

Phylogenetic analyses of *Eucryptorrhynchus* (Coleoptera: Curculionidae) reveal a cryptic species within eurytopic *E. scrobiculatus* (Motschulsky, 1854)

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Abstract. Economically important pest species commonly contain cryptic species, which can compromise the effectiveness of pest control. Therefore, accurately identifying pest species is the first step in developing an effective pest management strategy. In this study, we examined the forestry pest *Eucryptorrhynchus* Heller, 1937 from East and Southeast Asia. Cytochrome c oxidase subunit I (COI) sequences were used to infer the first phylogenetic hypothesis for this genus and to explore its potential cryptic diversity. Morphological examinations identified three species: *Eucryptorrhynchus scrobiculatus* (Motschulsky, 1854), *E. brandti* (Harold, 1880), and *E. khasiensis* Devi, Dey & Ray, 2016. Phylogenetic analysis revealed a monophyletic *E. brandti*, a distinct lineage corresponding to *E. khasiensis*, and two distinct clades of *E. scrobiculatus* belonging to different populations (one from Ningxia and the other from Southern China, Vietnam, and Taiwan). The haplotype network revealed 45 mutation steps between Ningxia and the Southern China, Vietnam, and Taiwan populations, and genetic distance analysis revealed a 9.76% Kimura 2-parameter (K2P) distance between these two clades. This distinct divergence supports the presence of two evolutionary lineages within *E. scrobiculatus*, suggesting the presence of a pair of morphologically cryptic species. We also provide a revised identification key to *Eucryptorrhynchus*, comprising five of the six described species. These findings indicate that the biology and taxonomy of *Eucryptorrhynchus* forest pests may be understudied, negatively affecting current pest management practices. A comprehensive bionomic survey, broader taxon sampling, and robust phylogenetic analysis are needed to fill the knowledge gaps and develop sophisticated management strategies for this well-known forestry pest.

Keywords. China, Vietnam, Taiwan, *Eucryptorrhynchus khasiensis*, *Eucryptorrhynchus scrobiculatus*, forestry pest

INTRODUCTION

Integrated pest management (IPM) has developed significantly over the past few decades as an environmentally friendly management strategy (Deguine et al., 2021). IPM involves establishing species-specific control strategies based on pest bionomics, which aims to reduce pesticide usage during pest control (Ehler, 2006). Therefore, basic taxonomic and bionomic research on pests is particularly important for establishing appropriate IPM strategies (Flint, 2012). Traditional methods of species identification rely on certain morphological characters. Although distinguishing some species morphologically is difficult or impossible, they differ genetically and display different biological preferences.

Consequently, insufficient taxonomic studies may have led to an underestimation of pest diversity, thus influencing current IPM strategies and affecting downstream commodity exports. Such cases have been reported for numerous pest species, including fruit flies (Diptera: Tephritidae) (Aluja et al., 2003; Hernández-Ortiz et al., 2004), western flower thrips (Thysanoptera: Thripidae) (Rugman-Jones et al., 2010), and click beetles (Coleoptera: Elateridae) (Andrews et al., 2020). These examples illustrate how pest diversity is commonly underestimated and comprehensive taxonomic studies are necessary, even for common pests.

The tree-of-heaven, *Ailanthus altissima* (Mill.) Swingle, is one of the most widely distributed plants across all continents except Antarctica. This species is native to China and North Vietnam, and was introduced to Europe and America between the mid-18th and mid-19th centuries (Hu, 1979; Kowarik & Säumel, 2007). The tree-of-heaven is an invasive species in temperate and Mediterranean ecosystems and has quickly spread throughout Europe and America (Trifilò et al., 2003). Additionally, the tree-of-heaven has various purposes for humans; for example, its wood can be used for furniture and household items, its bark and leaves can be used in traditional Chinese medicine, and it is regarded as one of the best ornamental plants in urban areas because of its hardy nature (Hu, 1979; McAvoy et al., 2014).

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At least 46 arthropods feed on the tree-of-heaven, of which weevils of the genus *Eucryptorrhynchus* Heller, 1937 can cause the most significant damage to this plant (Ding et al., 2006b). This genus comprises six species found in East Asia, Southeast Asia, India, and Papua New Guinea (Devi et al., 2016; Pancini, 2023). Two of the described species, viz., *E. scrobiculatus* (Motschulsky, 1854) and *E. brandti* (Harold, 1880), are well-known forestry pests widespread in East Asia. Both species feed on the leaves, buds, and petioles of the tree-of-heaven, and the larvae bore into the trunk, which causes illness and death of the plant (McAvoy et al., 2014; Yang et al., 2018; Wang et al., 2019). Due to their nature, both species are considered suitable biological control agents for the invasive tree-of-heaven in America (Ding et al., 2006a, b). However, we have limited knowledge about the host tree preferences of the other four species, viz., *E. papuanus* (Heller, 1915), *E. intermedius* (Heller, 1922), *E. khasiensis* Devi, Dey & Ray, 2016, and *E. cenninii* Pancini, 2023.

