ♀ 2 ♂, 1457-1, Napeup-ri, Aewol-eup, Jeju-si, Jeju-do, 33°26'4.0"N, 126°19'49.4"E, 01.V.2024, reared from *M. machili* on *M. thunbergii*, leg. Sangjin Kim.

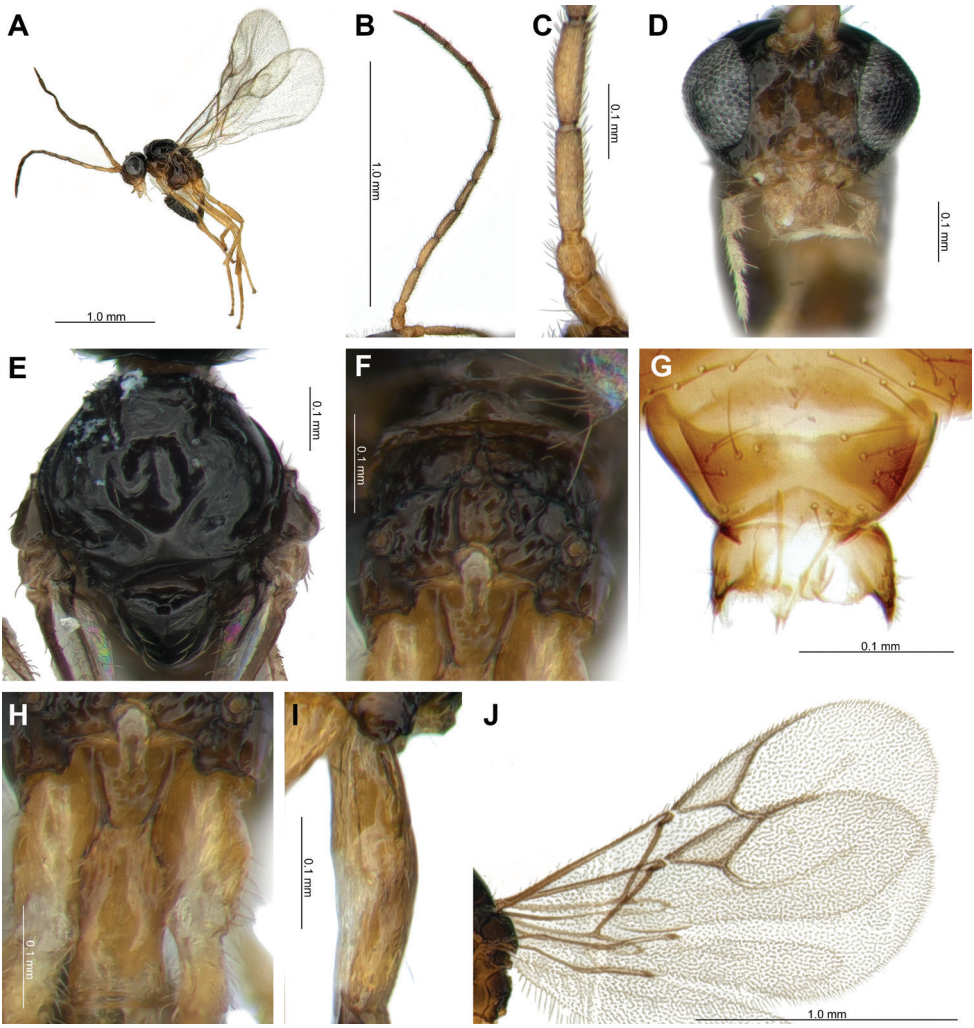

Figure 4. *Bioxys japonicus* male **A** habitus, lateral view **B** antenna **C** scape, pedicel, F1 and F2 **D** head **E** mesonotum **F** propodeum **G** male genitalia **H** dorsal view of petiole **I** lateral view of petiole **J** forewing.

Key to Korean genera of subtribe Trioxina

1 Petiole with primary (spiracular) and secondary tubercles; Ovipositor sheath with paired prongs and it start with two branches at the base; If fused at the base, at least present deep incision on the apical..... **2**

– Petiole with only primary (spiracular) tubercles; Ovipositor sheath with paired prongs and it start with two branches at the base; If fused at the base, at least bifurcated on apical two-thirds..... **Genus *Trioxys***

2 Paired prongs start with two branches at the base; female antennae with 10–13 segments..... **Genus *Binodoxys***

– Fused prongs start at base; female antennae with 11 segments **3**

3 Prongs are completely fused, short apical claw-like bristle present..... ..... **Genus *Bioxys***

– Prongs are fused, but possess a deep incision at the apex.....**Genus *Parabioxys***

