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Immature Stages of Genus *Hexatoma* (Diptera, Limoniidae) in the Korean Peninsula

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Abstract: The genus *Hexatoma* Latreille, 1809 is a large group of aquatic crane flies, with almost 600 species worldwide. The largest subgenus is *Eriocera* Macquart, 1838, which includes all nine species known from the Korean Peninsula. Molecular methods were used to associate *Hexatoma* larvae with their putative adult species from South Korea. Mitochondrial Cytochrome c Oxidase Subunit I (COI) gene fragment sequences (DNA barcodes) of recently collected adults of *H.* (*E.*) *gifuensis*, *H.* (*E.*) *ilwola*, *H.* (*E.*) *pernigrina*, and *H.* (*E.*) *pianigra* were compared with twelve sequences of *Hexatoma* larvae. The larvae of *H.* (*E.*) *pernigrina*, *H.* (*E.*) *pianigra*, and *H.* (*E.*) *gifuensis* were associated with their putative adults. The larvae of *H.* (*E.*) *gifuensis* and *H.* (*E.*) *pianigra* and the larvae and pupae of *H.* (*E.*) *pernigrina* are described and illustrated. The larvae of two species not associated with any adult are described, and their COI gene fragment sequences (DNA barcodes) are presented. This paper presents the morphological characteristics suitable for distinguishing larval species. A key for the identification of larvae of the genus *Hexatoma* on the Korean Peninsula has been compiled. *H* (*E.*) *sachalinensis* is recorded from the Korean Peninsula for the first time. Our study is the first contribution to the *Hexatoma* larvae taxonomy using phylogenetic analysis based on mitochondrial COI fragment (DNA barcode) and one of the first attempts to reveal phylogenetic relationships between *Hexatoma* species using molecular markers.

Keywords: crane fly; larvae; pupae; mtDNA; COI; South Korea; North Korea; integrative taxonomy

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1. Introduction

The globally distributed crane flies' genus *Hexatoma* Latreille, 1809 is one of the largest genera of the Tipuloidea. The genus includes 599 described species [1] and is particularly diverse in Asia, from which 365 species are known to date. The genus *Hexatoma* was erected by P.A. Latreille in 1809 when he described a single species, *Hexatoma nigra*. The main differences from other crane flies were antennae with a reduced number of flagellomeres and open discal cells of the wing. *Eriocera* was described as a separate genus by J. Macquart in 1838, which was based on closed discal cells. Now, we recognize six subgenera of extant *Hexatoma*, all of which have sexually dimorphic antennae with a reduced number of flagellomeres, some males having antennae that exceed the whole-body length by a few times. Most species of *Eriocera*, especially from the tropical and subtropical regions, have striking body and wing color patterns and are easily recognized among other crane flies. Partly because of that, identification usually was based on coloration rather than structures of the male terminalia, which are rather simple compared to other crane flies. Only recently was attention paid to the inner structures, such as the aedeagal complex, which has distinctive species-specific characteristics [2]. The first immatures of the genus *Hexatoma* were described by Alexander [3], and only the larval stages of two subgenera, *Eriocera* and *Hexatoma*, are known to date. The larvae of this genus have four spiracular lobes, a highly reduced (especially on the ventral side) head capsule, long maxillae, and a large sickle-shaped mandible. Their most distinctive features are the labrum, which has longer or shorter lateral lobes and frons divided into two plates. The pupae of this genus have a well-developed cephalic crest, which helps to distinguish them from other genera of the subfamily Limnophilinae. There are no characteristics of the last instar larvae suitable to distinguish between subgenera *Eriocera* and *Hexatoma*. Differences were found only in the pupal stage in which the male and female terminalia between those two subgenera are very different [4].

Hexatoma larvae are a common group in aquatic habitats on the Korean Peninsula, and a recent taxonomic study revealed that eight *Hexatoma* species belonging to only one subgenus, *Eriocera*, reside in the peninsula [2]. As many as five of them are probably endemic to the Korean Peninsula: *H.* (*E.*) *serenensis* Podenas, 2022 is known only from the northern part of the peninsula; *H.* (*E.*) *pianigra* Podenas, 2022 and *H.* (*E.*) *masaki* Alexander, 1924, is known only from the southern part of the peninsula; and *H.* (*E.*) *ilwola* Podenas, 2022 and *H.* (*E.*) *pernigrina* (Alexander, 1938) have been recorded from both the southern and northern parts.

Hexatoma (*Eriocera*) Macquart, 1838 is the largest subgenus of the genus *Hexatoma* and the only subgenus distributed worldwide, with the majority of species recorded in Asia [1]. All known larvae develop in water and are associated with lotic habitats [3–7]. Despite the fact that this group is very diverse, widespread, and may be important for the biological assessment of aquatic habitats, its immature stages are little known. Of the 563 known species of the subgenus *Eriocera*, only the larvae of ten species have been described to date [4].

The immature stages of seven species have been described from North America [3,4] and three species from East Asia [4]. Despite the small number of described larvae, it is evident that this group is characterized by a large diversity of larval morphological characteristics. Based on the morphological traits of larvae and pupae, such as the length and shape of the lateral lobes of the labrum, the sclerotization of the spiracular field, the length and position of the apical hairs of the spiracular lobes, the number and size of the horns of the cephalic crest, and the shape of the respiratory horns *Eriocera* are divided into four morphological groups [4]. The different structure of the spiracular field and the morphological characteristics of the head capsule indicate that *Eriocera* is not a monophyletic group, which was confirmed via a phylogenetic analysis carried out on the basis of adult traits [8].

