Additions to the genus Cratospila Foerster (Hymenoptera, Braconidae, Alysiinae) from South Korea

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Abstract
Two new species of the genus Cratospila Foerster, 1863 (Braconidae: Alysiinae), Cratospila albosignata sp. nov. and C. longivena sp. nov., are described and illustrated. In addition, the DNA barcode region of the mitochondrial cytochrome c oxidase subunit I (COI) of both species has been sequenced with three previously described species (C. albifera, C. luteocephala and C. syntoma). Alysia ponerola Papp, 2009 which was recorded from North Korea is transfered in Cratospila (C. ponerola (Papp, 2009) comb. nov.). All species validly recorded from Korea are included in a revised key.

Keywords
Alysiini, COI, Hymenoptera, new combination, new record, new species, taxonomy

Introduction
The subfamily Alysiinae is a large taxon of the family Braconidae, which includes two tribes, Alysiini and Dacnusini with 76 genera and 31 genera, respectively (Yu et al. 2016). Alysiinae contains over 2,440 valid species and the subfamily occurs worldwide

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(Yu et al. 2016), of which 180 species in 21 genera are listed in the National Species List of South Korea (NIBR 2021). Although, Alysiinae are very similar to members of the subfamily Opiinae both genetically and morphologically, the former can be distinguished from the latter easily by having the exodont (= non-overlapping) mandibles. Alysiinae is known as a group of koinobiont endoparasitoids of cyclorrhaphous dipterous larvae. They use the outward-curved teeth of the mandibles to break open the host puparium (Docavo et al. 2002). Some species are commercially utilized for biological control (Ozawa et al. 2001; Chabert et al. 2012).

The genus *Cratospila* Foerster, 1863, is a small and rather isolated taxon in the subfamily Alysiinae, including 18 species worldwide (Yu et al. 2016; Sohn et al. 2021). This genus is easy to be diagnosed by having the first flagellomere at least 1.5 times longer than the second flagellomere and often with white antennal segments apically. So far, four species have been known in the Oriental region but two species of them are doubtful. Bhat (1980) reported *C. curvabilis* from India and *C. bhutanensis* from Bhutan; considering the original description it is questionable if the first species belongs to *Cratospila*. Tobias (1990) described *C. alboapicalis* from Vietnam. Additionally, Wharton (2002) described six species (*C. confusa*, *C. difficis*, *C. dracula*, *C. elongata*, *C. masneri*, *C. neocirce* and *C. storeyi*) from Australia. *Cratospila circe* (Haliday, 1838) was reported from North Korea by Papp (1994) and also from Malaysia by Yaakop and Aman (2012), but both records are likely to concern one of the very similar local species of the genus (Sohn et al. 2021). So far, *C. circe* has been found only in the Western Palaearctic region. Checking figures of the holotype of *Alysia ponerola* Papp, 2009 (Papp 2009; NIBR 2012) made us aware that likely this species was classified into the wrong genus and should be transferred to *Cratospila* (*C. ponerola* (Papp, 2009) comb. nov.). Fortunately, it is different from the very similar and recently described *C. albifera* Sohn & van Achterberg, 2021 from S. Korea as indicated in the added key.

In this study, we present new morphological characters and the barcoding sequences of the COI region of both new species together with three previously described species (*C. albifera*, *C. luteocephala* and *C. syntoma*). Descriptions, diagnoses, species identification key, and photographs of the diagnostic characters of the new species are provided.

**Materials and methods**

Samples used in this study were collected with Malaise traps in South Korea at the Nebang-ri, Sudong-myeon, Nanyangju-si, Gyeonggi-do and Unilam Banilam, Jucheon-myeon, Jinan-gun, Jeollabuk-do. Sorting and preparation were done at the Animal Systematics Lab. (ASL), Department of Biology, Kunsan National University (KSNU) at Gunsan. For morphological identification, Wharton et al. (1997) and Zhu et al. (2017) were used. Morphological characters were observed with a Leica M205C stereo microscope. The Taxapad database (Yu et al. 2016) was used for references. We
followed the terminology of Wharton (2002) and van Achterberg (1993). The type specimens are deposited KNA (Korea National Arboretum).