Genetic relationships of the species in the subtribe Trioxina

We constructed a Bayesian tree using 28 sequences from 16 species, including an outgroup (Fig. 5). Our analysis revealed two main groups: Group A comprised *Trioxys liui*, *T. ulmi*, *T. remaudierei*, *T. pallidus*, *T. complanatus*, and *Bioxys japonicus*. Within this group, *T. liui* emerged as the sister group to *T. ulmi*, while *T. remaudierei* appeared as the sister group to *T. pallidus* and *T. complanatus*. Group B included *T. sunnysidensis*, *T. auctus*, *Binodoxys angelicae*, *B. centaurae*, *B. heraclei*, *B. revicornis*, *T. parauctus*, *B. communis*, *B. acalepha*e. Within this group, *T. sunnysidensis* was positioned as the sister group to *T. auctus*, and *B. angelicae* as the sister group to *B. centaurae*. *Binodoxys heraclei* positioned as the sister group to *T. brevicornis* and *T. parauctus*, and *B. communis* appeared as the sister group to *B. acalephae*. These genetic relationships align with previous findings by Čkrkić et al. (2021) and Kim et al. (2024).

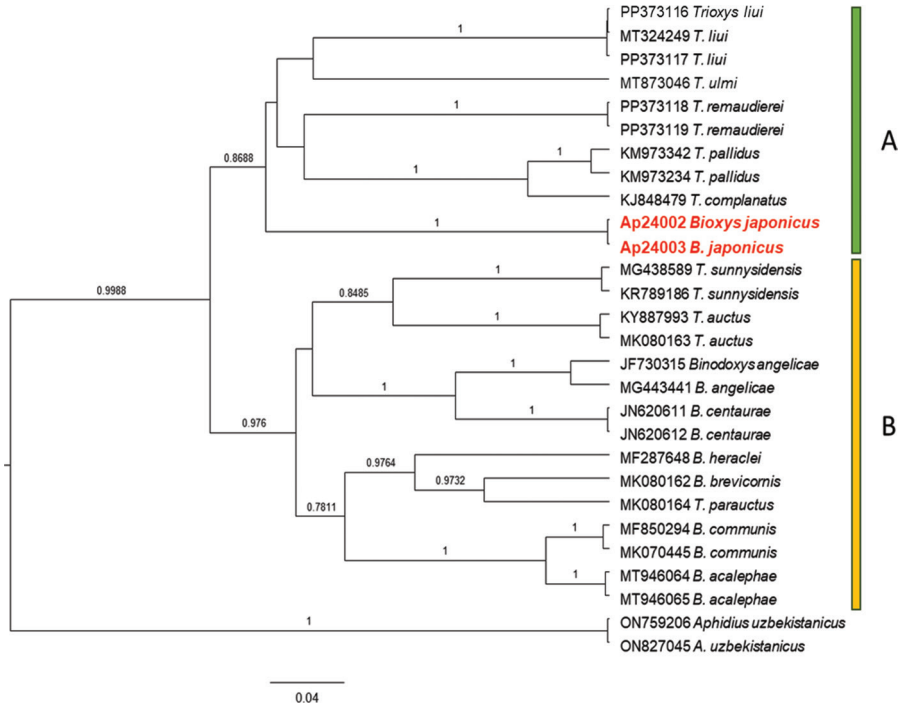

Figure 5. Bayesian tree constructed using COI barcode data. Bootstrap support values exceeding 50% are shown above the branches. *Aphidius uzbekistanicus* served as the outgroup. The scale bar represents the number of nucleotide substitutions per site. Gorup A indicate *Trioxys liui*, *T. remaudierei*, *T. pallidus*, *T. complanatus*, and *Bioxys japonicus*. Group B indicate *T. sunnysidensis*, *T. auctus*, *Bionodoxys angelicae*, *B. centaureae*, *B. heraclei*, *B. revicornis*, *T. parauctus*, *B. communis*, and *B. acalephae*.

Genetic distance analysis revealed intraspecific distances ranging from 0.000 to 0.027 (averaging 0.003), while interspecific distances spanned from 0.036 to 0.144 (averaging 0.112) (Table 3). Within Group A, the interspecific genetic distances between *B. japonicus* and the other species (*T. liui*, *T. ulmi*, *T. pallidus*, *T. remaudierei* and *T. complanatus*) ranged from 0.105 to 0.134, with an average of 0.121. Comparing *B. japonicus* to the species in Group B, interspecific genetic distances were found to be between 0.108 and 0.144, averaging 0.123.

