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## Article

# Integrated Identification and Genetic Diversity of the Invasive Clearwing moths (Lepidoptera: Cossidae: Sesiinae) in Korea

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**Simple Summary:** Climate change is accelerating the introduction of exotic pests along with an increase in international trade and travellers. This study is a case of a street tree planted as an urban green space for natural landscape, shade, and pollution prevention, which became a harbour for exotic pests. As an initial response to the exotic pests, damaging the urban landscape, this study was conducted to accurately identify the species and trace the route of introduction into Korea. Based on distinct morphological features and gene-based intra- and interspecific divergence analyses, the pest group was identified as two species, *Sphecodoptera sheni* and *Paranthrenella pinoakula* sp. n.. To track their movement in Korea, haplotype analyses revealed that *S. sheni* is likely to have spread to five other neighbouring areas, mainly in the Wonju and Goyang populations, while *P. pinoakula* sp. n. could only be identified in Wonju. Furthermore, the phylogenetic tree confirmed that *P. pinoakula* sp. n. is closely divergent from congeneric species and distantly related to *S. sheni*. Accurate species identification of pests is paramount to pest management strategies, and tracking migration routes is essential research for ongoing surveillance and pre-emptive action in high-infestation areas.

**Abstract:** The populations of clearwing moth borers in Korea have recently exploited and severe damage on pin oaks (*Quercus palustris* Munchh.). We conducted the field monitoring and the molecular analyses to identify them in an integrated manner. Morphological examination and molecular analyses on COI-gene based intra- and inter-specific genetic divergences (GD) were revealed that the borers were recognized as two invasive species, *Sphecodoptera sheni* and *Paranthrenella pinoakula* sp. nov.. The maximum intraspecific GD was found to be 1.9%, whereas the minimum interspecific GD was confirmed as 8.1%, with a distinct barcoding gap. Both MJ network and NJ tree were also showed that 18 haplotypes (HAPs) were detected from the 52 COI sequences. The borers were revealed total 17 HAPs: i) H1-H7 were detected from all seven regions with *S. sheni*; ii) Wonju and Goyang populations of *S. sheni* were revealed as more than three HAPs; iii) H7 was closely connected with H8 of Chinese population of *S. sheni*; iv) H9-H10 were detected as the other samples from Wonju population with *P. pinoakula* sp. n., and closely located with congeneric species. Maximum likelihood tree also revealed that *P. pinoacula* sp. n. nested within the congeneric species by genetically separating from the *S. sheni*.

**Keywords:** invasive insect pests; regional populations; genetic divergence; COI; street insect pests

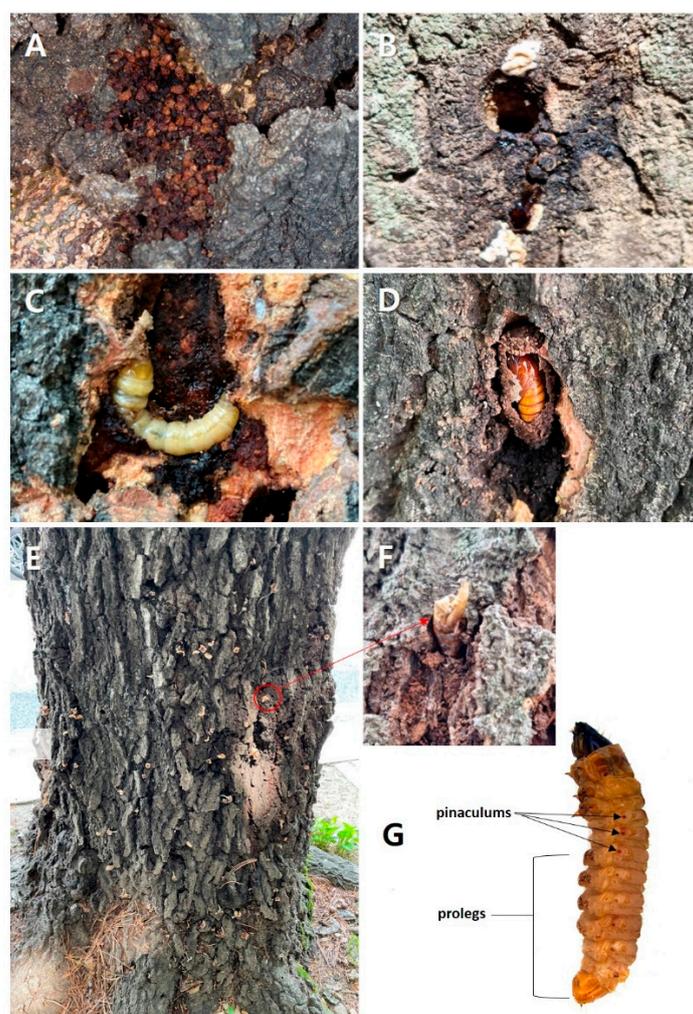
## 1. Introduction

Climate change presents a global threat to humanity [1,2]. Certain human activities, such as the expansion of worldwide trade and the increase in domestic and international travel, have led to unanticipated issues, such as the invasive species. Since 1900, 89 different invasive species have appeared in Korea, with 33 of them, or 38% of the total, being introduced in the last 19 years [3]. Moreover, extreme weather phenomena are not only spreading exotic pests but also triggering outbreaks of native insect pests in human habitats and forests. While the ecological function of urban street trees is providing natural scents and shade that act as a carbon sink in the urban areas, they also serve a special habitat for invasive forest pests.