The taxonomy and systematics of *Eucryptorrhynchus* are notably lacking. Heller (1937) distinguished this genus from *Cryptorrhynchus* Illiger, 1807 based on the presence of a sulcus on the dorsal margin of the eyes and distinct elytral humeri, recognising four species: *E. scrobiculatus*, *E. brandti*, *E. papuanus*, and *E. intermedius*. The taxonomy of *Eucryptorrhynchus* had remained largely unexplored for several decades until two recent studies described two additional species (Devi et al., 2016; Pancini, 2023). Nevertheless, the taxonomy and systematics of *Eucryptorrhynchus* remain considerably inadequate. For example, there is still a lack of a phylogenetic hypothesis of *Eucryptorrhynchus*, and the monophyly of described species remains untested. Additionally, Zhang et al. (2022) revealed distinct genetic structures within two forestry pest species in North China. Given the widespread distribution of these two species in East and Southeast Asia, there is a high potential for cryptic diversity within these common pests. In this study, we examined the morphology of *Eucryptorrhynchus* specimens from China, Taiwan, and Vietnam, and performed the first molecular analysis of the genus. This study aimed to investigate the phylogenetic relationships within *Eucryptorrhynchus*, test the spatial genetic structure of the two widespread forestry pests, and provide a preliminary survey of cryptic diversity within this genus.

MATERIAL AND METHODS

Species identification and specimen examination. In this study, we examined 26 specimens collected from 16 localities in Southern China, Taiwan, and Vietnam. Examined materials were deposited in the National Museum of Natural Science (NMNS), Taichung, Taiwan and the Taiwan Agricultural Research Institute (TARI), Wufeng, Taichung, Taiwan. The specimens were identified using keys and figures provided by Hong et al. (2011) and Devi et al. (2016). Morphological examinations were performed using

an SMZ 800N stereomicroscope. Prior to examination, the genitalia were removed from the abdomen and soaked in 1% KOH solution at 60°C for 10 minutes. Muscular tissues from the prothorax and mesothorax were removed and stored in 95% EtOH at -20°C. All images were obtained using a stereomicroscope with the SGviews software (Sage Vision Co., Ltd., Taiwan), combined with Helicon Focus 7.5.1. (Helicon Soft Ltd., Ukraine), and edited using Adobe Photoshop CS6 (Adobe Systems Incorporated, USA). The distribution map of *E. scrobiculatus* was produced by R package “ggplot2” (Wickham, 2016).

Laboratory procedures. We obtained sequences of the invertebrate barcode gene, cytochrome *c* oxidase subunit I (COI), from 21 specimens. Genomic DNA was extracted using a FavorPrep™ Tissue Genomic DNA Extraction Mini Kit (Favorgen, Pingtung, Taiwan). Sequences of COI locus were amplified using the universal primer-pair LCO1490-HCO2198/Maggie (Folmer et al., 1994; Hedin & Maddison, 2001). Amplifications were performed in a 25.5 µL reaction volume, including 3 µL of DNA template, 9 µL ddH₂O, 0.5 µL of each 10 µM primer, and 12.5 µL EmeraldAmp® MAX HS PCR Master Mix. The PCR conditions were as follows: initial denaturation at 94°C for 2 minutes, 35 cycles of denaturation at 94°C for 50 seconds, annealing at 54°C for 50 seconds, extension at 72°C for 50 seconds, and the final extension at 72°C for 7 minutes. PCR products were visualised by 1% agarose gel electrophoresis. The purification and sequencing of the PCR products were conducted at the National Yang Ming Chiao Tung University Cancer Progression Research Center, Taipei, Taiwan, using an ABI PRISM® 3730 sequencing system (Applied Biosystems). The sequences were translated into amino acids and checked for stop codons using Geneious 11.0.5 (Kearse et al., 2012).

Phylogenetic analyses. We included five sequences of two eurytopic species, *E. scrobiculatus* and *E. brandti*, from GenBank. In addition, three sequences of cryptorrhynchine species were included as outgroups: *Cryptorrhynchus lapathi* (Linnaeus, 1758), *Shirahoshizo juglandis* Zherikhin, 1991, and *Sybulus* sp. (accession numbers in Table S1). Sequence alignment was performed using MAFFT v.7. (Rozevicki et al., 2019). Alignment was checked for indels and stop codons using Geneious 11.0.5.

The maximum likelihood (ML) approach was implemented using RAxML v8.2.10 (Stamatakis, 2014) using the default GTRGAMMA substitution model. Node support was estimated using 1,000 bootstrap pseudo-replicates in a rapid bootstrap analysis. The topology was visualised using FigTree v1.4.4 (Rambaut, 2018), exported in the SVG format, and edited using Microsoft PowerPoint 2019 (Microsoft Corporation, USA). Genetic distances within and between species and clades were computed under the Kimura 2-parameter (K2P) substitution model in MEGA X (Kumar et al., 2018). A haplotype network was constructed using median-joining networks (Bandelt et al., 1999) in the PopART software (Leigh & Bryant, 2015) with default settings.

Table 1. The Kimura 2-parameter (K2P) distances within and between four major lineages of *Eucryptorrhynchus*.

Within species			
Population	Number	Mean	Standard error
<i>E. scrobiculatus</i> (Clade I)	15	0.0134	0.0029
<i>E. scrobiculatus</i> (Clade II)	2	0.0000	0.0000
<i>E. brandti</i>	8	0.0067	0.0021
<i>E. khasiensis</i>	1	NA	NA
Between species			
Species1	Species2	Mean	Standard error
<i>E. scrobiculatus</i> (Clade I)	<i>E. scrobiculatus</i> (Clade II)	0.0976	0.0137
<i>E. scrobiculatus</i> (Clade I)	<i>E. brandti</i>	0.1553	0.0172
<i>E. scrobiculatus</i> (Clade I)	<i>E. khasiensis</i>	0.1656	0.0192
<i>E. scrobiculatus</i> (Clade II)	<i>E. brandti</i>	0.1565	0.0182
<i>E. scrobiculatus</i> (Clade II)	<i>E. khasiensis</i>	0.1624	0.0175
<i>E. brandti</i>	<i>E. khasiensis</i>	0.1828	0.0204

RESULTS

Morphological examinations. See Figs. 1 & 2. Three species were identified in these specimens, corresponding to *E. scrobiculatus*, *E. brandti*, and *E. khasiensis*, respectively. Notably, morphological variations were observed in *E. scrobiculatus*, where the humeral calli varied from normal to distinctly protruding. Furthermore, several specimens represent the first regional records, including *E. scrobiculatus* from Taiwan and Vietnam, and *E. khasiensis* from Yunnan, China. Detailed information regarding the specimens is provided in the Taxonomy section.

