

A new species of the genus *Coccophagus* (Hymenoptera: Aphelinidae) associated with *Sphaerolecanium prunastri* (Hemiptera: Coccoidea) from the Tianshan Mountains, Xinjiang

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Abstract: To investigate the natural parasitoid enemies of *Sphaerolecanium prunastri*, a scale insect which harms wild fruit forests in western Tianshan, Xinjiang, branches seriously damaged by *S. prunastri* were collected and cultured in glass jars for obtaining natural parasitoids. *Coccophagus tianshanensis* Li & Yao **sp. nov.**, a new species of *Coccophagus* (Hymenoptera: Aphelinidae) was gained and identified based on the morphological and molecular data. Morphological characteristics of *C. tianshanensis* are described in detail, and COI and 28S sequences are determined. In addition, a key to species of the genus *Coccophagus* in the world parasitizing *S. prunastri* was established. This investigation has found a potential natural enemy of the *S. prunastri* in the wild fruit forests of the West Tianshan Mountains, Xinjiang, and establishes a new natural enemy for biocontrol of this pest.

Keywords: parasitoid; morphology; molecular data; taxonomy

新疆天山寄生杏树鬃球蚧（半翅目：蚧科）的食蚧蚜小蜂属一新种记述（膜翅目：蚜小蜂科）

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摘要: 杏树鬃球蚧在新疆西天山野果林对野杏已造成严重危害。为调查其寄生性天敌, 本文通过采集受害枝条和室内饲养, 获得了寄生蜂标本, 经形态学观察与 DNA 序列分析, 确定其为食蚧蚜小蜂新种, 将其命名为天山食蚧蚜小蜂 *Coccophagus tianshanensis* Li & Yao **sp. nov.**, 文中详细描述了其形态特征, 拍摄了局部特征照片, 提供了 COI 及 28S 序列, 同时编制了世界寄生杏树鬃球蚧的食蚧蚜小蜂属种检索表。本研究发现了新疆西天山野果林重要害虫杏树鬃球蚧的新天敌, 为进一步开展生物防治奠定了基础。

关键词: 寄生蜂; 形态学; 分子数据; 分类

Introduction

The genus *Coccophagus* was established by Westwood (1833, 1840) based on the type species *Entedon scutellaris* Dalman (1826). A total of 268 species of the genus has been

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recorded worldwide (Dixon 2021). Since 1833, *Coccophagus* has been combined with dozens of other genera, including *Paracharitopus* Brethes, *Onophilus* Brethes, *Aclerdaephagus* Sugonjaev, and *Euxanthellus* Silvestri (Mercet 1929a; De Santis 1948), thus becoming the largest genus in the family Aphelinidae (Hayat 1992, 1994). This genus is widely distributed worldwide and is economically important as a genus of parasitoids of scale insects that harm cash crops and have been spread from country to country (Compere 1931).

The species of *Coccophagus* are relatively diverse and intergrade (Girault & Dodd 1915), thus were frequently designated as encyrtids (Curtis 1829). The most notable feature of *Coccophagus* is the strigose mesoscutum and the scutellum with three pairs of setae or strigose (Husain & Agarwal 1982; Huang 1994). Within *Coccophagus*, species are distinguished mainly by the color of their mesosoma and legs. Most species are primary parasitoids and their main hosts are soft scale insects. Occasionally hyperparasitism occurs (Fischer & Agricola 1991).

In China, 38 species have been recorded (Liao *et al.* 1987; Huang 1994 ; Liu 2013; Chen 2017; Zhou *et al.* 2018; Wang *et al.* 2020) and most of them are associated with the scale coccoids. Liao *et al.* (1987) gave the first key containing nine Chinese species in this genus, and later a key comprising 33 Chinese species was established by Chen (2017). Research conducted by Zhou *et al.* (2018) for the purpose of clarifying the parasitoid-host associations in the genus *Coccophagus* conclusively delimited 17 morphospecies in China based on the COI barcode and the 28S-D2 rRNA region gene. This contained two newly-recorded species to China and five unidentified species. With this diversity of host scales, their parasitoid species should be abundant. Thus there is a need to continue taxonomic and systematic studies of Chinese species.