In this article, we describe the larvae of *H.* (*E.*) *gifuensis* Alexander, 1933, the larvae of *H.* (*E.*) *pianigra*, and the larvae and pupae of *H.* (*E.*) *pernigrina*. We also describe the larvae of two species of *Hexatoma*, not associated with the species, i.e., the adults. The obvious molecular and morphological differences suggest that these are larvae of other not identified species of the genus *Hexatoma*. Although we could not link them to a particular species, we present COI fragment sequences (DNA barcodes) that we hope will help the association of the larvae we described with the adult specimens in the future. These may be species that are new to science or species that have already been described but with unknown DNA barcodes.

This paper also provides a key to the described larvae of *Hexatoma* that are known to reside in the Korean Peninsula.

2. Materials and Methods

Most of the larvae and pupae of the genus *Hexatoma* were hand collected by a senior author in South Korea (2012–2022). Adults of *Hexatoma* (*Eriocera*) *gifuensis*, *H.* (*E.*) *pernigrina*, *H.* (*E.*) *ilwola*, *H.* (*E.*) *masaki*, and *H.* (*E.*) *pianigra* and 5 larvae of *Hexatoma* from the collection of the National Institute of Biological Resources (NIBR), Incheon, South Korea, were used for the DNA extraction and comparison of mitochondrial Cytochrome c Oxidase Subunit I (COI) gene fragment. A total of 143 larvae, 4 male pupae, 6 female pupae, and 5 adults were used in this study. Larvae and pupae are preserved in 70% ethanol. The larval head capsules were cleaned for several hours in heat, approximately 10% KOH solution, and temporary slides were made of gelatin glycerol. The spiracular fields of the larvae were excised, and temporary slides were made of gelatin glycerol. General photographs of larvae, pupae, and head capsules were taken with a Canon EOS 80D digital camera using a Canon MP-E 65 mm macro lens. Photographs of the details and the measurements of the head capsules were taken with a Leica DCM6 microscope with a Leica K3C camera using Las X core software. The length of the larvae was measured from the anterior end of the first thoracic segment to the apex of the longest spiracular lobes. The stigmal hairs were not measured. The length and width of the head capsule were measured before placing the larvae in KOH solution. The width was measured at the widest point of the head capsule, and the length was measured from the anterior end of the labrum to the end of the head capsule. All materials (except *H.* (*E.*) *sachalinensis*) were stored in the NIBR collection.

Molecular analysis was used to associate adult individuals with their putative larvae. A phylogenetic tree was constructed based on the mitochondrial COI representing DNA barcoding region (658 bp) sequence data from four adults, *H.* (*E.*) *gifuensis*, *H.* (*E.*) *pernigrina*, *H.* (*E.*) *pianigra*, and *H.* (*E.*) *ilwola* (*H.* (*E.*) *masaki* sequences were not obtained); twelve larvae belonging to *H.* (*E.*) *gifuensis*, *H.* (*E.*) *pernigrina*, *H.* (*E.*) *pianigra*; and three larvae of two *Hexatoma* species not associated with the adult. Total genomic DNA was extracted from the abdomen or legs of each specimen using a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). Standard PCR amplification and sequencing protocols were used to generate COI fragment sequences [9,10]. The target fragment of COI was amplified in 20 µL reactions containing AccuPower PCR PreMix (Bioneer Co., Daejeon, Korea), 1 U Top DNA polymerase, dNTPs (10 mM), Tris-HCl (pH 9.0), KCl (30 mM), MgCl2 (1.5 mM), 3 µL (5–50 ng) template DNA, and 1 µL of each primer (LCO1490 and HCO2198; 10 pM each) [11]. Amplification was performed using the following thermal cycling program: 94 °C for 4 min; 35 cycles of 94 °C for 0.5 min; 48 °C for 0.5 min; 72 °C for 1 min; and a final extension at 72 °C for 10 min. For the sample of *H.* (*E.*) *ilwola* (isolate CF129), the following thermal cycling program was used: 94 °C for 4 min; 35 cycles of 94 °C for 0.5 min; 48 °C for 1 min; 72 °C for 1.5 min; and a final extension at 72 °C for 10 min. PCR products were sequenced by Macrogen Inc. (Korea).

Before further analysis, DNA sequences for each specimen were aligned in the BioEdit Sequence Alignment Editor [11]. *Hexatoma* COI sequences were submitted to GenBank, and their accession numbers are OQ404918–OQ404933 (Table 1). Maximum likelihood (ML) phylogenetic tree was computed with MEGA X software [12] using all sites and Nearest-Neighbor-Interchange (NNI) heuristic search method with bootstrap test (1000 replicates). Best-fit model of evolution (GTR+G) was selected via the software jmodeltest 2.1.7 [13]. Genetic distances between examined species were calculated as proportion of differences (p-distances), as implemented in the program MEGA X [12]. We used mitochondrial COI sequence of *Ulomorpha longipenis* Kato and Kolcsar, 2020 as an outgroup (GenBank accession number OQ404934). Adult and larval associations were accepted when bootstrap values rose to 95–100% or clustered together in a monophyletic unit. The identified larvae were then compared morphologically to detect taxonomically informative traits.