Pin oak, *Quercus palustris* Munchh. (Fagaceae) was one of the most popular varieties to be planted as a domesticated street tree since the early 2000s in Korea (Figure 1). However, around 2014, signs of clearwing borer damage began to be reported from street trees of pin oaks in Korea. Our research team has been conducting nationwide monitoring for the past two years. Multiple pin oak populations planted as street trees show significant pest damage signs throughout Korea. The congruent symptoms were caused by the larvae, which bore holes in the xylem, crawled inside the tunnels to feed, and oozing milky sap with coarse reddish-brown excrement on the bark of pin oaks. The larvae attack the cambium layer of the xylem structure and create a pupal frame under barks. This leads to stunted tree growth and can cause decline. In addition, emerged adult moths leave conspicuous marks including pupal exuviae and holes on the bark that disturb the urban landscape. (Figure 2A-F). These larvae are often confused with beetle larvae due to the similarities on perforating feeding habit, large body size and milky coloration. However, the number of prolegs, development of pinaculums and other characteristics allow them to be classified as clearwing moth larvae (Figure 2G).



**Figure 1.** Panoramic view of a pin oak (*Quercus palustris* Munchh.) street trees. (A) Daegu metropolitan city; (B), Ilsan-gu, Goyang-si, Prov. Gyeonggi.



**Figure 2.** diverse damage on pin oak street trees by clearwing moths. (A) reddish-brown frass by larva; (B) milky sap damage from cavity in xylem made by larva; (C) xylem cambium fed by larva; (D) pupal frame in cambium; (E) left distinctive marks on the bark after emergence; (F) zoom in exuviae after larva emergence; (G) clearwing moth larva.

The clearwing moth family (Lepidoptera: Cossidae: Sesiinae) is a group of medium to large-sized that resemble adult bees or wasps, with 1,562 species and subspecies recorded worldwide [4], including many agricultural (e.g. *Nokona pernix*, *N. purpurea*, *N. regalis*, *Synanthedon bicingulata*, *S. haitangvora* etc.) and forestry pests (e.g. *Scasiba rhynchioides*, *Synanthedon quercus* etc.).

The study has two objectives. Firstly, we monitor and identify the insect pest species attacking pin oak street trees based on morphological and molecular analyses in Korea. Secondly, we intended the prediction of domestic routes of migration within Korea. To achieve the research objectives, we analyzed genetic diversity and haplotypes, and constructed a neighbor joining (NJ) tree in addition to planting history. Furthermore, phylogenetic analysis was used to infer the relationships between the insect pest groups.

## 2. Materials and Methods

### 2.1. Sampling

From 2022 to 2023, 41 individuals were collected by hands using pruning shears in seven regions (Bucheon, Goyang and Seongnam of Province Gyeonggi (GG); Chuncheon and Wonju of Prov. Gangwon (GW); Cheongju of Prov. Chungcheongbuk (CB); Daegu Metropolitan city and Gwangju Metropolitan city), planted with domestic pin oak street trees in Korea. Among the areas, the trees in

Goyang and Wonju were planted in early 2000s, while the others were planted within 5 years. In Gwangju Metropolitan city, we have only confirmed the insect pest damage, but no individuals were caught. Since they were in larval or pupal stages, they were reared in the falcon tube (200ml) individually to adults maintaining at 26-27°C, 12:12 (L:D), 60% humidity in the laboratory. For morphological identification, all individuals were examined their external characters and photographed under Leica Z16 APO stereomicroscope and Dhyana 400DC (4M) sCMOS camera (TUCSEN) with Mosaic Analysis Software. Genital slide vouchers were made according to Kim et al. [5]. All specimens and slide vouchers were deposited in the laboratory of Insect Phylogenetics and Evolution, Jeonbuk National University (IPE JBNU), Korea.

## 2.2. Molecular protocol

Total 52 sequences were included for molecular protocol. Of them, 50 sequences were used as ingroup: i) 9 sequences of 6 species (*Paranthrenella auriplena*, *P. cinnamoma*, *P. chrysophanes*, *P. formosicola*, *P. terminalia* and *Sphecodoptera sheni*) from BOLD public data (ANICF612-10, ANICF613-10, ANICF615-10, EPNG8497-14, EPNG8498-14, GBMNE5028-21, GBMNE61298-22, GSCMB812-12, LTOLB087-08); ii) 41 sequences of 2 species (*Sphecodoptera sheni* and *Paranthrenella pinoakula* **sp. n.**) from field survey. Two sequences of one species (*Anatrachyntis japonica*: Cosmopterigidae) used as out group.

DNA was extracted from the head or leg of each specimen using Tissue Genomic DNA Isolation Kit Mini in LaboPass™ DNA Purification Kit (Cosmo Genetech Co. Ltd., Republic of Korea), following the manufacturer's protocol. For PCR amplification for mitochondrial cytochrome oxidase subunit I (653 bp), the universal primer set, LCO 1490 (5'-GGTCAACAAATCATCATAAAGATATTGG-3') and HCO 2198 (5'-GCTACAACATAATAAGTATCATG-3') [6], was used in the following condition: initial denaturation at 94°C for 5 minutes, followed by 34 cycles of denaturation at 95°C for 1 minutes, annealing at 45.2°C for 1 minutes, extension at 72°C for 1 minutes, and a final extension at 72°C for 5 minutes. Amplification was performed using AccuPower PCR Premix (Bioneer, Daejeon, Republic of Korea) following the standard protocols. The PCR products were checked in 1.2% agarose gels, purified and sequenced at Macrogen, Inc. (Geumcheon-Gu, Seoul, Republic of Korea).

## 2.3. Data analysis

Intra-and inter-specific pairwise genetic distance and Neighbor joining (NJ) tree were conducted by using MEGA 7.0 under Kimura 2-parameter model [7]. To know evolutionary relationships among haplotypes of the sesiid species, the median joining (MJ) network was analyzed in Network ver. 10.2.0.0. A phylogenetic tree was constructed based on the haplotypes of Sesiinae detected in this study. Maximum likelihood (ML) tree was implemented by IQ-Tree 2.1.1 [8] with best-fit model, GTR+F+G4, according to the Bayesian information criterion [9,10]. Branch support values were produced by ultrafast bootstrap pseudoreplicates (UFB) [11] and a Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) [12]. Every test was with each 100,000 pseudoreplicates for UFB and SH-aLRT.