Maximum likelihood analysis. See Fig. 3. The full region of COI was amplified from 17 specimens, with sequence lengths ranging from 972 to 1212 bp. The barcode region of COI was sequenced from four specimens, with sequence lengths ranging from 613 to 647 bp. The maximum likelihood topology revealed three distinct clades and a separate single-taxon lineage. Notably, the node support value for all major branches were high (rapid bootstrap values > 90), with only two exceptions having moderate support (rapid bootstrap values > 70, but < 90). The ML topology indicated that *E. brandti* is the most distant lineage in comparison to the other two *Eucryptorrhynchus* species, and *E. khasiensis* is more closely related to *E. scrobiculatus*. In terms of the eurytopic species, *E. brandti* and *E. scrobiculatus*, the ML topology revealed relatively short branch lengths among specimens with different geographical origins for *E. brandti*. In contrast, specimens of *E. scrobiculatus* were grouped into two distinct clades, each with strong node support (rapid bootstrap values of 97 for Clade I and 100 for Clade II). Specifically, Clade I contained specimens from Southern China, Vietnam, and Taiwan, whereas Clade II included specimens from Ningxia, North China. Notably, a subtle genetic structure was found

in Clade I, corresponding to specimens originating from distinct geographical populations (Southern China, Vietnam, and Taiwan).

Genetic distance. See Table 1. Genetic distance analysis revealed minimal genetic variation within species or clades among the specimens, with the highest value identified in Clade I of *E. scrobiculatus* (1.34%). In contrast, there was substantial genetic divergence between species or clades, with the smallest K2P distance observed between Clade I and Clade II of *E. scrobiculatus* (9.76%), while all other comparison pairs exhibited K2P distances exceeding 15%. Additionally, since the ML topology revealed the genetic structure of specimens from Southern China, Vietnam, and Taiwan, we calculated the genetic distance between these two geographical populations, revealing a limited interpopulation divergence (K2P) of 2.32%.

Haplotype network. See Fig. 4. Haplotype network analysis, which used only sequences from *E. scrobiculatus*, revealed three phylogeographical populations distributed in Ningxia, Southern China, Vietnam, and Taiwan. A total of 12 mutations were observed between the Southern China, Vietnam, and Taiwan populations, whereas 45 mutations were detected between the Ningxia and Southern China, Vietnam, and Taiwan populations.

TAXONOMY

Eucryptorrhynchus scrobiculatus (Motschulsky, 1854)
(Figs. 1A, B, 2A, B)

Material examined. TAIWAN: 1 female (ex. 15; accession number: PP697477) (NMNS), Wuling Farm [武陵農場],