A Leica DMC2900 digital camera and a Leica M205 C microscope (Leica Geosystems AG) were used for photography and several pictures being taken for each height using multi-focusing 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.

Extraction of DNA was done in ASL, KSNU. Whole genomic DNA was extracted from the specimens by using a DNeasy Blood & Tissue kit (QIAGEN Inc., Dusseldorf, Germany) following the manufacturer’s protocol. In order to conserve morphologically complete voucher specimens, DNA extraction method was used slightly modified from ‘non-destructive method’ by Favret (2005) and ‘freezing method’ by Yaakop et al. (2009). In the original protocol, the sample was crushed or wounded, and then soaked with 180 μl of buffer ATL + 20 μl of proteinase, following by three hours over incubation at 55 °C. In the slightly modified DNA extraction methods, samples were soaked with 180 μl of buffer ATL + 20 μl of proteinase K without destroying the sample, followed by 10 minutes incubation at 55 °C and then kept in a freezer at -22 °C overnight. After that the general protocol was used for the remaining steps. The primer set of LCO-1490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) and HCO-2198 (5’-TAAACTTCAGGGTGACCAAATCA-3’) was used to amplify approximately 658 bp as the partial front region of the COI. The polymerase chain reaction (PCR) products were amplified by using AccuPowerH PCR PreMix (BIONEER, Corp., Daejeon) in 20 μl reaction mixtures containing 0.4 μM of each primer, 20 μM of the dNTPs, 20 μM of the MgCl₂, and 0.05 μg of the genomic DNA template. PCR amplification was performed using a GS1 thermo-cycler (Gene Technologies, Ltd., U.K) according to the following procedure: initial denaturation at 95 °C for 5 min, followed by 34 cycles at 94 °C for 35 sec; an annealing temperature of 48 °C for 25 sec; an extension at 72 °C for 45 sec, and a final extension at 72 °C for 5 min. The PCR products were visualized by electrophoresis on a 1.5% agarose gel. A single band was observed, purified using a QIAquick PCR purification kit (QIAGEN, Inc.), and then sequenced directly using an automated sequencer (ABI Prism 3730 XL DNA Analyzer) at Macrogen Inc. (Seoul, South Korea).

Sequence alignment was performed in MEGA version 7 (Kumar et al. 2016) with ClustalW method. To estimate the pairwise genetic distances, the P-distance model was conducted using MEGA version 7.

**Results**

Total of 630 bp of the COI locus were sequenced for *Cratospila albosignata* sp. nov. (GenBank accession no. ON504323), *Cratospila longivena* sp. nov. (GenBank accession no. ON504322), *C. albifera* Sohn & van Achterberg, 2021 (GenBank accession no. MW376064), *C. luteocephala* Sohn & van Achterberg, 2021 (GenBank accession no. MW376065).
MW376065) and C. syntoma Sohn & van Achterberg, 2021 (GenBank accession no. MW376066). Pairwise genetic distances were calculated by using ‘P-distance’ model with option for pairwise deletion; C. albosignata differed by 7% from C. longivena, by 7% from C. albifera, by 9% from C. luteocephala and by 6% from C. syntoma (Table 1).

Taxonomy

**Cratospila Foerster, 1863**

Figs 1, 2


**Diagnosis.** First flagellomere 1.5–2.1 times longer than second (Figs 1B, 2B), most species with 8–13 white segments in apical part of antenna (unknown of *C. longivena*, but has reddish brown head, morphologically related to *C. albifera* and has according to the COI analysis a derived position compared to other species), face with setae (Figs 1E, 2E), eye slightly oval, clypeus protruding anteriorly (Figs 1E, 2E), clypeus large, triangularly shaped and ventrally truncate, mandible with three teeth, second tooth narrow and sharp, maxillary palp with six segments, as long as mesosoma; notauli at least present anteriorly, scutellar sulcus distinct, precoxal sulcus medially deeply impressed and coarsely crenulate, more or less reduced anteriorly and posteriorly (Figs 1G, 2G); fore wing (Figs 1C, 2C) vein 2-SR slightly bent, vein 3-SR shorter than vein 2-SR; veins 2-SR+M and r-m not sclerotized, hind wing vein 1-M shorter than vein 1r-m; first tergite longer than second (Figs 1H, 2H).