## 3. Results

### 3.1. Morphological identification

Two sesiid borers were identified as two species, *Sphecodoptera sheni* and *Paranthrenella pinoakula* **sp. nov.**, based on adult and genitalic characteristics.

#### 3.1.1. Taxonomic accounts

##### **Genus *Sphecodoptera* Hampson, [1893]**

*Sphecodoptera* Hampson, [1893]: 189. Type species. *Sphecia repanda* Walker

*Sphecia* Hampson, 1919: 80.

*Sesia Spatenka, Lastuvka, Gorbunov, Tosevski & Arita, 1993: 87*

*Spherodoptera Matsumura, 1931: 1017.*

*Scasiba Matsumura, 1931: 8.*

***Sphecodoptera sheni* (Arita & Xu, 1994)**

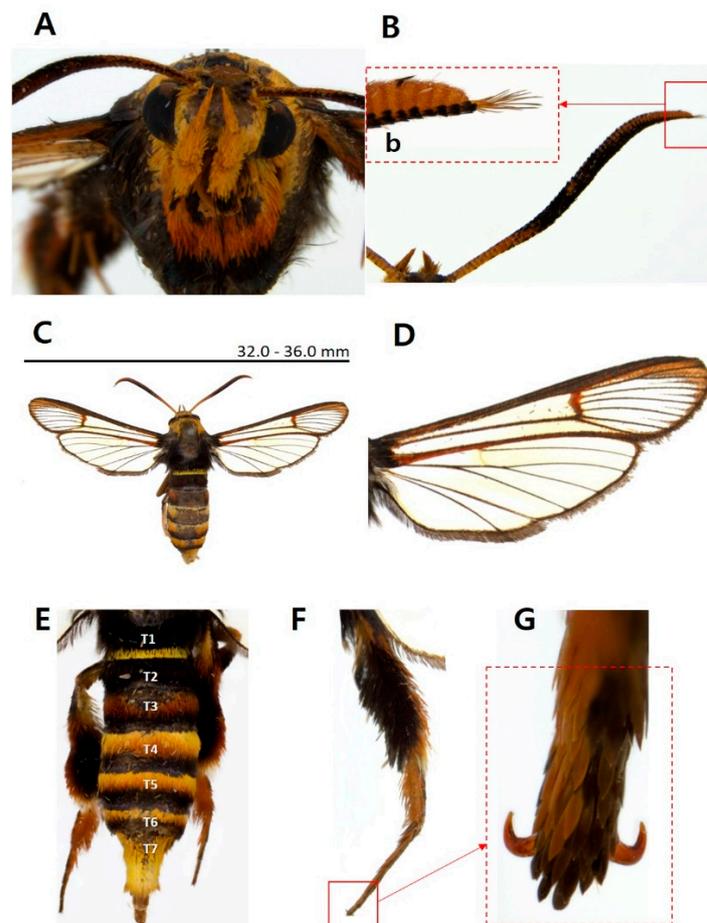
*Sesia sheni* Arita & Xu, 1994: 61. Type locality. Nanjing, Jiangsu, China

*Scasiba caryavora* Xu & Arita, 1994: 2.

*Sphecodoptera sheni* Kallies, Arita, Owada & Wang, 2014: 587.

*Material examined.* Korea. GW: 2♀, Wonju, Moosil-dong 1710, 8.VII.2020, J-K. Jung; 2♂, 1♀, ditto, 12.VII.2022, J-K Jung, gen. slide no. IPE04, IPE05/S.Kim; 5exs, Chuncheon, Woodoo-dong 1121, 12.V.2023, B. Lee. GG: 9 exs, Seongnam, Bungdang-gu, Sampyong-dong, 15.VII.2022, JK Jung; 5, Goyang, Ilsan, Juyeob-dong 152, 6.V.2023, B. Lee; 6, Bucheon, Yeokgok-dong, 3. VI. 2023, B. Lee. CB, 2, Chungju, Heungdeok-gu, 28. IV.2023, S. Kim. Daegu, 6, Jung-gu, Gukchaebosang-ro 139gil 1, 8-12.IV.2023. I. Park.

*Diagnosis.* This species is superficially close to *Scasiba rhynchioides* in having yellowish scales on thorax, but it can be easily recognized by the 1<sup>st</sup> tergite of abdomen black with a narrow and yellow posterior band and the 2<sup>nd</sup> and 3<sup>rd</sup> tergites dark brown with reddish brown centrally (Figure 3E). The male genitalia of *S. sheni* is also differentiated from *S. rhynchioides* by a finger-like processus on the saccular margin antemedially (Figure 5E). Female genitalia are also distinguished from that by the long and narrow ovate corpus bursae bearing distinct signum (Figure 5G-H).



**Figure 3.** Adult of *Sphecodoptera sheni*. (A) Head; (B) flagellum of antenna; (Ba) apical part of flagellum; (C) entire view; (D) wings; (E) abdomen; (F) hind leg; (G) tarsal claw of hind tarsus; scale bar indicates wingspan.

*Host Plants.* *Carya illinoensis* (Wang.) K. Koch (Juglandaceae); *Quercus suber* Linnaeus, *Q. variabilis* Blume, *Q. acutissima* Carruthers and *Castanea mollissima* Blume (Fagaceae) [13,14]; *Quercus palustris* Münchh. (this study).

*Distribution.* Korea (GG, GW, CB, Daegu) (this study), China (Jiangsu) [14].

*Remark.* Of the pin oak street borers, the large-sized sesiids were identified as *S. sheni* in this study. This species was found in Korea for the first time since it was reported from China. According to Arita et al. [14], this species has been reported as a pest of North American native pecan trees planted as street trees in Nanjing, China. Based on investigation of number of pupal exuviae, *S. sheni* was higher than other sessid borer density and their distribution on tree was aggregated between ground to 1m in heights.