Discussion

Previous research by Kim et al. (2024) noted that *Trioxys remaudierei* exhibits a fused prong at the base, potentially indicative of ancestral evolutionary traits. Furthermore, *Bioxys japonicus* appears to display a more distinct ancestral trait with a clearly fused prong. However, this study, limited by sample size, could not establish a clear correlation between these taxa. In our genetic analysis, we recovered *Bioxys japonicus* as a sister to *Trioxys* in group A. Interestingly, members of the *Trioxys* group possess only primary tubercles on their petiole, whereas *Bioxys* and *Binodoxys* species exhibit both primary and secondary tubercles. In the genetic analysis, *Bioxys* species clustered with *Trioxys* species in group A, rather than with *Binodoxys* species, despite sharing similar characteristics (Group A). Notably, some *Trioxys* species (*T. sunnysidensis*, *T. auctus*, *T. parauctus*) clustered with *Binodoxys* species, indicating an incongruence between morphological traits and the genetic relationships.

We attempted to determine the genetic position of *Parabioxys* among four closely related genera by including its sequence in our analysis. However, DNA extraction was unsuccessful due to the age of the specimen. In the original description (Starý and Schlinger 1967), *Bioxys* was recorded as a parasitoid of Callipterine aphids on *Ficus* sp. Subsequent records (Takada 1968; Chou and Chou 1999) documented its parasitism of *Machilaphis machili* on *Machilus thunbergii*. Until 1994, the subfamilies Calaphidinae Oestlund, 1919 and Phyllaphidinae Herrich-Schaeffer, 1857, to which *M. machili* belongs, were treated as a single subfamily (Quedneau and Remaudière 1994). This suggests that the Calaphidinae aphids mentioned in the earlier records (Starý and Schlinger 1967) might actually belong to the subfamily Phyllaphidinae.

Group A primarily comprises species parasitic to the aphid subfamily Calaphidinae. However, two exceptions were noted: *T. complanatus* (KJ848479) and *B. japonicus*. While the Genbank sequence for *T. complanatus* (KJ848479) lacks specific host data, this species is known to parasitize aphids from both Calaphidinae (two genera) and Aphidinae Latreille, 1802 (three genera) subfamilies (Yu et al. 2016). In contrast, *B. japonicus* specifically parasitizes members of the Phyllaphidinae. Group B is predominantly composed of species parasitic to the aphid subfamily Aphidinae. However, this group also includes sequences without host data, such as *T. sunnysidensis*, *T. auctus*, *B. angelicae*, *B. centaurae*, and *B. communis* (MK070445). Despite the lack of specific host information in our dataset, these species are preliminarily

Table 3. Genetic distances among Trioxina spp. based on COI sequences analysis.

	Binodoxys acalephae (n = 2)	*B. angelicae* (n = 2)	*B. brevicornis*	*B. centaurae* (n = 2)	*B. communis* (n = 2)	*B. heraclei*	*Trioxys auctus* (n = 2)	*T. complanatus*	*T. liui* (n = 3)	*T. pallidus* (n = 2)	*T. parauctus*	*T. remaudierei* (n = 2)	*T. sunnysidensis* (n = 2)	*T. ulmi*	*Bioxys japonicus* (n = 2)	*Aphidius uzbekistanicus* (n = 2)
Binodoxys acalephae (n = 2)	(0.002)															
angelicae (n = 2)	0.097	(0·027)														
B. brevicornis	0.095	0.107	(0.000)													
B. centaurae (n = 2)	0.103	0.080	0.113	(0.000)												
B. communis (n = 2)	0.036	0.100	0.109	0.106	(0.004)											
B. heraclei	0.092	0.109	0.096	0.110	0.093	(0.000)										
Trioxys auctus (n = 2)	0.089	0.110	0.119	0.118	0.092	0.116	(0.006)									
T. complanatus	0.116	0.126	0.137	0.128	0.112	0.118	0.123	(0.000)								
T. liui (n = 3)	0.105	0.119	0.132	0.121	0.119	0.129	0.115	0.119	(0.000)							
T. pallidus (n = 2)	0.117	0.127	0.136	0.122	0.124	0.123	0.129	0.057	0.121	(0.016)						
T. parauctus	0.087	0.106	0.074	0.102	0.088	0.096	0.108	0.133	0.133	0.111	(0.000)					
T. remaudierei (n = 2)	0.115	0.118	0.144	0.122	0.126	0.137	0.115	0.121	0.114	0.119	0.118	(0.000)				
T. sunnysidensis (n = 2)	0.093	0.078	0.111	0.102	0.093	0.096	0.081	0.120	0.113	0.132	0.108	0.107	(0.005)			
T. ulmi	0.111	0.119	0.120	0.118	0.124	0.126	0.113	0.114	0.107	0.112	0.118	0.105	0.101	(0.000)		
Bioxys japonicus (n = 2)	0.112	0.112	.0144	0.137	0.118	0.133	0.118	0.133	0.119	0.134	0.125	0.116	0.108	0.105	(0.000)	
Aphidius uzbekistanicus (n = 2)	0.144	0.149	0.142	0.149	0.144	0.149	0.133	0.165	0.145	0.167	0.136	0.145	0.137	0.136	0.144	(0.000)

recorded as parasitoids of Aphidinae. These patterns suggest a potential genetic association between the genera *Trioxys*, *Binodoxys*, and *Bioxys* and their host aphids. To better understand the relationships among these taxa, future research should integrate morphological, molecular, and ecological data while expanding the sample size.