We use the terminology of Oosterbroek and Theowald [14] and McAlpine [15] for morphological features.

Table 1. Korean *Hexatoma* GenBank Accession numbers.

Species Name	GenBank Accession Number	Adult/Larva
Hexatoma (*Eriocera*) *gifuensis*	OQ404918	Larva
Hexatoma (*Eriocera*) *gifuensis*	OQ404919	Adult
Hexatoma (*Eriocera*) *ilwola*	OQ404920	Adult
Hexatoma (*Eriocera*) *pernigrina*	OQ404921	Larva
Hexatoma (*Eriocera*) *pernigrina*	OQ404922	Larva
Hexatoma (*Eriocera*) *pernigrina*	OQ404923	Larva
Hexatoma (*Eriocera*) *pernigrina*	OQ404924	Larva

Table 1. *Cont.*

Species Name	GenBank Accession Number	Adult/Larva
Hexatoma (*Eriocera*) *pernigrina*	OQ404925	Adult
Hexatoma (*Eriocera*) *pianigra*	OQ404926	Larva
Hexatoma (*Eriocera*) *pianigra*	OQ404927	Larva
Hexatoma (*Eriocera*) *pianigra*	OQ404928	Larva
Hexatoma (*Eriocera*) *pianigra*	OQ404929	Larva
Hexatoma (*Eriocera*) *pianigra*	OQ404930	Adult
Hexatoma sp. 1	OQ404931	Larva
Hexatoma sp. 2	OQ404932	Larva
Hexatoma sp. 1	OQ404933	Larva

3. Results

3.1. Molecular Phylogeny

The maximum likelihood (ML) phylogenetic analysis grouped the twelve larval and four adult sequences into six well-supported clades, which allowed the association of the South Korean larval specimens with the putative adults (Figure 1).

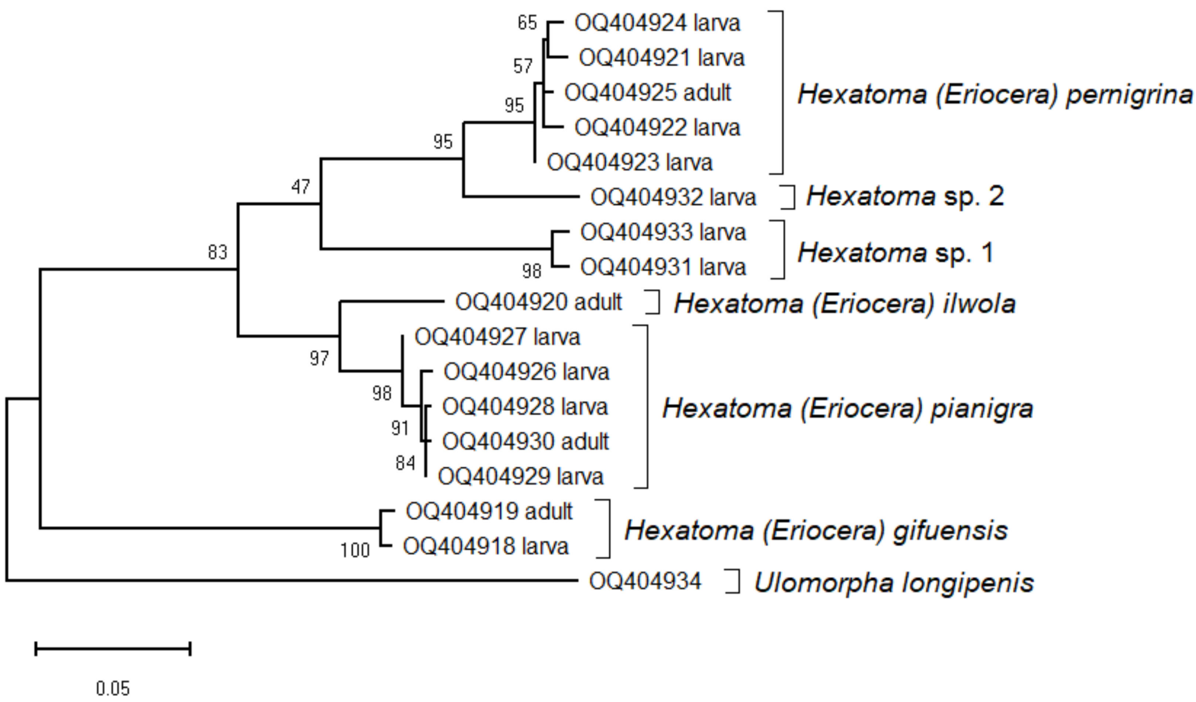

Figure 1. Maximum likelihood phylogenetic tree constructed using mitochondrial COI gene sequences (682 bp) of Korean *Hexatoma* species with *Ulomorpha longipenis* as the outgroup. Bootstrap values (1000 replicates) are indicated in the tree. Species names, life cycle stages, and GenBank accession numbers are given.