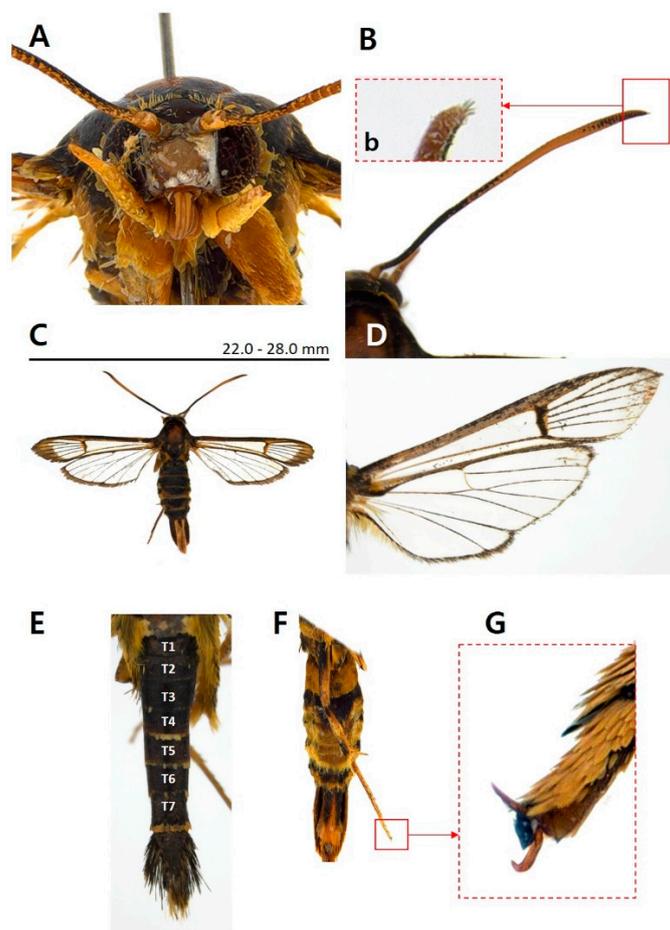
### Genus *Paranthrenella* Strand, 1916

*Paranthrenella* Strand, 1916: 47. Type Species. *Paranthrenella formosicola* Strand

#### *Paranthrenella pinoakula* sp. nov. Kim

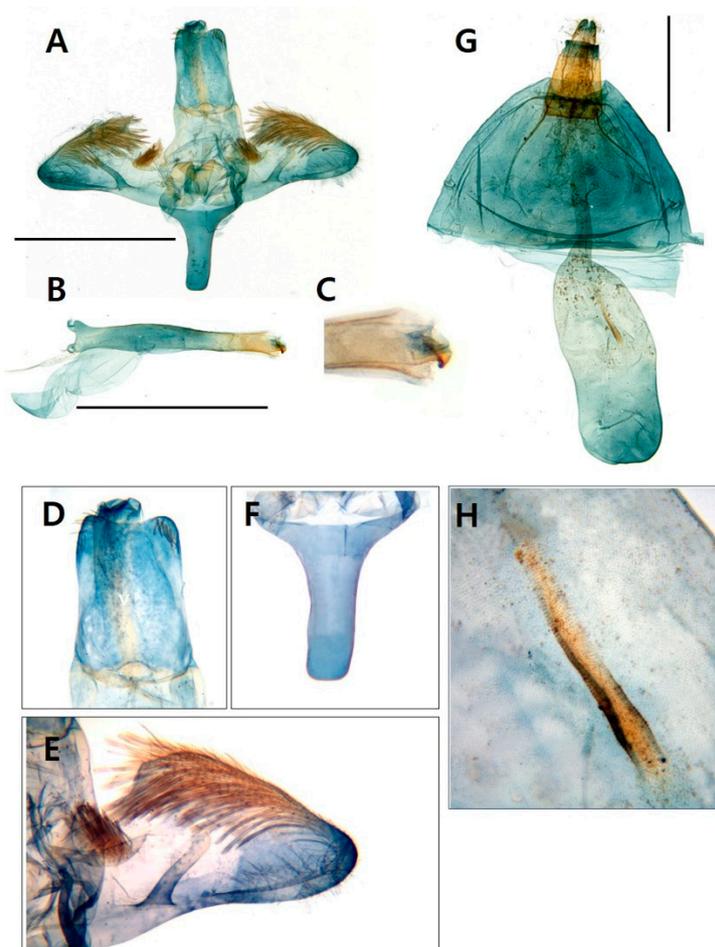
*Type Material.* Holotype: One male, Korea, GW, Wonju, Moosil-dong 1710, 2.VII.2022, JK Jung, gen. slide no. IPE03/ S. Kim; Paratypes: two males, ditto, 8.VII.2020, JK Jung.

*Diagnosis.* This species is externally similar to *Paranthrenella formosicola* in having a long and slender abdomen (Figure 4E). However, it can be readily distinguished from the former due to the lesser development of yellow scales between the thorax and tegula (Figure 4C), as well as the abdomen dorsally (Figure 4E). Except for the 4th and 7th tergite, the presence of yellow scales on the posterior margin of each tergite is not noticeable. In contrast, *P. formosicola* has thickly developed yellow markings on the posterior margin of the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> tergites in male and almost entire tergites in female. The male genitalia is also differentiated from that by the asymmetrical valva including a ridge line and aedeagus bearing four small cornuti at apex.



**Figure 4.** Adult of *Paranthrenella pinoakula* sp. nov. (A) Head; (B) flagellum of antenna; (Ba) apical part of flagellum; (C) entire view; (D) wings; (E) abdomen; (F) hind leg; (G) tarsal claw of hind tarsus; scale bar indicates wingspan.