**Biology.** Rather small genus, of which the biology is unknown.

**Distribution.** Cosmopolitan, except Neotropical region.

**Key to species of *Cratospila* Foerster from Korea**

1 Mesoscutum medio-posteriorly and scutellum reddish brown; notauli on middle of mesoscutum comparatively coarsely crenulate; pterostigma rather slender and narrowly yellow basally; vein 1-SR+M of fore wing slightly sinuate; mesosoma 1.5–1.6 times longer than high in lateral view and anterior half of propodeum less sloping; propodeum less extensively rugose medially; antennal sockets comparatively close to level of inner side of eyes; [head in dorsal view yellowish brown] .................................................................2

- Mesoscutum medio-posteriorly and scutellum black; head in dorsal view more transverse and at least posteriorly darkened; notauli on middle of
Review of the genus *Cratospila* (Hymenoptera, Braconidae, Alysiinae) from S. Korea

mesoscutum narrowly crenulate; pterostigma rather robust and brown basally; vein 1-SR+M of fore wing nearly straight; mesosoma 1.4–1.5 times longer than high in lateral view and anterior half of propodeum largely sloping; propodeum more extensively rugose medially; antennal sockets more removed from level of inner side of eyes .........................................................4

Minimum width of face 0.9 times its height (measured from lower rim of antennal socket to upper medio-dorsal margin of clypeus); vein r of fore wing approx. 3 times longer than wide; first subdiscal cell of fore wing approx. 7.5 times longer than wide; [colour of apical antennal segments unknown] ...........

................................. *C. luteocephala* Sohn & van Achterberg, 2021

– Minimum width of face 1.2 times its height; vein r of fore wing 4–5 times longer than wide; first subdiscal cell of fore wing 4–5 times longer than wide; [antenna of ♀ with 10–11 white segments] ........................................3

First tergite about twice as long as wide apically; eye in dorsal view approx. 2.4 times longer than temple and head in dorsal view more transverse (Fig. 2 in Papp 2009); apical antennal segment dark brown; vein r of fore wing less oblique (Fig. 9 l.c.) ..........................................................

................................. *C. ponerola* (Papp, 2009) comb. nov.

– First tergite approx. 2.8 times longer than its apical width; eye in dorsal view approx. 1.9 times longer than temple and head in dorsal view less transverse (Fig. 1D in Sohn et al. 2021); apical antennal segment white; vein r of fore wing more oblique (Fig. 1C l.c.) .................. *C. albifera* Sohn & van Achterberg, 2021

Vein 2-SR 1.8–1.9 times longer than vein 3-SR; first subdiscal cell approx. 8 times longer than wide; minimum width of face 0.95 times its height; [pedicellus entirely yellow; head (except posteriorly) yellowish brown; antenna of ♀ with 10–11 white or ivory segments and apical segment dark brown, pale part 4.6 times longer than apical dark brown part] ....................................................

................................. *C. ejuncida* Sohn & van Achterberg, 2021

– Vein 2-SR 1.4–1.5 times longer than vein 3-SR; first subdiscal cell 5–6 times longer than wide; minimum width of face 1.1–1.3 times its height............5

Eye in dorsal view approx. 1.6 times longer than temple; vein r of fore wing about as long as wide; head black dorsally; pedicellus partly infuscated; [minimum width of face 1.1 times its height].....................................................

................................. *C. syntoma* Sohn & van Achterberg, 2021

– Eye in dorsal view 2.3–2.8 times longer than temple; vein r of fore wing about twice as long as wide; head reddish brown or blackish brown dorsally; pedicellus yellow.................................................................................6

First tergite comparatively slender (Fig. 2F), approx. 3.5 times longer than its apical width; minimum width of face 1.1 times its height; head blackish brown dorsally ......................................................... *C. longivena* sp. nov.