We did not find further sequences of identified *Hexatoma* samples from the Korean Peninsula in the Barcode of Life Data Systems (BOLD) and GenBank databases, so this phylogenetic tree is based solely on our material. We obtained adult sequences of the South Korean species *H.* (*E.*) *gifuensis*, *H.* (*E.*) *ilwola*, *H.* (*E.*) *pernigrina*, and *H.* (*E.*) *pianigra*. However, the sample of *H.* (*E.*) *masaki* was not sequenced successfully. The sequences of *H.* (*E.*) *pernigrina* and *H.* (*E.*) *pianigra* adults corresponded to four larval sequences each. One larval sequence corresponded to the adult of *H.* (*E.*) *gifuensis*. There were no larval sequences matching the sequence of the *H.* (*E.*) *ilwola* adult. Three larval sequences formed two separate clades, but they were not linked to any adult species analyzed in this study (Figure 1). The genetic distances as well as the morphological differences between them and other *Hexatoma* species clearly confirm that they are separate species (Table 2). Pairwise genetic distances between four identified *Hexatoma* species ranged from 4.4% between *H.* (*E.*) *pianigra* and *H.* (*E.*) *ilwola* to 13.05% between *H.* (*E.*) *ilwola* and *H.* (*E.*) *pianigra* with *H.* (*E.*) *gifuensis* (Table 2). Pairwise genetic distances between unidentified larvae and four identified *Hexatoma* species ranged from 9.09% between *H.* (*E.*) sp. 1 and *H.* (*E.*) *pernigrina* to 10.26% between *H.* (*E.*) sp. 1 and *H.* (*E.*) *ilwola* with *H.* (*E.*) *gifuensis*, and from 4.55% between *H.* (*E.*) sp. 2 and *H.* (*E.*) *pernigrina* to 14.08% between *H.* (*E.*) sp. 2 and *H.* (*E.*) *gifuensis* (Table 2). Pairwise genetic distances between *H.* (*E.*) sp. 1 and *H.* (*E.*) sp. 2 were from 9.38% to 9.68%.

Table 2. Pairwise genetic distance for COI gene sequences between Korean *Hexatoma* species (calculated in MEGAX as proportion of differences (p-distances).

Species	Pe1	Pe2	Pe3	Pe4	Pe5	Sp2	Sp11	Sp12	Pn1	Pn2	Pn3	Pn4	Pn5	Gf1	Gf2	Iw
Pe1																
Pe2	1.03															
Pe3	1.17	1.32														
Pe4	0.88	1.03	0.88													
Pe5	0.88	1.03	0.88	0.59												
Sp2	5.43	5.28	5.13	4.82	4.55											
Sp11	9.38	9.38	9.09	9.09	9.09	9.68										
Sp12	9.38	9.38	9.09	9.09	9.09	9.38	1.03									
Pn1	8.50	8.94	8.8	8.06	8.06	9.24	9.68	9.82								
Pn2	9.09	9.53	9.38	8.65	8.65	9.38	9.82	9.97	0.88							
Pn3	9.09	9.24	9.38	8.65	8.65	9.24	10.12	10.26	0.88	0.59						
Pn4	9.24	9.38	9.53	8.8	8.8	9.38	9.97	10.12	0.73	0.44	0.15					
Pn5	9.38	9.53	9.68	8.94	8.94	9.53	10.12	10.26	0.88	0.59	0.29	0.15				
Gf1	12.61	12.46	12.76	12.46	12.17	12.32	13.93	13.93	12.61	13.05	12.6	12.76	12.9			
Gf2	12.61	12.46	12.76	12.46	12.17	12.46	13.78	14.08	12.32	12.9	12.32	12.46	12.61	0.73		
Iw	9.53	9.97	9.82	9.09	9.09	9.53	10.41	10.26	4.4	4.99	5.28	5.13	5.28	12.9	13.05	

Abbreviations: Pe1—*Hexatoma* (*Eriocera*) *pernigrina* larva (OQ404924); Pe2—*Hexatoma* (*Eriocera*) *pernigrina* larva (OQ404921); Pe3—*Hexatoma* (*Eriocera*) *pernigrina* larva (OQ404922); Pe4—*Hexatoma* (*Eriocera*) *pernigrina* adult (OQ404925); Pe5—*Hexatoma* (*Eriocera*) *pernigrina* larva (OQ404923); Sp2—*Hexatoma* sp. 2 larva (OQ404932); Sp11—*Hexatoma* sp. 1 larva (OQ404933); Sp12—*Hexatoma* sp. 1 larva (CF64OQ404931); Pn1—*Hexatoma* (*Eriocera*) *pianigra* larva (OQ404927); Pn2—*Hexatoma* (*Eriocera*) *pianigra* larva (OQ404926); Pn3—*Hexatoma* (*Eriocera*) *pianigra* adult (OQ404930); Pn4—*Hexatoma* (*Eriocera*) *pianigra* larva (OQ404929); Pn5—*Hexatoma* (*Eriocera*) *pianigra* larva (OQ404928); Gf1—*Hexatoma* (*Eriocera*) *gifuensis* adult (OQ404919); Gf2—*Hexatoma* (*Eriocera*) *gifuensis* larva (OQ404918); Iw—*Hexatoma* (*Eriocera*) *ilwola* adult (OQ404920).