*Description.* Male (Figure 4). Wingspan. 22.0-28.0 mm; forewing length 10.0-12.5 mm; body length 17.0-19.5 mm. Head: frons pale brown; vertex dark brown; occipital fringe yellow; scape of antenna yellow entirely, shorter than 1/2 diameter of eye; flagellum blackish brown antemedial and apical parts, tinged with yellow postmedial part dorsally, short setose at apex, yellow ventrally; labial palpus entirely yellow; 3<sup>rd</sup> segment of labial palpus 2/3 length of 2<sup>nd</sup> segment. Thorax: patagium blackish brown, mixed with pale brown laterally; tegula blackish brown with yellow scales on posterior margin; mesothorax blackish brown with two spots of yellow scales on posterior margin; metathorax blackish brown. Forewing hyaline; costal margin blackish brown; dorsal margin yellowish brown; wing base blackish brown tinged with bright yellow; bright yellow between radius vein 2 (R2) and R3; cilia yellowish brown; forewing venation: radius 4 (R4) stalked with R5 anteromedially; anterior transparent area (ATA) extended to 3/4 of forewing; posterior transparent area (PTA) extended to 4/5 of forewing; apical 1/3 exterior transparent area tinged with yellowish brown scales. Abdomen blackish brown with yellow scales noticeably at posterior margin of tergite 4 (T4) and T7.



**Figure 5.** Genitalia of *Sphecodoptera sheni*. (A) Male genitalia; (B) aedeagus; (C) apical part of aedeagus including cornutus; (D) uncus; (E) a finger-like process on the saccular margin of valva; (F) saccus; (G) Female genitalia; (H) signum in corpus bursae; scale bar: 0.25 mm.

Male genitalia (Figure 6). Tegumen-uncus complex broad; scopula androconialis well-developed, slightly shorter than the length of tegumen-uncus complex; crista gnathi lateralis large, a

cup-shaped; crista gnathi medialis relatively narrow, oval. Valvae elongated, oval, asymmetrical; right valva with asymmetric ridge line from basal crista sacculi to apex, rather dense setose followed the ridge from base to 1/3 of valva; left valva without ridge. Saccus with bifurcate terminal margin, a reverse blunt fork-shaped, as long as vinculum. Aedeagus as long as valva, gradually narrow from base to apex; vesica curved at sub-apex; bearing four cornuti.

Female genitalia. Unknown.

*Host Plants.* *Quercus palustris* Münchh. (this study).

*Distribution.* Korea (GW) (this study).

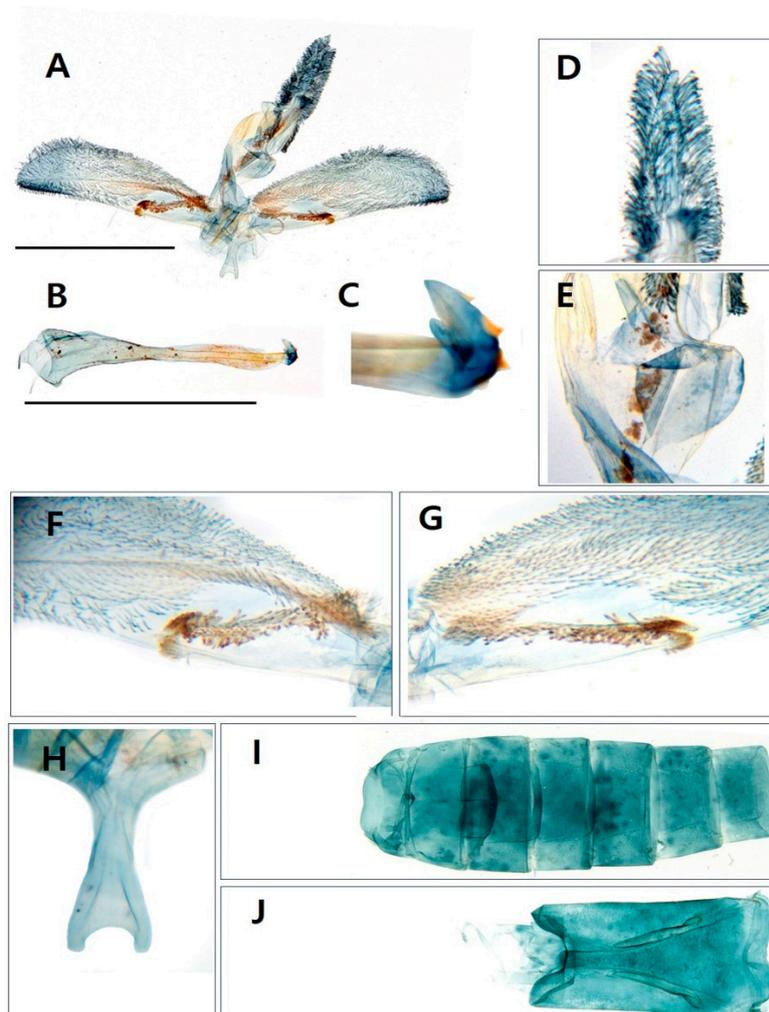
*Etymology.* The species name is derived from a common name of the host plant (*Quercus palustris*), *pinoak-* plus a Latin diminutive suffix, *-ula*, referring to the insect pest borer of the pin-oak.

*Remark.* In the pin oak street insect pests, the small-sized borers were identified as new species, *P. pinoakula* sp. n., in this study. This species was only found from WonJu (GW) in Korea, and their densities were relatively lower compared to *S. sheni*.