– First tergite comparatively robust (Fig. 1F), approx. 2.9 times longer than its apical width; minimum width of face 1.3 times its height; head reddish brown dorsally ......................................................... *C. albosignata* sp. nov.
Cratospila albosignata Sohn & van Achterberg, sp. nov.
https://zoobank.org/DF862519-BCFF-4322-BA83-591378EF63B1
Fig. 1A–I


Description. Holotype, ♀, length of body 2.8 mm in lateral, length of antenna 4.5 mm and of fore wing 2.7 mm.

Colour. Body (Fig. 1A) black, but head (Fig. 1A), first tergite and mesonotum entirely reddish brown; antenna yellowish brown basally, medially dark brown, subapically white (11 flagellomeres); mandible pale orange.

Head (Fig. 1D): Width of head 1.6 times its median length in dorsal view. Antenna 1.6 times longer than body, 32 segmented. First flagellomere 2.1 times longer than second and 8.7 times longer than wide. Compounded eye slightly oval, in lateral view 1.2 times as long as wide. Minimum width of face (Fig. 1E) 1.3 times its height (measured from ventral rim of antennal sockets to upper margin of clypeus). Eye in dorsal view 2.3 times as long as temple. Ocello-ocular line (OOL) 3.5 times longer than diameter of anterior ocellus; OOL : antero-posterior ocellar line (AOL) : postero-ocellar line (POL) = 30 : 8 : 13. Stemmaticum concave. Vertex smooth and with polished stripe. Mandible pale yellow with three teeth, first tooth lobe-shape, second tooth narrow and sharp with reddish brown tip. Maxillary palp white and approx. as long as mesosoma.

Mesosoma: Mesosoma 1.8 times longer than wide in dorsal view; 0.7 times longer than wide in lateral view. Mesoscutum (Fig. 1G) with medio-posterior depression; notauli distinctly impressed anteriorly, not reaching medio-posterior depression; scutellar sulcus with six carinae; in lateral view, ventral of mesopleuron and metapleuron with setae. metanotum sculptured. Propodeum (Fig. 1H) 0.5 times longer than wide, anterior half of propodeum smooth, posterior of median carina wrinkled; precoxal sulcus (Fig. 1F) deep and distinct, with more than 14 carinae, propodeum not curved dorsally in lateral view. Fore wing (Fig. 1C) 2.4 times as long as wide in maximum length; pterostigma 3.9 times longer than wide; vein r of fore wing 3 times longer than wide; vein 2-SR slightly bent; vein 2-SR+M and r-m not sclerotized; 2-SR: r : 3-SR = 17 : 3 : 12; first discal cell of fore wing approx. 1.3 times longer than wide; first subdiscal cell of fore wing approx. 5 times longer than wide. Hind wing vein M+CU : vein 1-M = 11 : 1.

Leg: Hind coxa compressed and grooved; hind coxa 1.4 times longer than hind trochanter; hind femur 4.2 times longer than wide and 0.7 times longer than hind tibia; hind tibia as long as hind tarsus.

Metasoma: First tergite striate and narrow, 2.9 times longer than its apical width and dark brown, T1:T2 = 52:39. Setose part of ovipositor sheath (Fig. 1I) 0.4 times as long as mesosoma, 0.5 times as long as hind tibia and with long setae.

Male. Unknown.

Distribution. South Korea.

Etymology. Named after the conspicuous white apex of the ♀ antenna: “albo” is derived from “albus” (Latin for white) and “signata” is derived from “signatus” (Latin for marked).
Review of the genus *Cratospila* (Hymenoptera, Braconidae, Alysiinae) from S. Korea

**Cratospila longivena** Sohn & van Achterberg, sp. nov.

https://zoobank.org/13631339-38B5-4DF1-9C13-A3E03E5E78E2

Fig. 2A–I


**Comparative diagnosis.** Differ from other South Korean species of *Cratospila* by having the first tergite very long (3.5 times longer than its apical width; 2.5–2.9 times...
in other species). Unfortunately, some apical segments of antenna are missing, but COI analysis apparently showed that it is genetically close to *C. syntoma*.