Acknowledgements

This work was supported by a grant from the Honam National Institute of Biological Resources (HNIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (HNIBR202401220) and supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBRE202404) and supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2022R1A2C1091308) Also used photos from Starý collection in IECA (Institute of Entomology Czech Academy of Sciences).

References

Berkovitch A, Belfer I, Hassin Y, Landa E (2009) Diffraction imaging by multifocusing. Geophysics 74: WCA75-WCA81. https://doi.org/10.1190/1.3198210

Bouckaert R (2010) DensiTree: making sense of sets of phylogenetic trees. Bioinformatics 26(10): 1372–1373. https://doi.org/10.1093/bioinformatics/btq110

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu CH, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Computational Biology 10: e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Chang YD, Youn YN (1983) On fourteen unrecorded species of Aphidiidae (Hym.) from Korea. Research reports of environmental science and technology 1: 16–22.

Chen JH, Shi QX (2001) systematic studies on aphidiidae of China (Hymenoptera: Aphidiidae). Fujian Science and Technology Publishing House, Fujian: 122–123.

Chou LY (1999) New records of six Braconids (Hymenoptera: Braconidae) from Taiwan. Journal of Agricultureal Research of China 48: 64–66.

Čkrkić J, Petrović A, Kocić K, Tomanović Ž (2021) Insights into phylogenetic relationships between *Trioxys* Haliday, 1833 and *Binodoxys* Mackauer, 1960 (Hymenoptera, Braconidae, Aphidiinae), with a description of a new species of the genus *Trioxys*. Zoosystema 43: 145–154. https://doi.org/10.5252/zoosystema2021v43a8

Davidian EM (2016) A new genus and new species of subfamily Trioxinae (Hymenoptera: Aphidiidae) from the Far East of Russia. Zootaxa 4205(5): 475–479. https://doi.org/10.11646/zootaxa.4205.5.5

Ferreira MAR, Suchard MA (2008) Bayesian analysis of elapsed times in continuous-time Markov chains. Canadian Journal of Statistics 36: 355–368. https://doi.org/10.1002/cjs.5550360302

Folmer O, Black M, hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology Biotechnology 3: 294–299.

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20: 1160–1166. https://doi.org/10.1093/bib/bbx108

Kim S, Sohn J, Kim H (2024) Two new records of the genus *Trioxys* (Hymenoptera, Braconidae, Aphidiinae) parasitic on bamboo aphids from South Korea. Biodiversity Data Journal 12: e118599. https://doi.org/10.3897/BDJ.12.e118599

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

NIBR (2023) National list of species of Korea. National Institute of Biological Resources. 01.29.2024 ed.

Quedneau FW, Remaudière G (1994) Le genre sud-américain Neuquenaphis E. E. Blanchard, description de deux nouvelles espèces et définition de nouvelles sous-familles d'Aphididae (Homoptera). Bulletin de la Société entomologique de France 99: 365–384. https://doi.org/10.3406/bsef.1994.17076

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032

Starý P (1975) A Checklist of the Far East Asian Aphidiidae. Beiträge Zur Entomologie 25(1): 53–76.

Starý P, Rakhshani E, Havelka J, Tomanović Ž, Kavallieratos NG, Sharkey M (2010) Review and Key to the World Parasitoids (Hymenoptera: Braconidae: Aphidiinae) of Greenideinae Aphids (Hemiptera: Aphididae), Including Notes on Invasive Pest Species. Annals of the Entomological Society of America 103: 307–321. https://doi.org/10.1603/AN09127

Starý P, Schlinger EI (1967) A revision of the Far East Asian Aphidiidae (Hymenoptera). Springer science&Business Media, The Hague, 32–33. https://doi.org/10.1007/978-94-017-6337-0

Takada H (1968) Aphidiidae of Japan (Hymenoptera). Insecta Matsumurana 30: 92–102.

Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44: W232–W235. https://doi.org/10.1093/nar/gkw256

Yaakop S, van Achterberg C, Ghani I, Zuki A (2013) Freezing Method as a New Non-Destructive Modification of DNA Extraction. Pertanika Journal of Tropical Agricultural Science 36: 373–392.