### 3.2. Molecular analyses

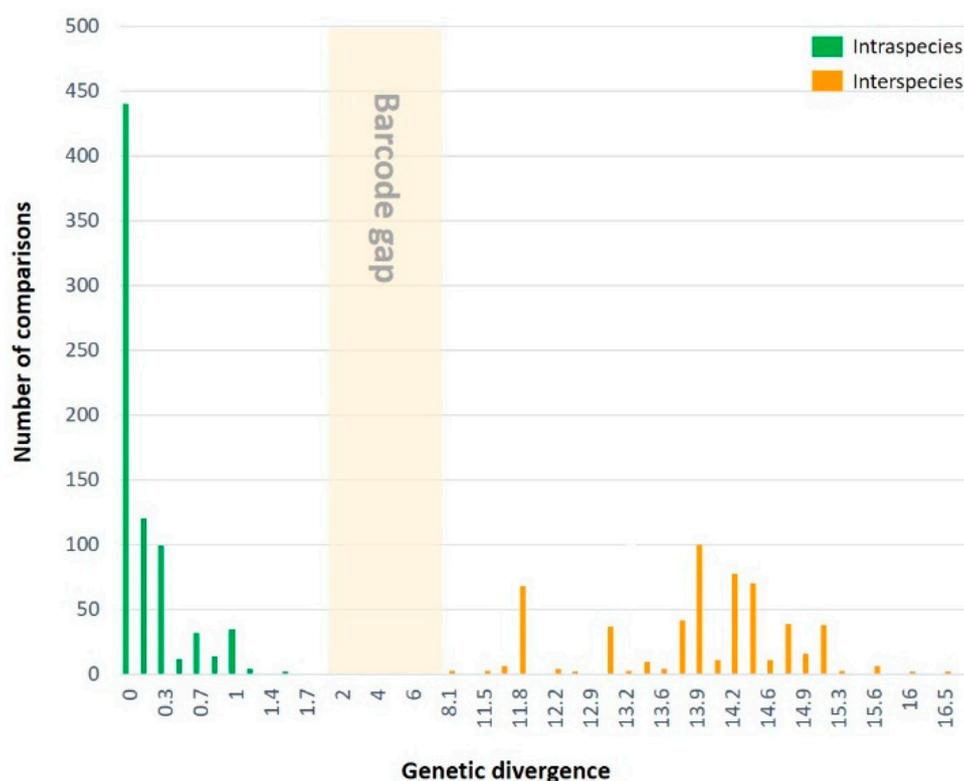
#### 3.2.1. Genetic divergence and distribution

Genetic divergences within all species included in this study are presented in Table 1. Except for four species (*Paranthrenella auriplena*, *P. cinnamoma*, *P. formosicola* and *P. terminalis*) which having only one sequence, a total of 647 comparison pairs of four species (*S. sheni*, *P. pinoakula* sp. n., *P. chrysophanes* and *Anatrachyntis japonica*) were calculated within the species. The intraspecific genetic distance (GD) averaged 0.3% and the maximum intraspecific divergence did not exceeding 1.9%. These of *S. sheni* and *P. pinoakula* sp. n. were 1.7% and 0.3% respectively.



**Figure 6.** Genitalia of *Paranthrenella pinoakula* sp. nov. (A) Male genitalia; (B) aedeagus; (C) apical part of aedeagus including cornute; (D) scopula androconialis; (E) medial gnathos flap; (F) right valva with a ridge line; (G) right valva; (H) saccus; (I) sternum I-VII segments; (J) sternum VIII segmenbbt; scale bar: 0.25 mm.

The highest value of intraspecific GD was found in *P. chrysophanes* with 1.9%. The interspecies GD was analyzed with 564 comparison pairs. The minimum interspecific GD among all seven species was calculated to be 8.1% between *P. auriplena* and *P. chrysophanes*, while the maximum interspecific genetic value was 16.8% (averaging 13.8%) between *P. terminalis* and *P. chrysophanes*. The distribution of their genetic divergence is shown in Figure 7. All species analyzed in this study exhibited a distinct barcode gap between the maximum of intraspecific GD and the minimum of interspecific GD in the COI gene.



**Figure 7.** Distribution of genetic divergences based on the Kimura-2 parameter model for COI sequences according to taxonomic levels. 647 comparison pairs within the species and 564 comparison pairs between the species were calculated.

### 3.2.2. NJ Tree analysis

NJ tree analysis, a heuristic method based on genetic distance, showed that *S. sheni* and *P. pinoakula* sp. n., branched into clade A and B, respectively (Figure 8). In clade A, Chinese *S. sheni* individual was the first to branch out and followed by the Wonju (GW) population. And then, each population of Goyang (GG) and Chuncheon (GW) were branched off, followed by the populations of Bucheon (GG) and Cheongju (CB), and all diverse populations were claded together. All individuals of *P. pinoakula* sp. n. were nested within the congeneric species in clade B. Of them, the *P. pinoakula* sp. n. was also genetically close to *P. formosicola* which was the allied species having similar morphology.



**Figure 8.** Neighbor joining tree of COI gene of Sesiid species. Numbers under the branches are bootstrap percentages (%).

**Table 1.** Genetic divergences within all species included in this study.

1. Different taxonomic levels				
Taxonomic level (No. of Comparison Pairs)	K2P Pairwise distances			
	Maximum	Minimum	Average	
Within species (647)	1.9	0.0	0.3	
Between species (564)	16.8	8.1	13.8	
2. Intraspecific genetic divergence				
Species	Comparison pairs (CP)	Intraspecific genetic divergence		
		Maximum	Minimum	Average
<i>Sphecodoptera sheni</i>	637	1.7	0.0	0.2