**Description.** Holotype, ♂, length of body in lateral view 2.9 mm, and of fore wing 2.8 mm.

**Colour:** Body (Fig. 2A) black, head dorsally blackish brown, remainder of head, first tergite and mesonotum entirely reddish brown; antenna yellowish brown basally, medially dark brown (apical part of antenna missing, but according to notes made in Netherlands with at least 7 white segments).

**Head** (Fig. 2D): Width of head 1.5 times its median length in dorsal view. First flagellomere 1.6 times longer than second and 7.3 times longer than wide; most of antenna lost during transport from Netherlands to Korea. Compounded eye slightly oval and glossy; in lateral view 1.2 times as long as wide. Width of face (Fig. 2E) 1.1 times its height (measured from ventral rim of antennal sockets to upper margin of clypeus). Face with long setae and glabrous. Eye in dorsal view 2.8 times as long as temple. Ocello-ocular line (OOL) 3.6 times longer than diameter of anterior ocellus; OOL: antero-posterior ocellar line (AOL) : postero-ocellar line (POL) = 32 : 8 : 13. Stemmaticum concave and with setae. Mandible entirely pale orange, with three teeth, second tooth narrow and sharp with dark brown tip, and separated from first tooth and third tooth. Third tooth with carina in ventral view. Medial length of mandible 1.6 times its maximum width. Labrum 0.7 times longer than maximum width. Maxillary palp 0.8 times longer than mesosoma.

**Mesosoma:** Mesosoma 2.0 times longer than its maximum width in dorsal view and 1.4 times its height in lateral view. Mesoscutum (Fig. 2G) with medio-posterior depression; notauali chain-shaped, nearly complete but not reaching medio-posterior depression; scutellar sulcus with six distinct carinae; in lateral view mesopleuron smooth and glossy, apical parts with setae; metapleuron smooth with setae; metanotum sculptured; small basal bump on hind coxa. Propodeum (Fig. 2H) 0.6 times longer than wide, anterior half of propodeum smooth, posterior of median carina strongly wrinkled; precoxal sulcus (Fig. 2F) deep and distinct, with about eight carinae, propodeum curved in lateral view. Fore wing (Fig. 2C) 2.4 times as long as wide; pterostigma long and narrow, 3.2 times longer than wide; vein r of fore wing 3.5 times longer than wide; vein 2-SR slightly bent; vein 2-SR+M and r-m not sclerotized; 2-SR: r : 3-SR = 11 : 2 : 7; first subdiscal cell of fore wing approx. 5 times longer than wide. Hind wing M+CU : 1-M = 22 : 4.

**Table 1.** COI pairwise genetic distances between the three *Cratospila* spp. from South Korea.

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<th><em>C. albosignata</em></th>
<th><em>C. longivena</em></th>
<th><em>C. albifera</em></th>
<th><em>C. luteocephala</em></th>
<th><em>C. syntoma</em></th>
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<td></td>
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<td><em>C. longivena</em></td>
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<td>0.000</td>
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<tr>
<td><em>C. luteocephala</em></td>
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<td>0.092</td>
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<td><em>C. syntoma</em></td>
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Figure 2. (A–I) *Cratospila longivena* sp. nov.♀ A habitus, lateral view B antennae C wings D head, dorsal view E head, front view F mesosoma, dorsal view G mesosoma, lateral view H anterior half of metasoma, dorsal view I ovipositor sheath, lateral view.

**Leg:** Hind coxa compressed and grooved; hind coxa 1.2 times longer than hind trochanter; hind femur 5.5 times longer than wide and 0.7 times longer than hind tibia; hind tibia as long as hind tarsus.

**Metasoma:** First tergite striate and narrow, 3.5 times longer than apical width, T1:T2= 5:3. Setose part of ovipositor sheath (Fig. 2I) 0.5 times as long as mesosoma, 0.5 times as long as hind tibia and with long setae.

**Male.** Unknown.
**Distribution.** South Korea.

**Etymology.** Named after the comparatively long vein \( r \) of the fore wing: “longi” is derived from “longus” (Latin for long) and “vena” is Latin for vein.

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**References**