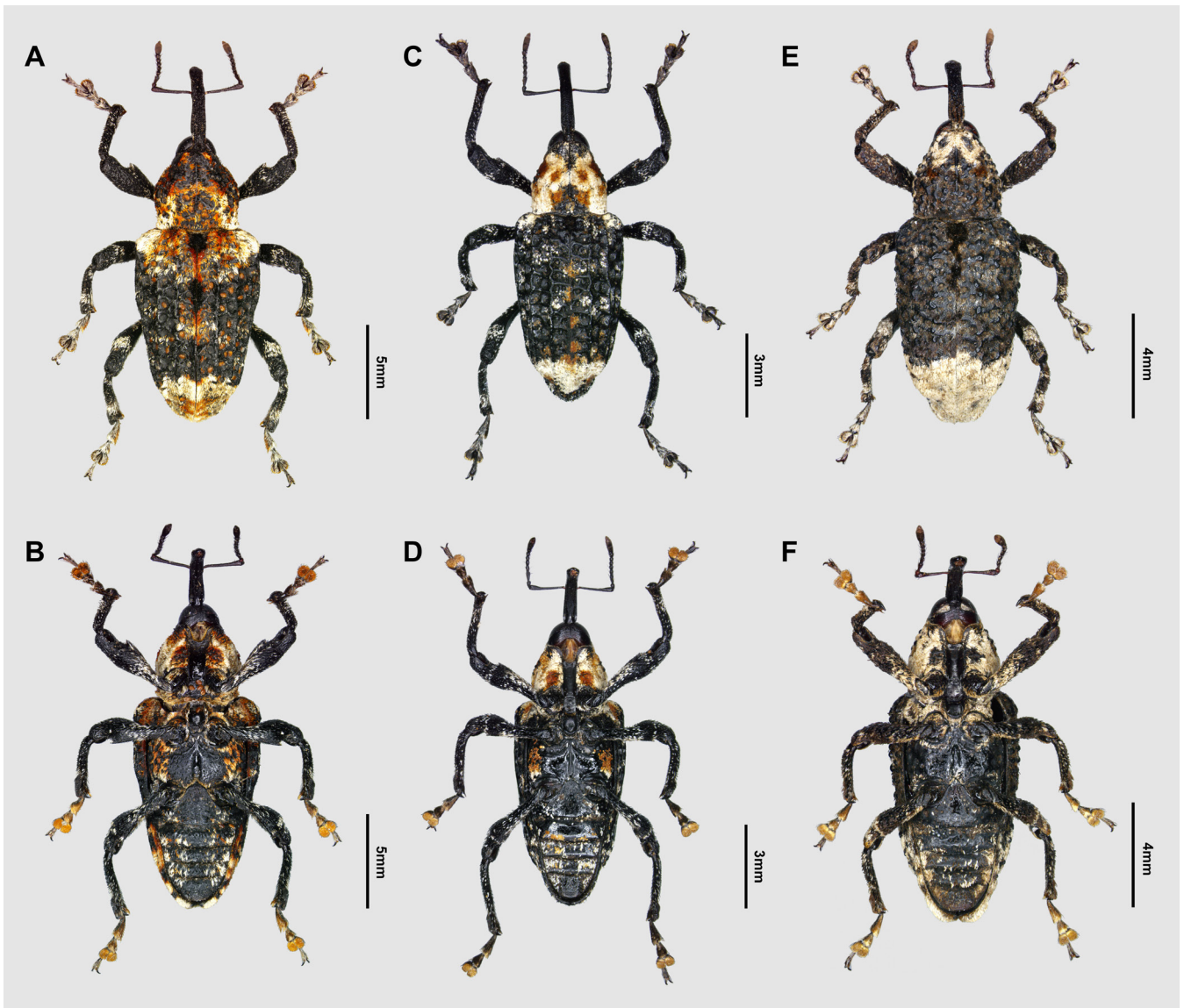


Fig. 1. Habitus of *Eucryptorrhynchus* species. *E. scrobiculatus* (Motschulsky, 1854) from Taiwan: A, dorsal and B, ventral views; *E. brandti* (Harold, 1880): C, dorsal and D, ventral views; *E. khasiensis* Devi, Dey & Ray, 2016: E, dorsal and F, ventral views.

Heping District, Taichung City, coll. W-Z Tseng, 23 July 2015; 1 male (NMNS), Mamei [馬美], Jianshih Township, Hsinchu County, N24.682502 E121.295629, coll. Y-T Lai, 11 August 2018; 1 ex. (ex. 13; accession number: PP697493) (NMNS), Newjiayang [新佳陽], Heping District, Taichung City, N24.247667 E121.228694, coll. B-H Ho, 7 June 2019; 1 ex. (ex. 14; accession number: PP697494) (NMNS), Kuanyun Mountain Villa [觀雲山莊], Xiulin Township, Hualien County, N24.1874 E121.3395, coll. B-H Ho, 7 June 2019; 3 ex. (ex. 12; accession number: PP697492) (NMNS), Sihyuan Wind Gap [思源埡口], Datung Township, Yilan County, N24.394508 E121.362836, coll. L-W Yeh, 6 August 2019. **CHINA, Guangxi:** 1 male (ex. 1; accession number: PP692124) (TARI), Yangmeiao [楊梅坳], Rongshui Miao Autonomous County, Liuzhou, coll. Y-T Wang, 4 August 2015; 1 male (ex. 3; accession number: PP697478) (TARI), Maoershan [貓儿山], Xing'an County, Guilin, coll. Y-T Wang, 17 August 2015. **Guizhou:** 1 male (ex. 4; accession number: PP697479) (TARI), Huixiangping [回香坪], Guanling Buyei and Miao Autonomous County,

Anshun, coll. Y-T Wang, 23 July 2016. **Yunnan:** 1 male (ex. 9; accession number: PP697480) (TARI), Sengang [森當], Gongshan Derung-Nu Autonomous County, Nujiang of the Lisu Autonomous Prefecture, coll. Y-T Wang, 10 June 2016; 1 female (ex. 11; accession number: PP697481) (TARI), Naqialuo [那恰洛], Gongshan Derung-Nu Autonomous County, Nujiang of the Lisu Autonomous Prefecture, coll. Y-T Wang, 12 June 2014; 4 males, 1 female (ex. 5, 6, 7, 8, 10; accession number: PP697485, PP697486, PP697484, PP697483, PP697482) (TARI), Nageluo [娜格洛], Gongshan Derung-Nu Autonomous County, Nujiang of the Lisu Autonomous Prefecture, coll. Y-T Wang, 15 June 2016. **VIETNAM:** 1 male (NMNS), Hà Giang Province, coll. local collector, June 2017; 1 ex. (ex. 2; accession number: PP697495) (NMNS), Phan Xi Păng Mountain, Tam Đường District, Lai Châu Province, N22.349444 E103.770833, coll. S. Alekseenko, 10 April 2019.

Distribution. China (Anhui, Beijing, Fujian, Gansu, Guizhou, Hebei, Henan, Hubei, Hunan, Jiangsu, Liaoning, Qinghai,