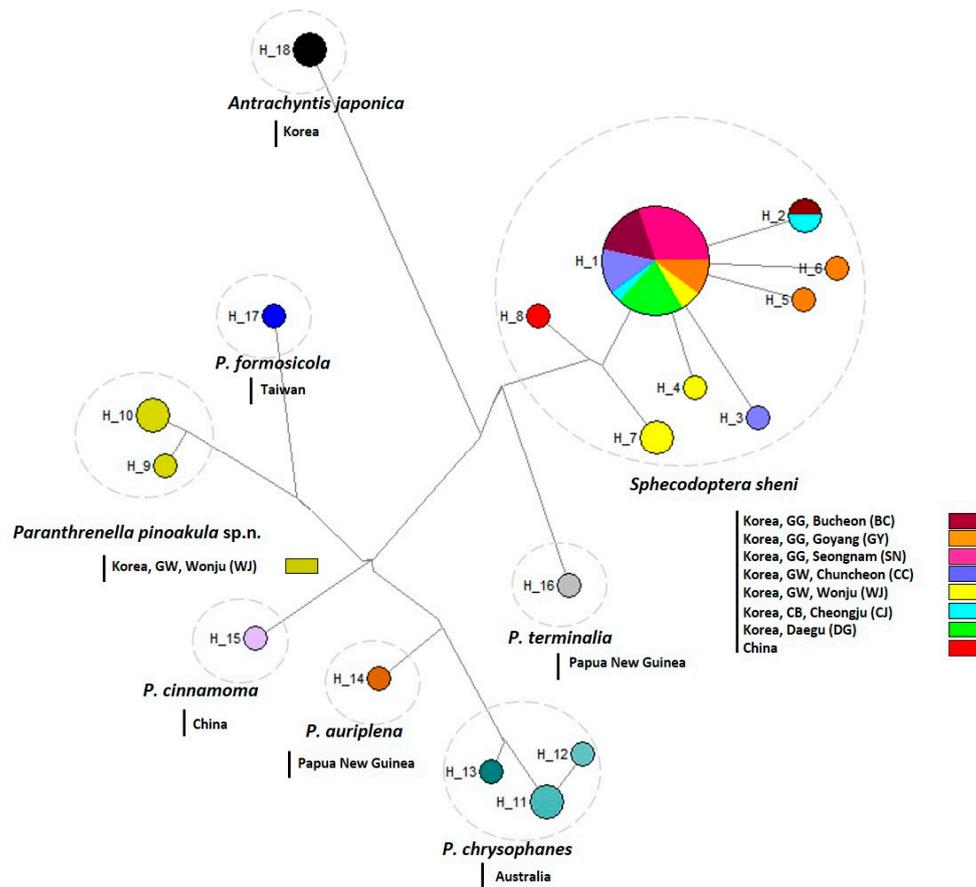
<i>Paranthrenella pinoakula</i> sp.n.	3	0.3	0.0	0.2
<i>Paranthrenella auriplena</i>	-	-	-	-
<i>Paranthrenella cinnamoma</i>	-	-	-	-
<i>Paranthrenella chrysophanes</i>	6	1.9	0.0	0.9
<i>Paranthrenella formosicola</i>	-	-	-	-
<i>Paranthrenella terminalis</i>	-	-	-	-
<i>Anatrachyntis japonica</i>	1	0.0	0.0	0.0

### 3. Interspecific genetic divergence

species	<i>Sphecoptera sheni</i>	<i>P. pinoakula</i> sp. n.	<i>P. auriplena</i>	<i>P. cinnamoma</i>
<i>Sphecoptera sheni</i>		CP=114, 14.6(14.2-15.3)	CP=38, 14.2(13.9-14.9)	CP=38, 15.1(14.9-15.8)
<i>Paranthrenella pinoakula</i> sp.n.			CP=3, 13.9(13.9-13.9)	CP=3, 13.0(13.0-13.0)
<i>Paranthrenella auriplena</i>				CP=1, 11.5(11.5-11.5)
<i>Paranthrenella cinnamoma</i>				
<i>Paranthrenella chrysophanes</i>				
<i>Paranthrenella formosicola</i>				
<i>Paranthrenella terminalis</i>				
<i>Anatrachyntis japonica</i>	CP=78, 11.8(11.5-12.5)	CP=6, 14.9(14.9-14.9)	CP=2, 13.7(13.7-13.7)	CP=2, 15.6(15.6-15.6)
species		<i>P. chrysophanes</i>	<i>P. formosicola</i>	<i>P. terminalis</i>
<i>Sphecoptera sheni</i>		CP=152, 14.0(13.7-14.9)	CP=38, 13.1(12.9-13.7)	CP=38, 13.7(13.4-14.4)
<i>Paranthrenella pinoakula</i> sp.n.		CP=12, 13.4(13.0-13.7)	CP=3, 11.8(11.7-12.0)	CP=3, 14.3(14.2-14.4)
<i>Paranthrenella auriplena</i>		CP=4, 8.2(8.1-8.4)	CP=1, 13.2(13.2-13.2)	CP=1, 14.9(14.9-14.9)
<i>Paranthrenella cinnamoma</i>		CP=4, 138(13.7-13.9)	CP=1, 12.2(12.2-12.2)	CP=1, 14.4(14.4-14.4)
<i>Paranthrenella chrysophanes</i>			CP=4, 14.4(14.2-14.6)	CP=4, 16.5(16.3-16.8)
<i>Paranthrenella formosicola</i>				CP=1, 12.2(12.2-12.2)
<i>Paranthrenella terminalis</i>				
<i>Anatrachyntis japonica</i>		CP=8, 15.6(15.3-16.0)	CP=2, 14.9(14.9-14.9)	CP=2, 14.1(14.1-14.1)

### 3.2.3. Haplotype analysis

The MJ network analysis presented that eighteen haplotypes were detected from the 52 COI sequences (Figure 9). A total of 36 sequences of Korean clearwing borers were revealed total ten haplotypes. H1-H8 haplotypes of 34 sequences were recognized as *Sphecoptera sheni*, and H9-H10 of three sequences were revealed as *Paranthrenella pinoakula* sp. n.