Herein, we describe a new species, *Coccophagus tianshanensis* Li & Yao **sp. nov.**, that parasitizes the scale insect *Sphaerolecanium prunastri* (Fonsec) (Hemiptera: Coccoidea) found on *Armeniaca vulgaris* Lamarck and *Malus sieversii* (Ledeb.) (Rosales: Rosaceae) (Editorial Committee of Flora of China 1994), the main tree species in the wild fruit forests in the western Tianshan Mountains of Xinjiang, China. This region is the largest and most dense growing area in Europe and Asia. Because its wild resources are considered an important gene pool for breeding and improving fruit tree varieties globally, it plays a significant role in the protection of wild plants. However, in the last decade, the wild fruit forests have shown a severe decline, likely caused by pest attacks, human activity, and forest aging (Cui 2018), with some individuals of certain tree species dying. One of the main pests is the scale insect, which is generally harmful to *A. vulgaris* and has been found on *M. sieversii* in recent years (Zhang *et al.* 2012; Peng *et al.* 2018). This outbreak has extended into wild fruit forests in Tianshan Mountains since 2018, mainly attacking the wild fruit source *A. vulgaris* and causing many individuals to die. Therefore, a parasitoid should be a potential natural enemy agent for the biological control of this important pest.

Furthermore, its host scale is a serious pest widely distributed in Europe, North America, and Asia, including many provinces of north China. This scale is polyphagous and attacks many plants in the families Rosaceae, Rhamnaceae, Poaceae, and Aizoaceae (Wang *et al.* 2021). Currently nine species of the genus *Coccophagus* associated with *S. prunastri* in the world have been recorded (Noyes 2021): *C. differens* Yasnosh, 1966, *C. excelsus* Erdos, 1956, *C. lycimnia* (Walker, 1839), *C. palaeolecanii* Yasnosh, 1957, *C. proximus* Yasnosh, 1966, *C.*

pulchellus Westwood, 1833, *C. scutellaris* (Dalman, 1826), *C. semicircularis* (Foerster, 1841), and *C. spartanus* Japoshvili and Karaca, 2002. Of these, *C. lycimnia* and *C. scutellaris* are found in China. Consequently, a key to these species is necessary for the purpose of species identification. In addition, the phylogenetic relationships, based on the new species and all species whose sequences are available in GenBank, were investigated based on the mtDNA COI gene and the 28S rRNA gene in the present.

Material and methods

Sample collection and preservation

Branches of *A. vulgaris* infested with scale insects were collected on 27 May 2018 from Jiaolesai in Kuerdening County, Ili Kazakh Autonomous Prefecture, Xinjiang (43°14.58'N, 82°51.82'E) and placed in 4.0 × 8.5 cm glass jars and reared at 25°C and 60% humidity in the Plant Quarantine Lab 3 at Ecology and Nature Conservation Institute, Chinese Academy of Forestry (CAF), Beijing, China. The newly emerged adult parasitoids were killed directly in 75% alcohol for morphological examination (Qin 1954) and 100% alcohol for molecular studies, then stored at -20°C. As this species is weakly sclerotized, the specimens were dried with carbon dioxide at a critical point for slide mounting. From May to June 2021, we also obtained specimens of this new species from the same host attacking *M. sieversii* in the same region. All individuals are deposited in the CAF.

Taxonomic studies

All specimens were examined under a ZEISS Stemi 508 stereo microscope. The selected parasitoid specimens were card- and slide-mounted in Canada balsam for detailed examination (Platner *et al.* 1999). Images of card-mounted specimens were generated using Helicon Focus software (Helicon Soft Limited, Kharkiv, Ukraine) after generating an image stack with ZEISS Primo star fitted with a Canon DS126801 digital camera.

Specimens used for scanning electron microscopy (SEM) were first dried with a Leica EM CPD 300 critical point dryer and affixed to aluminum SEM stubs with double-sided adhesive tabs. Stub-mounted specimens were sputter-coated with gold particles from all angles to ensure complete coverage using a HITACHI E-1010. SEM images were captured using a Hitachi/S4800 desktop unit. All images were processed using PHOTOSHOP CS6.

Morphological terminology follows Gibson *et al.* (1997). Additional abbreviations used are F1-3 for funicular segments and C1-3 for clava segments. We examined multiple characteristics traditionally used to identify the species of *Coccophagus* (Howard 1895; Compere 1931), and compared this new species with two similar congeneric species, *C. lycimnia* (Walker) and *C. cowperi* Girault, based on these characteristics. The characteristics were recorded from the holotype, with the average value calculated after three measurements.