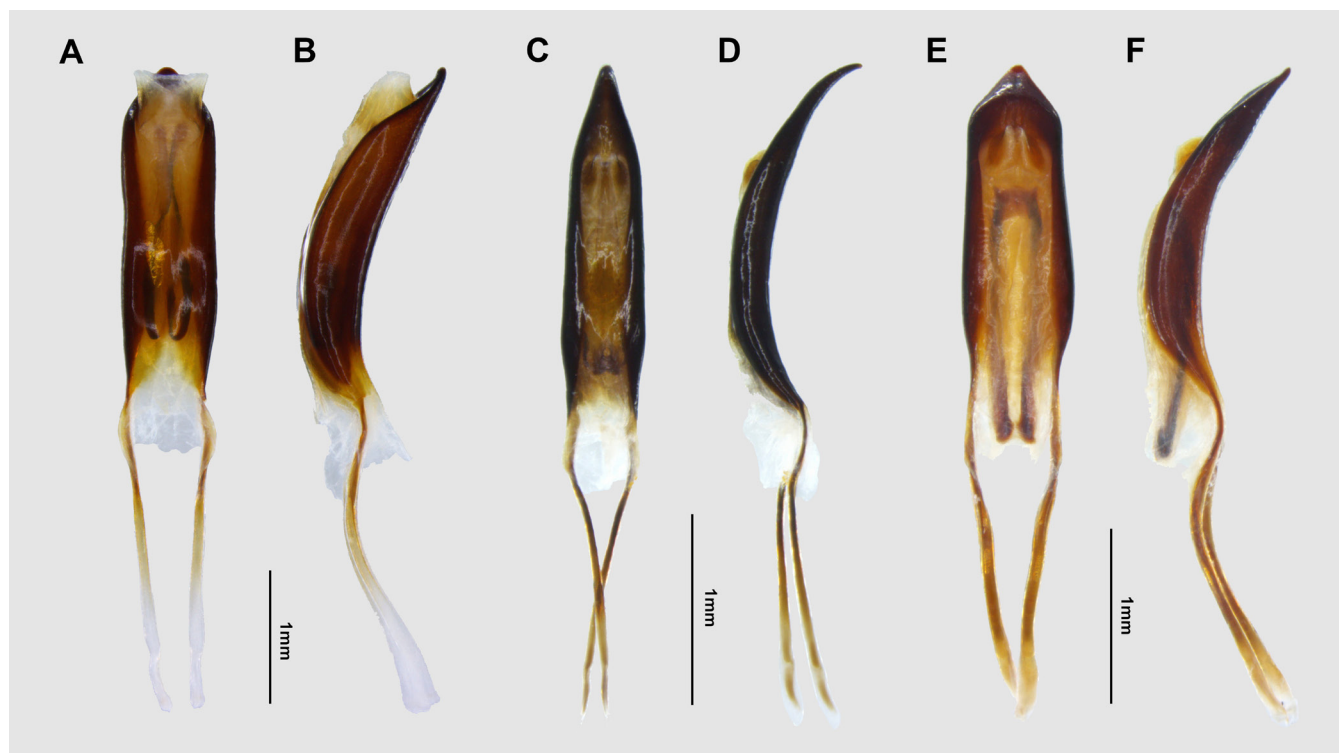


Fig. 2. Male genitalia of *Eucryptorrhynchus* species. *E. scrobiculatus* (Motschulsky, 1854) from Taiwan: A, dorsal and B, lateral views; *E. brandti* (Harold, 1880): C, dorsal and D, lateral views; *E. khasiensis* Devi, Dey & Ray, 2016: E, dorsal and F, lateral views.

Sichuan, Shaanxi, Shanghai, Shandong, Tianjin, Zhejiang), North Korea, South Korea (Alonso-Zarazaga et al., 2017).

Remarks. *E. scrobiculatus* was previously considered a junior synonym of *E. chinensis* (Olivier, 1791) (Alonso-Zarazaga & Lyal, 1999). Nevertheless, the original combination of *E. chinensis*, *Curculio chinensis* Olivier, 1791, is a junior homonym of *Curculio chinensis* Linnaeus, 1758 (= *Callosobruchus chinensis* (Linnaeus, 1758), a species within Chrysomelidae). Alonso-Zarazaga (2013) addressed this issue by assigning *E. scrobiculatus* as the valid name.

***Eucryptorrhynchus brandti* (Harold, 1880)**
(Figs. 1C, D, 2C, D)

Material examined. CHINA, Guizhou: 1 male 1 female (ex. 1, 2; accession number: PP697490, PP697489) (TARI), Huixiangping [回香坪], Guanling Buyei and Miao Autonomous County, Anshun, coll. Y-T Wang, 23 July 2016. Yunnan: 1 female (TARI), Dadi [巴迪], Diqing Tibetan Autonomous Prefecture, coll. Y-T Wang, 30 June 2014; 1 female (ex. 4; accession number: PP693364) (TARI), Wangzanka Hotel [旺咱卡酒店], Diqing Tibetan Autonomous Prefecture, coll. Y-T Wang, 8 July 2016; 2 females (ex. 3, 5; accession number: PP697487, PP697488) (TARI), Nageluo [娜格洛], Gongshan Derung-Nu Autonomous County, Nujiang of the Lisu Autonomous Prefecture, coll. Y-T Wang, 15 June 2016.

Distribution. China (Anhui, Beijing, Gansu, Hebei, Heilongjiang, Henan, Hubei, Jiangsu, Liaoning, Sichuan, Shaanxi, Shanghai, Shandong, Shanxi), Japan, North Korea,

Russia (Far East), South Korea (Alonso-Zarazaga et al., 2017). New records: China (Guizhou, Yunnan).

***Eucryptorrhynchus khasiensis* Devi, Dey & Ray, 2016**
(Figs. 1E, F, 2E, F)

Material examined. CHINA, Yunnan: 1 male (ex. 1; accession number: PP697491) (TARI), Bangda [邦达], Mang City, coll. Y-T Wang, 8 September 2015.

Distribution. India (Khasi hills) (Devi et al., 2016). New record: China (Yunnan).

Identification key. We provided a revised identification key that covers five of the six described *Eucryptorrhynchus* species. For the morphological comparisons of two species, *E. papuanus* and *E. cenninii*, we had to rely on the original descriptions due to the unavailability of specimens for examination. Additionally, we were unable to include *E. intermedius* in the key, due to the unavailability of specimens for examination and the limited information available in the original description of this species.