ML phylogenetic tree revealed two divergent lineages, which are supported by larval morphological characteristics. The first clade or "gifuensis" branch, including the only species *H.* (*E.*) *gifuensis*, is characterized as follows: long, narrow, sickle shaped, outwardly directed, densely covered with long hairs lateral lobes of the labrum, posteriorly tapering clypeus, long antenna, and a maxilla more than twice as long as the mandible. They also have four long setae on the ventral side of the penultimate segment, with setae 1–2 and 3–4 very close to each other. Their ventral lobes of spiracular disc bear "Y"-shaped sclerite, the inner branch of which is fused with its opposing sclerite. The rest of the Korean species belong to another clade, larvae of which are characterized as being short and broad at the base, membranous, and sparsely covered with setae lateral lobes of the labrum and having posteriorly broadly rounded clypeus, short antennae, and maxillae less than twice as long as the mandible. They also have four long setae on the ventral side of the penultimate segment, with all setae equidistant from each other. Their ventral lobes of spiracular disc bear "Y"-shaped sclerite, separated from its opposing sclerite.

3.2. Systematics

Key to the Last Instar Larvae of the Genus *Hexatoma* from the Korean Peninsula

1. The lateral lobes of the spiracular field have a narrow, stripe-shaped sclerite (Figure 4A,B). The ventral lobes bear a "Y"-shaped sclerite, the inner branch fused with its

opposing sclerite (Figure 4A,B). The median labrum border laterally extended into a setose, saw-shaped lobe directed outward (Figure 3A,B). 2

–The lateral lobes of the spiracular field have a narrow sclerite distinctly wider at the base or "Y"-shaped sclerite (Figures 8, 16, 19 and 22). The ventral lobes bear a "Y"-shaped sclerite, separated from its opposing sclerite. The median labrum lobes are short, broadly rounded, fleshy, and scarcely covered with setae (Figures 7A, 15A,B, 18A,B and 21A,B). 3

2. The dorsal inner branch of the ventral sclerite is fused with its opposing sclerite at a pointed angle [6] (Figure 44). Marginal hairs of the ventral lobes are located only on the apices of the lobes. ***Hexatoma* (*Eriocera*) *ussuriensis*** Alexander, 1934 (North Korea)

–The dorsal inner branch of the ventral sclerite fused with its opposing sclerite at an obtuse angle (Figure 4A,B). The apices of the ventral lobes bear long hairs, whereas the rest of the lobes are fringed with short hairs (Figure 4A,B).

Hexatoma* (*Eriocera*) *gifuensis Alexander, 1933

3. The lateral lobe of the spiracular field with a "Y"-shaped sclerite, with the outer branch of it considerably shorter than the inner branch (Figure 16).

Hexatoma* (*Eriocera*) *pianigra Podenas, 2022 (South Korea)

–The sclerite on the lateral lobe of the spiracular field is not bifurcated (Figures 8, 19 and 22). 4

4. Each lateral lobe bears a narrow, dark sclerite, expanding into a rhomb at the base. The dorsal margin bears two tufts of long hairs separated by a narrow bare area (Figure 19).

Hexatoma (Eriocera) sp. 1

–Each lateral lobe bears narrow, dark, sclerite, expanding into a triangle at the base. The dorsal margin without tufts of long hairs (Figures 8 and 22). 5

5. The ventral lobe has a dark, narrow sclerite, with both branches of the "Y"-shaped sclerite similar in length [6] (Figures 26 and 33). 6

–The ventral lobe has a dark, narrow sclerite, with the outer branch of the "Y"-shaped sclerite considerably longer than the inner branch (Figures 8 and 22). 7

6. The apical hairs on the ventral lobes are similar in length. Spiracles are small and widely separated; the distance between them is more than three times the diameter of a spiracle [6] (Figure 26).

Hexatoma* (*Eriocera*) *sachalinensis (Alexander, 1924) (North Korea)

–The apex of the ventral lobe has a a few longer setae, almost twice as long as the lobe. The distance between the spiracles is about 1.5 times the diameter of a spiracle [6] (Figure 33).

Hexatoma* (*Eriocera*) *stackelbergi Alexander, 1933 (North Korea)

7. The apical hairs on the ventral lobes are similar in length. The distance between the spiracles is more than five times the diameter of a spiracle (Figure 22).

Hexatoma (Eriocera) sp. 2

–The apex of the ventral lobe has a few longer setae, almost twice as long as the lobe. The distance between the spiracles is more than three times the diameter of a spiracle (Figure 8).

Hexatoma (Eriocera) pernigrina Alexander, 1938

Hexatoma (Eriocera) gifuensis Alexander, 1933

Figures 2–5

Diagnosis. Larvae: the maxilla is long (more than twice as long as the mandible). The apical part of the ventral spiracular lobe has a few very long dark setae. The lateral lobe with narrow, stripe-shaped sclerite is slightly wider at the base. The ventral lobe of the spiracular field has a broad "Y"-shaped sclerite, and the dorsal inner branch is fused to the opposite sclerite. A mature larva is about 21.0–25.0 mm long.

Description. The length of the last instar larvae is 21.0–25.0 mm, and the width is 3.0–4.2 mm. The body is covered with short yellowish-brown hairs, which give the body a golden color (Figure 2A,B). The terminal segment is covered with long hairs, especially on the outer surface of the spiracular lobes. All thoracic and first abdominal segments are short and wide.