DNA extraction, amplification, and sequencing

DNA was extracted from two female specimens using the TIANamp Genomic DNA Kit (Tiangen Biotech [Beijing] Co., Ltd., Beijing, China) following the manufacturer's protocol. The COI gene, primer sequences and PCR protocols followed Folmer *et al.* (1994) and Paul *et al.* (2003), respectively. The 28S gene amplification was performed using standard three-step

polymerase chain reaction in 25- μ L reaction volumes. The protocol optimized from Yao *et al.* (2018) is as follows: initial denaturation for 5 min at 94°C, followed by 35 cycles at 94°C for 45 s, 46°C for 45 s, 72°C for 1 min, and a final extension for 5 min at 72°C; primer pair: 28S-F3633 (forward) (Rugman *et al.* 2010) and 28S-b (reverse) (Whiting *et al.* 1997). The sequencing reaction was carried out using the ABI BigDye Terminator version 3.1 cycle sequencing kit on an ABI 3730XL (SinoGenoMax, Beijing, China).

Sequence alignment and phylogenetic analysis

Sequencing quality was determined with CHROMAS 1.62 (Helensvale 2000). DNA sequence assembly was conducted using DNAMAN V6 (Lynoon 2005) and SEQMAN Pro software (Burland 2000) with default settings. The sequence information was retrieved using Sequence Manipulation Suite (Stothard 2000) from <http://www.bio-soft.net/sms/> and NCBI. The alignments were performed in MAFFT online service (<https://mafft.cbrc.jp/alignment/>), and FASTA files formatted were imported into MEGA7.0 (Kumar *et al.* 2016) for subsequent shear analysis. Phylogenetic trees were constructed containing the new sequences and all available sequences of species within *Coccophagus* from GenBank, and with *Aphelinus varipes* plus *Aphytis lepidosaphes* (Aphelinidae) as outgroups. Maximum likelihood analysis was performed using PHYML version 3.0 software (Guindon *et al.* 2010) under a GTRGAMMA substitution model and bootstrap analysis with 1000 replicates of COI and 28S rDNA sequence data. The phylogenetic trees were viewed and edited using FIGTREE v1.4.4 software (Rambaut 2018; Rachel 2006).

Taxonomy

Coccophagus tianshanensis Li & Yao **sp. nov.** (Fig. 1)

Description. Body length (including exerted ovipositor) 1.22 mm (Fig. 1A).

Color. Body dark. Eyes hairy. Antennae light brown with darker clava. Most of mesosoma dark brown, with the post two-thirds of scutellum pale lemon-yellow. Coxae dark brown with paler apex; femora yellow except brown middle part; fore and mid tibiae yellow, with the fore appearing darker than the mid tibiae; hind tibia yellow except nearly 1/3 of the upper portion, which is brown; tarsi yellow with darker tips. Wings hyaline except for the brown marginal, submarginal, and postmarginal veins; stigmal veins light brown; metasoma dark brown (Fig. 1A).

Head. Head in anterior view 0.88 \times as long as broad (Fig. 1B). The front vertex with raised reticulation; setae sparse, with two rows of pilose along the preorbital. The ocelli diameter is nearly 0.46 \times the distance between the ocelli and inner eye margin; the eyes slightly greater than the distance between the malar, while the malar space nearly 0.4 \times the head; the distance between the torulus suborbicular and the vertex 0.76 \times the front face height. The scrobe depression inverted-V-shaped, and the distance from its divergence to the vertex 0.68 \times the front face. Antennae (Fig. 1C) with the scape not reaching the anterior ocellus, oblate, and slightly broad medially with length 1.5 \times its maximum breadth; F1 nearly rectangular, 2.4 \times as long as broad, while the F2 and F3 are mostly quadrate; F2 slightly longer; F1 : F2 : F3 = 6 : 5 : 4; clava with three segments with noticeably oblique truncating; and C1 : C2 : C3 = 8 : 8 : 7.