Key to the species of *Eucryptorrhynchus* (Heller, 1937)

1. Pronotum sparsely covered with whitish or reddish-brown vestiture, with two patches of reddish-brown, erect scales on the middle.....2
- Pronotum densely covered with whitish or reddish-brown vestiture, without erect scales.....3
2. Forehead normal; whitish and reddish-brown scales dense on pronotum, elytral base, and apical third of elytra; elytra with two patches of reddish-brown scales on the base and middle of

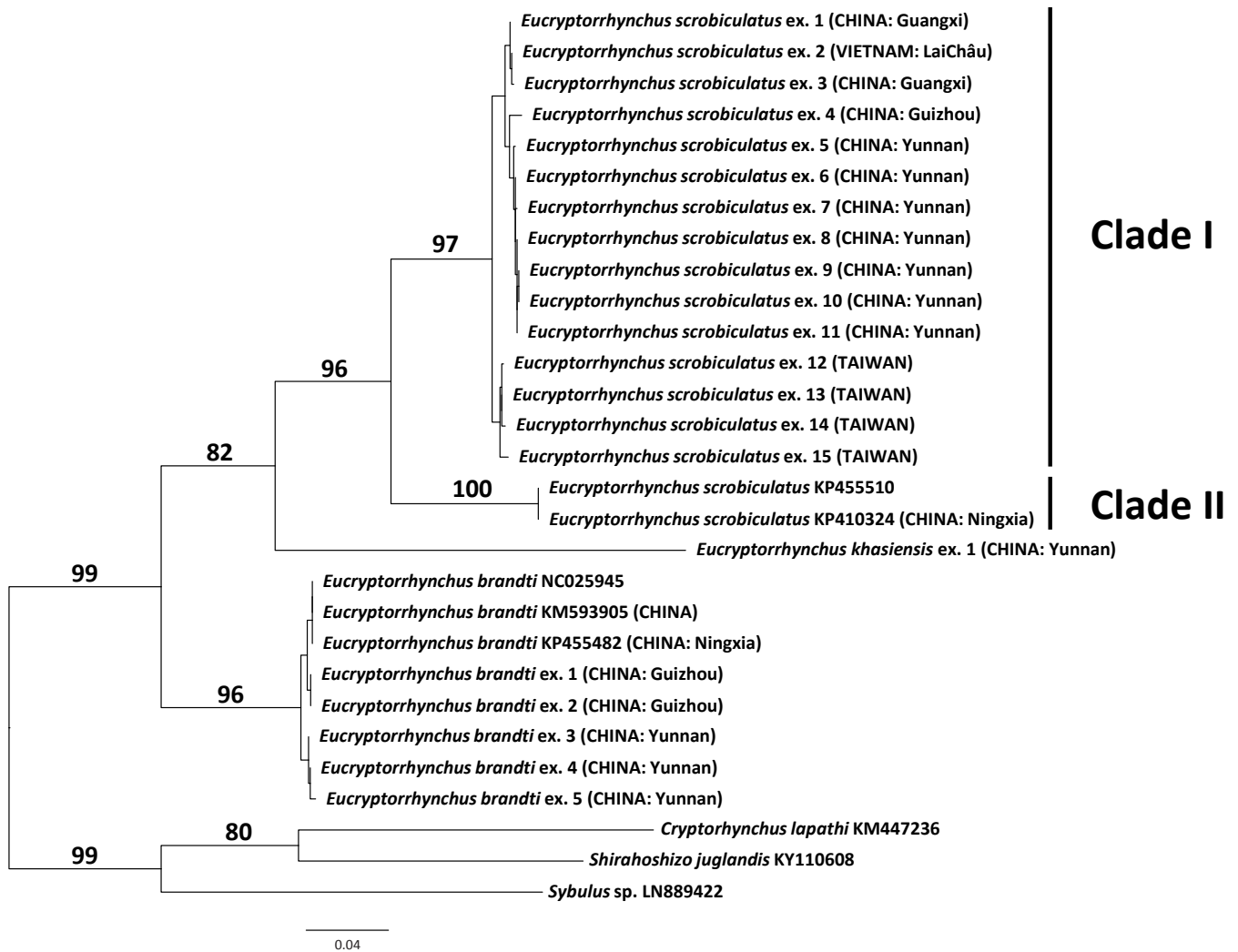


Fig. 3. A maximum likelihood COI gene tree of *Eucryptorrhynchus* from China, Taiwan, and Vietnam. The numbers above nodes indicate rapid bootstrap supports. Scale bar = expected number of substitutions per site.

- interstria I; genital sclerites cylindrical, not reaching the base of pedon.....*E. scrobiculatus* (Motschulsky, 1854)
- Forehead pointed; whitish scales dense on pronotum apex and apical third of elytra; elytra with a patch of black scales on the basal fourth of interstria I; genital sclerites cylindrical, exceeding the base of pedon.....*E. khasiensis* Devi, Dey & Ray, 2016
- 3. Pronotum with distinct tubercles on postero-basal margin; reddish-brown scales dense on pronotum and basal and apical parts of elytra*E. cenninii* Pancini, 2023
- Pronotum without distinct tubercles; whitish scales on pronotum and elytra4
- 4. Pronotum narrowed anteriorly, side bisinuate; elytra with several patches of reddish scales on interstria I; metafemora not reaching the apex of elytra.....*E. brandti* (Harold, 1880)
- Pronotum subconical, side rounded; elytra with a patch of whitish scales in the middle of interstria I; metafemora exceeding apex of elytra..... *E. papuanus* (Heller, 1915)

DISCUSSION

In this study, we used COI sequences to examine specimens of *Eucryptorrhynchus* from China, Taiwan, and Vietnam, and reconstructed a mitochondrial COI gene tree. The results reveal three well-supported clades along with one

distinct lineage. One clade corresponded to *E. brandti*, while the single-taxon lineage corresponded to *E. khasiensis*. In contrast, two clades were detected within *E. scrobiculatus*. Subsequent genetic distance and haplotype network analyses revealed substantial divergence between these two clades, supporting the existence of cryptic species. These findings demonstrate the lack of research on this genus and highlight the need for comprehensive taxonomy, life history, and bionomy research on common forestry pests.