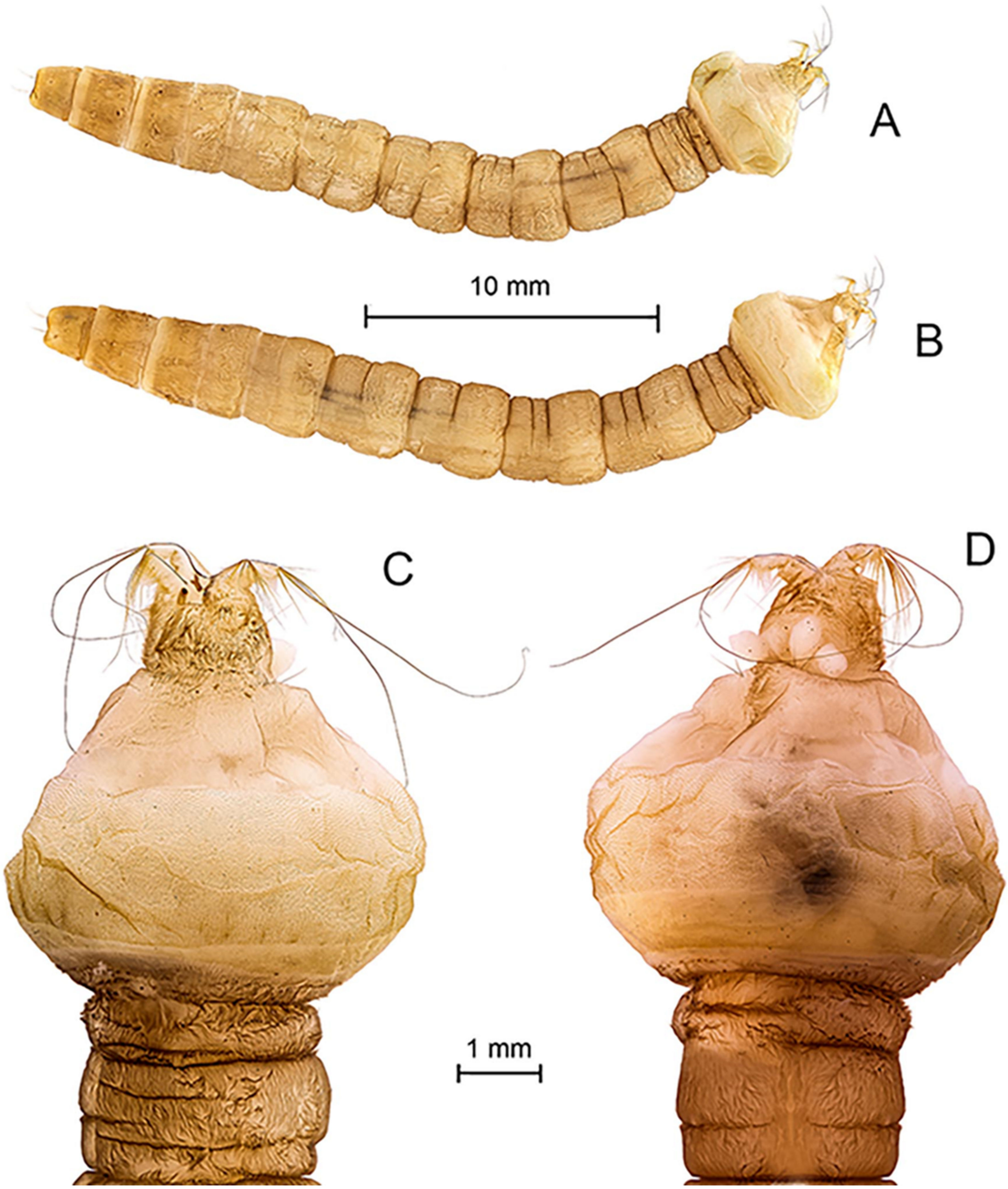

Figure 2. Larva of *Hexatoma* (*Eriocera*) *gifuensis* Alexander, 1933. (**A**) General view, dorsal aspect. (**B**) General view, ventral aspect. (**C**) Terminal segment, dorsal view. (**D**) Terminal segment, ventral view.

The length of abdominal segment II is similar to its width. Abdominal segments III to VI are 1.5 times as long as wide. Abdominal segment VII is twice as long as wide. Terminal segment constricted. The penultimate segment is inflated (Figure 2C), covered with shorter hairs forming long transverse rows with four long setae on the ventral side, 1–2 and 3–4, which are very close together (Figure 2B).

The head capsule is 2.5–2.7 mm long and 1.2–1.3 mm wide. It is an elongated oval in shape, depressed dorsoventrally and reduced (Figure 3A). The medial part of the labrum extended into a setose, sickle-shaped lobe directed outward (Figure 3B). One sensory papilla has a long seta, and another sensory papilla has a short seta with a prominent tubercle with sensory pegs behind them on the anterior part of the labrum. There are several short sensory structures near the posterior-lateral side of the labrum.