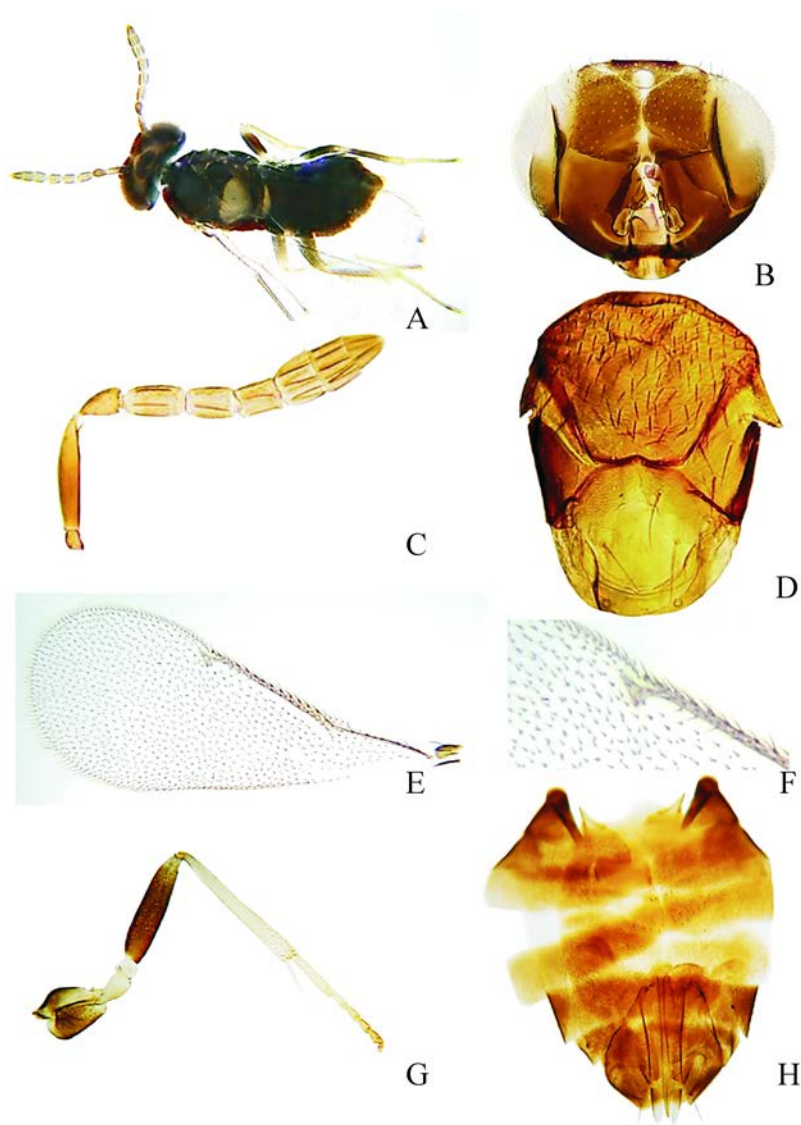


Figure 1. *Coccophagus tianshanensis* Li & Yao **sp. nov.** ♀. A. Body (killed by 75% alcohol), dorsal view; B. Head, anterior view; C. Antenna; D. Mesosoma, dorsal view; E. Forewing; F. Stigma vein; G. Mid leg; H. Metasoma and ovipositor, ventral view.

Mesosoma. Mesoscutum with raised reticulation and pilose, notauli present (Fig. 1D); both axilla sub equilateral, the distance between axillae approximately equal to the width, each of bearing two long bristles; the length ratio of mesosoma and metasoma (excluding ovipositor) 29 : 36; mesosoma 1.2× as long as broad. The length ratio of the scutellum and mesoscutum 32 : 29; scutellum with inverted acute triangle and three pairs of long bristles, the ratio of the distances between each seta row to posterior margin of scutellum 4 : 11, 1 : 2, 14 : 3, respectively. Forewing (Fig. 1E) 1.94× as long as broad, linea calva interrupted by six setae in the middle; angle between stigma and postmarginal vein less than 90°; expanded uncus

nearly as long as stigma; submarginal vein with even setae; marginal vein 6.5× as long as stigmal vein (Fig. 1F). Hind wing 3.67× as long as broad, the length ratio of submarginal, marginal, and postmarginal veins 19 : 24 : 2. All tarsi with five segments; tibia and femur delicately pilose; mid metafemur 4× as long as spur (Fig. 1G).

Metasoma. Metasoma nearly oval, reticulated, and 1.12× as long as broad; cerci originate at nearly 2/3 of final petiole. Ovipositor barely projects beyond apex of metasoma (Fig. 1H). Scaly lines on dorsal plate with slender and sparse bristles, ventral surface transverse, distinct from metasoma segment 4, and each scale with 1–4 spikes; however, metasoma segment 8 scales without spikes. Gaster segments 3–7 with a row of bristles in each segment.