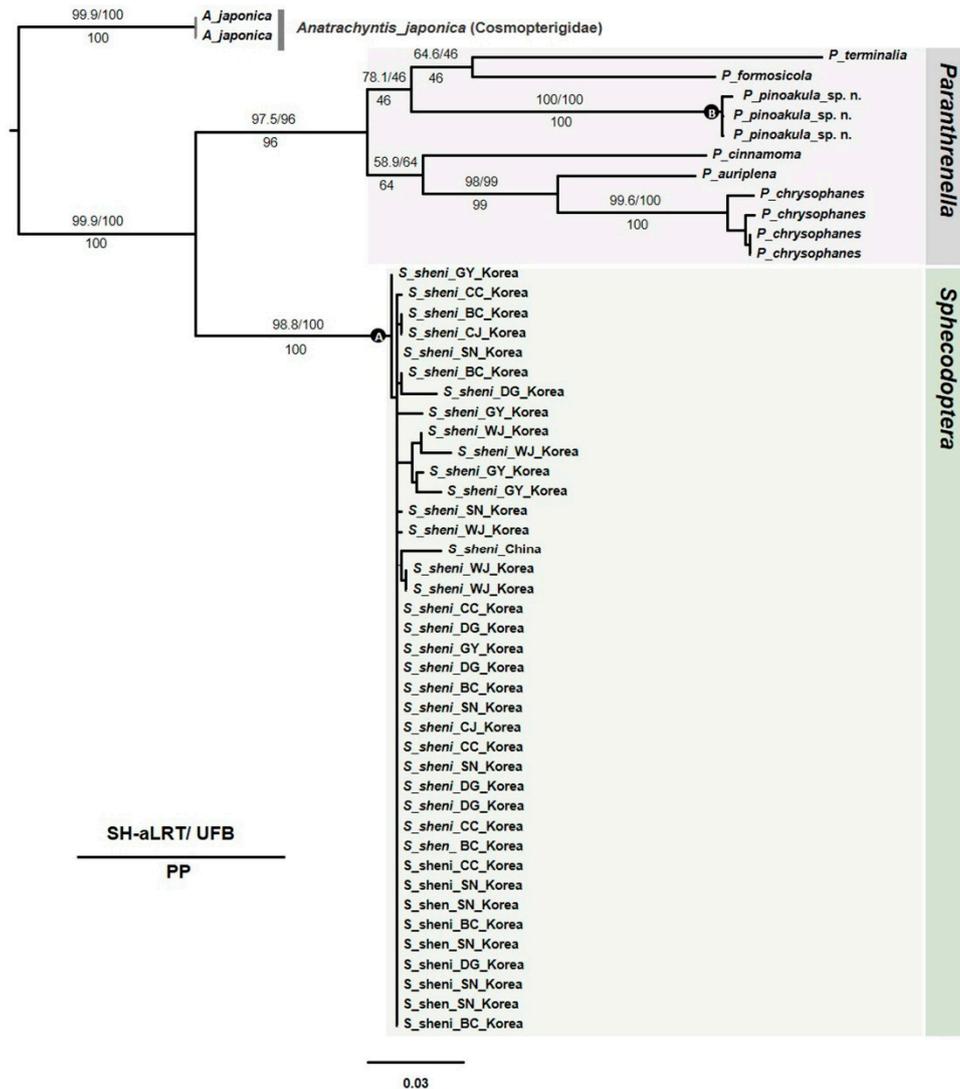


**Figure 9.** Median joining network about 18 haplotypes of the 52 COI sequences. The pie size is proportional to the haplotype frequency. A dotted circle means same species.

H1-H7 were detected from Korean samples (BC, GY, SN, CC, WJ, CJ, DG) with large-sized borers, *S. sheni*. WJ and GY populations of *S. sheni* were revealed as three haplotypes, and among them, H7 haplotype (WJ) was closely connected with H8 haplotype of Chinese *S. sheni*. BC and CJ populations of that werzze presented as two haplotypes, H1 and H2. CC population of that was recognized as two haplotypes, H1 and H3, whereas SN and Daegu populations were detected in only H1. H9 and H10 haplotypes were detected as the other Korean Wonju population of small-sized borers, *P. pinoakula* sp. n., and closely located with each congeneric species.

### 3.2.4. Phylogenetic tree

Although one gene is not enough to analyze a phylogenetic tree, Maximum Likelihood tree (MLtree) was constructed to show the phylogenetic relationship between the species and individual under species-level. The MLtree is derived from a statistical method based on evolutionary models including assumptions about the probability distribution of the data. As a result, all individuals of the large-sized clearwing moth borer, *Sphecodoptera sheni*, were separated from the other *Paranthrenella* spp. (Figure 10, clade A: Sh-aLRT/ UFB/ PP: 98.8/100/100). The small-sized clearwing moth borer, *P. pinoakula* sp. nov. was also phylogenetically claded within the genus *Paranthrenella* (Figure 10, clade B: Sh-aLRT/ UFB/ PP: 100/100/100). The species is closely located to the clade of *P. formosicola* and *P. terminalia*.



**Figure 10.** Maximum likelihood tree of Sesiid species. Each node marked with three types of supporting value: Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT): upper left; Ultrafast bootstrap (UFB) pseudoreplicates: upper right; Maximum likelihood (ML) bootstrap: below the branch.

#### 4. Discussion

The purpose of this study is twofold. The first objective is to identify the recent outbreak of invasive insect pests attacking pin-oaks, one of the popular street tree species planted in Korea. The pin-oak borers are actually referred to as a variety of clearwing moths, e.g. *Scasiba rhynchioides* (Butler), *Synanthedon bicingulata* (Staudinger), *S. quercus* (Matsumura) that are similar in appearance. In morphological identification based genitalic characters, pin-oak street tree borers identified as *Sphecodoptera sheni* and *Paranthrenella pinoakula* sp.nov..

We unexpectedly confirmed that there are two invasive species and that they are distinguished by body size. COI gene-based molecular identification also supported the two clearwing borers showing a distinct barcode gap within and between species. Haplotype analysis suggests that *S. sheni* may expand its distribution from China. The outbreak was centered in WJ (GW) and GY (GG) in having each three haplotypes, to neighboring areas of CC (GW), BC (GG) and CJ (CB) with each two haplotypes. Relatively, both SN (GG) and Daegu populations are detected to H1 haplotype, inferring a recent outbreak. On the other hand, for *P. pinoakula* sp. nov., only the WJ (GW) population was confirmed. In addition, our phylogenetic analysis (MLtree) shows that the two borer groups are

phylogenetically distant, indicating that they may be occurred by host preference of the species rather than the occurrence of phylogenetically related groups.

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