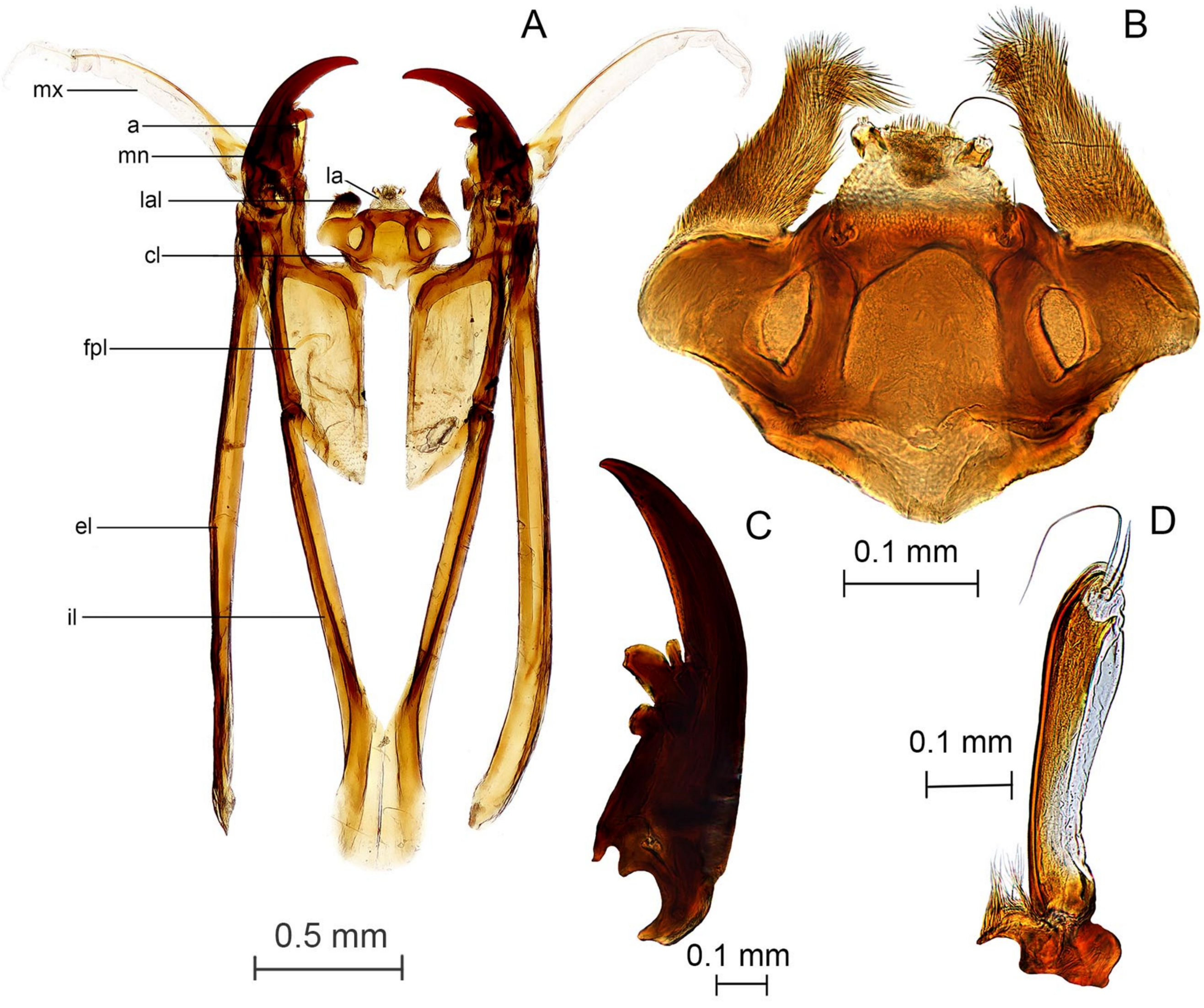

Figure 3. Larva of *Hexatoma* (*Eriocera*) *gifuensis* Alexander, 1933. (**A**) General view of head capsule, dorsal aspect, mx—maxilla, a—antenna, mn—mandible, la—labrum, lal—labral lobes, cl—clypeus, fpl—frontal plate, il—internolateralia, el—externolateralia. (**B**) Clypeolabrum, dorsal aspect. (**C**) Left mandible, dorsal view. (**D**) Left antenna, dorsal view.

The labrum is completely covered with tufts of short setae (Figure 3B). The clypeus is separated from the labrum. The clypeus rhomboid has a posterior end tapering to a sharp point. (Figure 3A,B). The frons consists of two large rectangular lateral plates: the anterior part is sclerotized, and the posterior part is membranous (Figure 3A). The mandible is sickle shaped, with one sharp, curved apical tooth (Figure 3C) and three small teeth at the base; the first tooth is short, narrow, and blunt; the second tooth is twice as long as the first tooth, wide and sharp; the third tooth is blunt, twice as short as the second. The basal segment of the antenna is elongated, cylindrical, and more than four times as long as wide, with the apical part directed outward (Figure 3D). It bears long and short setae on the apical part; the long seta is more than twice as long as the short seta. The apical segment is short and sculptured, its length equal to the diameter of the basal segment of the antenna. A cushion of long hairs is situated at the base of the antenna.