Male. Unknown.

Holotype. ♀, **China**, Xinjiang, Ili: Kuerdening, Jiaolesai; 43°14.58'N, 82°51.82'E; 27-V-2018; Yanxia YAO leg., ex *S. prunastri* (Fonsc) on *A. vulgaris*. **Paratypes.** 4♀, **China**, with the same location as the holotype, 26-V-2021, Zirao LI leg., ex *S. prunastri* (Fonsc) on *M. sieversii*.

Etymology. The specific epithet is derived from the place where it was found.

Host. *S. prunastri* (Fonsc) on *A. vulgaris* and *M. sieversii*.

Remarks. This new species is morphologically similar to *C. lycimnia* and *C. cowperi* but can be distinguished by features as follows: for *C. lycimnia*, according to Howard (1895), the last half of the mesoscutellum and the center of the metanotum are bright lemon-yellow, and F2 shorter than F3 (Howard 1895; Huang *et al.* 2004), while for *C. tianshanensis*, the last two-thirds of the scutellum are bright yellow, and F2 is longer than F3; for *C. cowperi*, similar to *C. lycimnia*, the only difference is that *C. cowperi* has darker, hairier wings and paler fore and mid femora than those of *C. lycimnia* (Gahan 1927), while the primary difference with *C. tianshanensis* is still based on the mesoscutellum color and the length of F2 and F3.

Key to female species of *Coccophagus* associated with *Sphaerolecanium prunastri*

1. Scutellum with a pair of long and strong setae apically, scattered with bristles..... 2
- Scutellum with three pairs of long and strong setae apically, scattered with or without bristles 6
2. Club longer than F2 and F3 together 3
- Club shorter than F2 and F3 together..... 5
3. All coxae almost black; scape three times longer than width..... *C. proximus* Yasnosh
- Front coxae pale at least, mid and hind coxae and hind femur black; scape usually more than three times longer than width 4
4. Scutellum with apical two-fifths yellow, except for black spot at tip; marginal vein subequal in length to submarginal vein, head rather coarsely punctulate..... *C. semicircularis* (Foerster)
- Scutellum yellow except its base, and not infrequently blotched with black at tip; marginal vein plainly longer than submarginal vein, head without coarsely punctulate..... *C. scutellaris* (Dalman)
5. Scutellum entirely scattered with short bristles; antennae brown and scape dark brown, legs pale yellow except hind femora almost black *C. spartanus* Japoshvili & Karaca
- Scutellum partially scattered with bristles; antennae pale, legs pure pale yellow except the tarsi of the fore legs slightly brownish..... 7
6. Postmarginal vein longer than stigmal vein; scutellum with the apical nearly 1/5 nearly as densely setose as the mesoscutum; body 1.25 mm *C. pulchellus* Westwood
- Postmarginal vein almost absent; scutellum with basal position scattered with several short bristles; body 0.78–0.95 mm *C. excelsus* Erdos

7. Scutellum proximal 2/3 scattered with bristles except three pair of long setae, and darkness or yellow in the top *C. palaeolecanii* Yasnosh
- Scutellum without bristles except three pairs of long setae, and pure yellow or at least 1/2 yellow 8
8. Scutellum pure yellow; pronotum apically 2/3 with bristles; scape less than 4× longer than width
..... *C. differens* Yasnosh
- Scutellum part yellow; the whole plate of pronotum with bristles; scape more than 4× longer than width 9
9. F2 longer than F3; scutellum with the apical 2/3 bright yellow, and metanotum black
..... *C. tianshanensis* Li & Yao **sp. nov.**
- F2 shorter than F3; scutellum with the apical 1/2 bright lemon-yellow, and metanotum yellow in the middle *C. lycimnia* (Walker)

Molecular data

After trimming the low-quality bases from the edge, the data matrix consisted of 608 base pairs for the COI gene (GenBank: OL742795), 780 base pairs for 28S D2 + D3 (GenBank: OK094480.1) and 530 for the 28S D2 (GenBank: OL712410) gene. For COI, the average compositions of the nucleotides A, T, G and C bases were 30.8%, 41.3%, 16.1% and 11.8%, respectively. A + T content was rich and accounted for 72.2% of the nucleotides. For 28S D2 + D3, the average compositions of the nucleotides A, T, G, and C were 19.4%, 21.4%, 32.1%, and 27.1%, respectively. G + C content was rich and accounted for 59.2%. For 28S D2, A, T, G, and C bases were 20.9%, 21.1%, 26.4%, and 31.5%, respectively, and the richer G + C content was 57.9%.