Cryptic diversity in the common forestry pest *E. scrobiculatus*. The genetic distance threshold is a fast and widely used method for proposing preliminary species hypotheses (e.g., Agnarsson et al., 2016; Lin et al., 2022). It has been proposed that the genetic distance threshold for weevils ranges from 3% for Cryptorrhynchinae (Astrin et al., 2012) to 9.18% for Entiminae (Ma et al., 2022). Even with wide thresholds for weevils, we observed a high genetic distance between the Northern (= Clade II) and Southern (=Clade I) populations of *E. scrobiculatus* (Table 1). Furthermore, the haplotype network revealed a distinct haplotype in northern China (Fig. 4). These findings are aligned with Zhang et al. (2022), indicating that *E. scrobiculatus* exhibits a distinctive genetic structure across

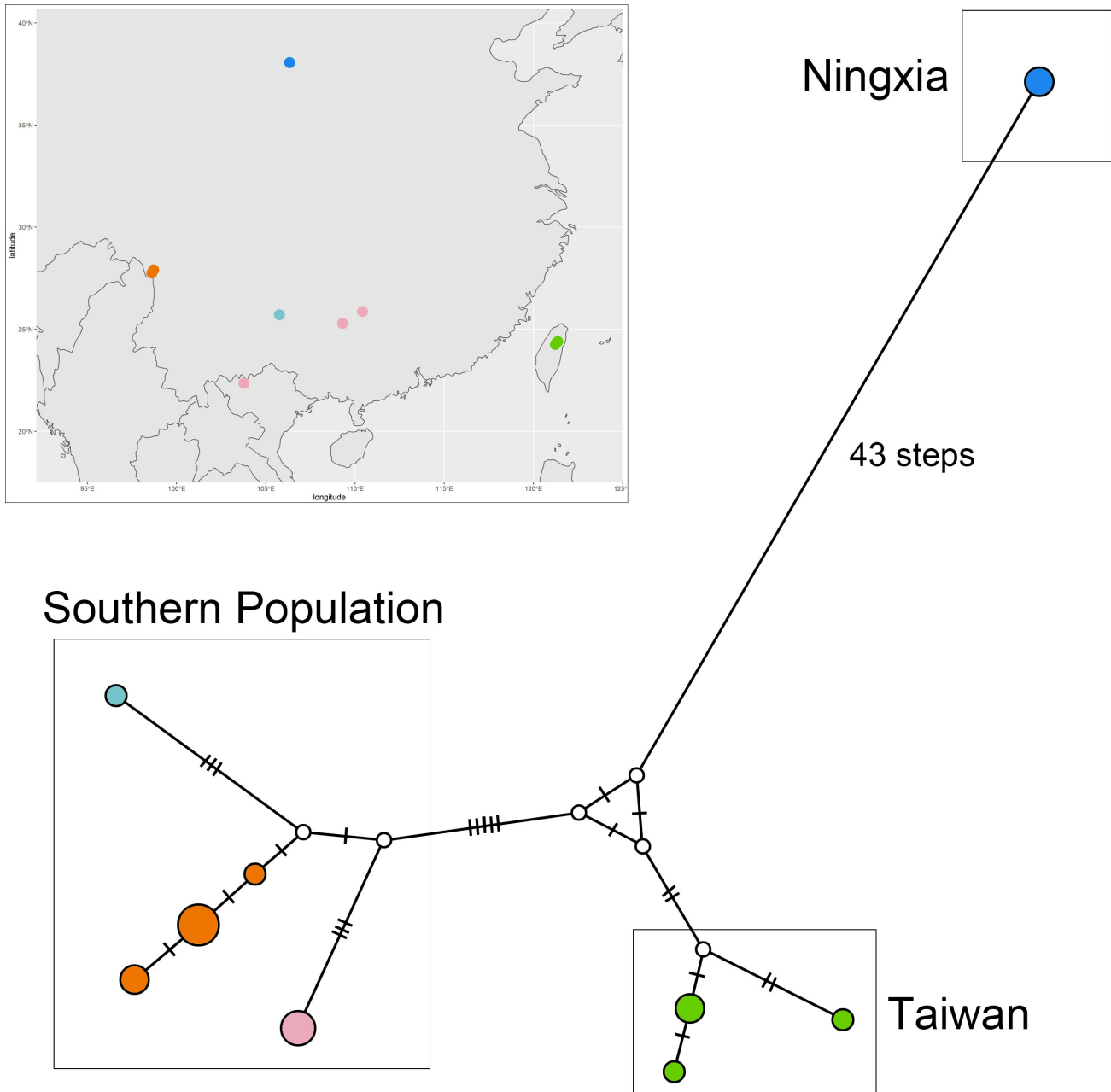


Fig. 4. Haplotype network of *Eucryptorrhynchus scrobiculatus*. Coloured circles indicate the localities; uncoloured indicate unobserved haplotypes; circle size reflects the number of haplotypes.

its distribution and supports the existence of cryptic species within this eurytopic forest pest, which warrants further investigation. Notably, the elytral humeri shape may differ between this morphologically similar species pair, with populations from Clade II (North China) likely having pointed humeri (Hong et al., 2011: fig. 105), while specimens of Clade I (Southern China, Vietnam, and Taiwan) have rounded humeri (Fig. 1A, B). However, a specimen from Guangxi, belonging to Clade II, exhibited pointed elytral humeri. As a result, we cannot conclude whether the shape of the elytral humeri serves as a diagnostic character to distinguish these two genetically divergent species. A detailed morphological examination of specimens from both clades is necessary to uncover potential morphological differences and lead to a formal species description on this cryptic species.