In total, 15 species of the genus *Coccophagus* had 28S sequences available in GenBank. Among them, 12 species also had COI sequences. Phylogenetic analyses were therefore performed to investigate the phylogenetic relationships with our new species, the above species, and two outgroup species. As a result, a phylogram (Fig. 2) based on the 28S D2 sequence demonstrates the close evolutionary relationship among *C. tianshanensis*, *C. lycimnia*, and *C. cowperi* which clustered into a clade. Also, the phylogram (Fig. 3) based on COI sequence demonstrates the close evolutionary relationship between *C. tianshanensis* and *C. lycimnia* which cluster into a clade. Due to lack of the COI sequence of *C. cowperi*, we could not investigate the relationships among those three species based on COI gene. However, the evolutionary relationship between our species and its sister species *C. lycimnia* was supported by both of our datasets. In addition, the four species *C. semicircularis*, *C. scutellaris*, *C. tianshanensis* **sp. nov.** and *C. lycimnia*, with the same host insect *S. prunastri*, became two pairs of sister species in their respective clade, and did not cluster into a same clade.

Discussion

The new species, *C. tianshanensis* Li & Yao **sp. nov.** described in this paper was obtained from the scale *S. prunastri* attacking the wild apricot and apple trees in China. This new species is morphologically similar to the other two congeneric species, *C. lycimnia* and *C. cowperi*. However, *C. lycimnia* parasitizes many other species of Hemiptera excluding *S. prunastri* (Howard 1881, 1895), while *C. cowperi* attacks *Saissetia oleae*, *Parasaissetia nigra*, and *Coccus hesperidum* (Burks 1967). In addition, both our morphological examination and molecular analysis strongly support that it is a significantly different species.

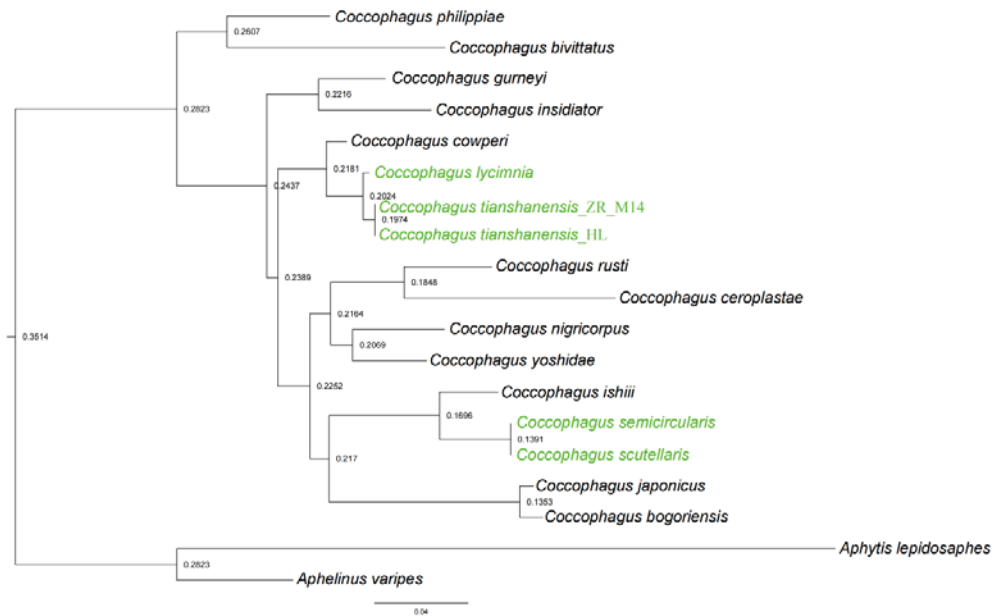


Figure 2. Maximum likelihood analysis of species of *Coccophagus* based on 28S gene with 1000 replicates bootstraps. Green represents the species associated with *Sphaerolecanium prunastri* presented in the key.

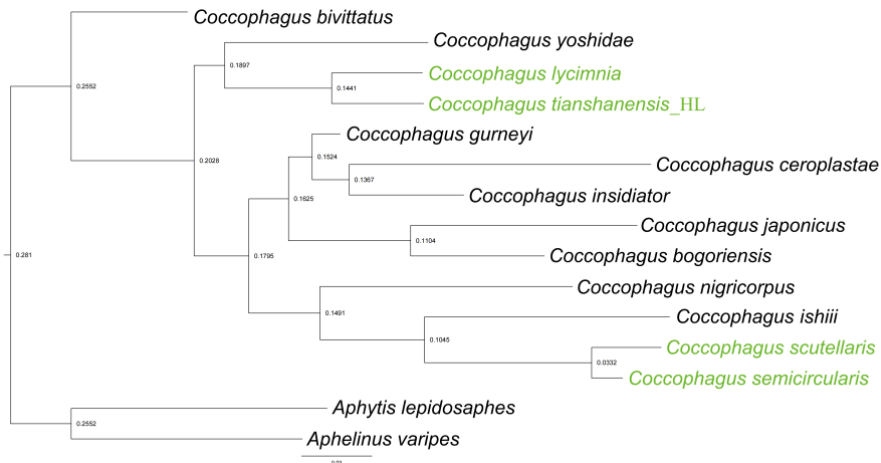


Figure 3. Maximum likelihood analysis of splices of *Coccophagus* based on COI barcode with 1000 replicated bootstraps. Green represents the species associated with *Sphaerolecanium prunastri* presented in the key.

The difficulty of species classification within the genus *Coccophagus* is typically reflected in the identification history of *C. lycimnia*. It was first discovered and reported in Northern Ireland in 1839 (Walker *et al.* 1839) but was assigned to the genus *Aphelinus* at the time. Later, it was mistakenly designated as a new species by Howard in 1881 and 1889 (Howard 1881, 1889); this was self-corrected in 1895 by Howard (1895) and corrected by Compere in 1931. Moreover, it was listed as *Platygaster lecanii* of Platygastroidea by Fitch in

1859, but was revised to *C. lycimnia* in 1929 (Mercet 1929b). Gahan (1925), Mercet (1929b), and Ferrière (1965) also contributed to the amalgamation of *C. lycimnia* synonyms. There was difficulty in deciding if *C. lycimnia* and *C. cowperi* were to be split into two separate species. In 1917, Girault first discovered and reported *C. cowperi* in Uganda (Girault 1917); however, it was misidentified as *C. lecanii*, a synonym of *C. lycimnia* by Gahan (1925, 1927) and Masi (1907) on several occasions, and was corrected by Burks in 1967. As such, *C. lycimnia* and *C. cowperi* differ only slightly morphologically, mainly according to the color (Gahan 1927). In the case of *C. tianshanensis*, the color distribution of the scutellum and postmarginal vein can clearly distinguish it from the former two species. The zigzag history of *C. lycimnia* and *C. cowperi* typically reflects the difficulty and confusion of species determination within the genus *Coccophagus*.

Such difficulty was also reflected in the key to the species of the genus which usually has branches based on only with one color character of a part of the body (Liao *et al.* 1987; Myartseva 2006). In our key, we try to provide more characters beyond body color. As a result, our new species is a sister to *C. lycimnia* based on morphology, and this relationship is also validated by our molecular phylogeny analysis in which they are sister species. In addition, our phylogeny dendrograms comprising 15 species (28S) and 12 species (COI) respectively, contain only four species which were present in the key: *C. semicircularis*, *C. scutellaris*, *C. tianshanensis* **sp. nov.**, and *C. lycimnia*. Fortunately, both dendrograms strongly support the sister relationship not only between *C. tianshanensis* **sp. nov.** and *C. lycimnia*, but also between *C. semicircularis* and *C. scutellaris*, which suggests our key is credible. Moreover, two dendrograms depicting the genetic phylogeny show that the species from the same host did not cluster into the same clade, as was found in the previous study (Zhou *et al.* 2018). This indicates the parasitoids possibly are not host specific and the evolutionary history among these parasitoids probably was outside of its host.

Owing to the significant economic importance of host plants and pest insects, the parasitoid should be considered as a potential natural enemy for the bio-control of this serious scale. Therefore, its biology and artificial propagation methods should be further explored in the future.

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