Impact on pest management strategies and distribution range of *E. scrobiculatus*. Traditional pest taxonomy relies on morphology; however, single-discipline methods can easily underestimate pest diversity. For example, the South American fruit fly, *Anastrepha fraterculus* (Wiedemann, 1830), is an important citrus pest that has restricted the export of Mexican citrus (Clarke & Schutze, 2014). However, Aluja et al. (2003) noted that Mexican populations do not feed on citrus and Hernández-Ortiz et al. (2004) asserted that Mexican populations deserve to be a distinct species. This phenomenon has also been observed in other groups, including several well-known pests, such as western flower thrips (Thysanoptera: Thripidae) (Rugman-Jones et al., 2010), click beetles (Coleoptera: Elateridae) (Andrews et al., 2020), and bark beetles (Coleoptera: Curculionidae) (Gomez et al., 2018; Cognato et al., 2020). These morphologically indistinct

or indistinguishable species may exhibit distinct biological and genetic characteristics, resulting in dramatic effects on downstream pest-management strategies and the export of international commodities.

Biological research and pest control strategies for *E. scrobiculatus* heavily rely on studies of the Ningxia population in North China (Yu et al., 2012; Ji et al., 2017; Yang et al., 2018; Zhang et al., 2019; Yang & Wen, 2021). It is likely that our discovery of cryptic species in this important pest will challenge current pest management strategies because of the potential biological differences between this genetically distinct species pair. For example, based on our preliminary observations, Taiwanese populations were not associated with the endemic tree-of-heaven variant *Ailanthus altissima* var. *tanakae* (Hayata) Kaneh. & Sasaki. Therefore, we cannot exclude the possibility that the heaven tree is not the primary host for the southern populations. Further bionomic studies are essential to verify the pest status and evaluate the effectiveness of current pest control strategies for this cryptic species.

The reassessment of the distribution of *E. scrobiculatus* is equally important. According to Alonso-Zarazaga et al. (2017), *E. scrobiculatus* is widely distributed in China and neighbouring countries. Our preliminary results suggest that the cryptic species identified in this study likely has a relatively southern distribution. Therefore, we propose a reassessment of the occurrence records of *E. scrobiculatus*, especially in southern Chinese provinces and Southeast Asia. The challenge of this re-evaluation may be heightened by the translocation of the tree-of-heaven, with molecular evidence suggesting human-mediated translocation occurs in *E. scrobiculatus* from North China (Zhang et al., 2022). Therefore, a comprehensive field survey, incorporating both morphology and molecular analyses, is necessary to elucidate the distributions of this significant forestry pest.

Phylogenetic relationship of genus *Eucryptorrhynchus*.

The current study presents the first phylogenetic hypothesis for *Eucryptorrhynchus* (Fig. 3). *E. brandti* is most distantly related to the monophyletic *E. khasiensis* and *E. scrobiculatus* clade. Some morphological characteristics corresponded with the phylogenetic hypothesis. For example, the pronotum of *E. khasiensis* and *E. scrobiculatus* have a pair of erect reddish-brown tuft scales, and the pedon apex is broader (Figs. 1A, E, 2A, E), suggesting both characters are synapomorphic for the two species. Additionally, we suggest a potential closer relationship between *E. brandti* and two unexamined species, *E. papuanus* and *E. cenninii*, based on the shared dense vestiture on the pronotum. However, the evaluation of this hypothesis awaits further taxon sampling. Furthermore, the phylogenetic hypothesis provides a potential explanation for the sympatric distribution of *E. scrobiculatus* and *E. brandti*. Ji et al. (2017) revealed microhabitat differentiation between these two species; however, the underlying mechanisms were not discussed. The relatively distant relationship in the current phylogeny may imply long-term evolutionary effects on the microhabitat preference of these two sympatric weevils.

Conclusions. Effective pest management strategies require accurate pest identification (Flint, 2012; Barzman et al., 2015). Therefore, the lack of taxonomic knowledge increases the potential for cryptic species, and thus influences the effectiveness of downstream pest control approaches. In recent decades, the development of molecular species identification techniques has provided practical approaches for the detection of cryptic species. In this study, we performed molecular analyses on *Eucryptorrhynchus* from East and Southeast Asia to determine the cryptic diversity of these well-known pests. These results indicate that the three East Asian species are distinct lineages, whereas a cryptic species pair was detected in the eurytopic *E. scrobiculatus* (Motschulsky, 1854). The presence of cryptic species within *E. scrobiculatus* suggests a potentially distinct biological preferences that could threaten the current IPM for this forestry pest. Furthermore, the discovery of the cryptic diversity of economically important weevils demonstrates that taxonomic research is insufficient, even for widespread pests. Therefore, further taxonomic and bionomic studies of this genus are essential, not only to explore species diversity, but also to develop effective pest management strategies for these common forestry pests.

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APPENDIX

Table S1. List of the sequences downloaded from GenBank, including the accession numbers and localities.

Species	GenBank accession number	Locality
<i>Eucryptorrhynchus scrobiculatus</i>	KP455510	China
	KP410324	Ningxia, China
<i>Eucryptorrhynchus brandti</i>	KP455482	Ningxia, China
	KM593905	China
	NC025945	unknown
<i>Cryptorrhynchus lapathi</i>	KM447236	Germany
<i>Shirahoshizo juglandis</i>	KY110608	Russia
<i>Sybulus</i> sp.	LN889442	unknown