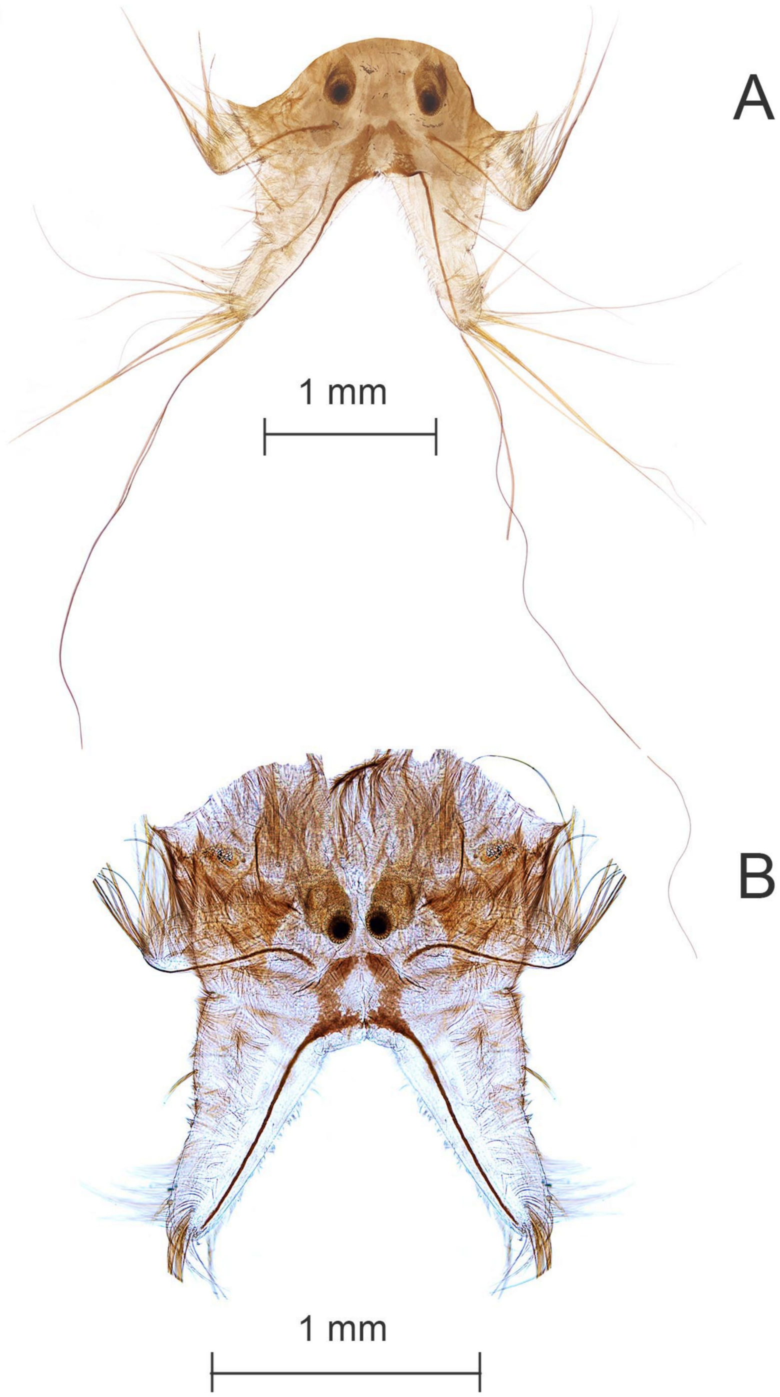

Figure 4. Larva of *Hexatoma* (*Eriocera*) *gifuensis* Alexander, 1933. (**A**,**B**) General view of spiracular field (in (**B**), longer hairs from the spiracular lobes have been cut off in the image).

The maxilla is twice as long as the mandible and consists of two unequal lobes (Figure 3A). The inner lobe of the maxilla is very short, almost seven times as short as the outer lobe. It bears a sclerite at the base and a sensory papilla at the apex. The outer lobe of the maxilla is long and curved outward; the basal part is sclerotized, and the apical part bears only a narrow elongate sclerite. Cardo is reduced. The hypopharyngeal part of the head capsule is membranous. The posterior part of the head capsule consists of one pair of rod-shaped internolateralia (dorsal) and one pair of rod-shaped externolateralia (lateral). The posterior part of the externolateralia is slightly bent inwards. The internolateralia is straight and diagonal until the apical fifth, then diverges slightly (Figure 3A).

Anal division. A spiracular field is surrounded by four (lateral and ventral) lobes (Figure 4A,B). Both pairs are elongated, and the ventral lobe is 1.5 times as long as the lateral lobe. The lateral lobe is almost 2.5 times as long as the width of the base. The ventral lobe is almost three times as long as the width of the base. The spiracular field is bordered with short light brown hairs except for the apical part of the lobes. The apical part of the lateral lobe is covered with long, dark brown hairs, slightly longer than the lobe. The apical part of the ventral lobe has a few dark elongate hairs, which are more than four times as long as the lobe itself. The lateral lobe has a very narrow, strap-shaped sclerite, slightly wider at the base. The ventral lobe has a very dark and narrow sclerite, which becomes very broad and branched at the base; the outer branch is darker than the inner branch; the inner branch is fused with its opposite sclerite. There is a pair of sensory structures located at the edge of the spiracular field above each spiracle and a pair of sensory structures located at the dorsal edge of the spiracular field (Figure 5). Spiracles are small, round, and widely spaced; the area between them is more than three times the diameter of a spiracle.

The anus is surrounded by four short, white, fleshy anal papillae (Figure 3B). Lobes are oval in shape and uniform in size.

Pupa. Unknown.

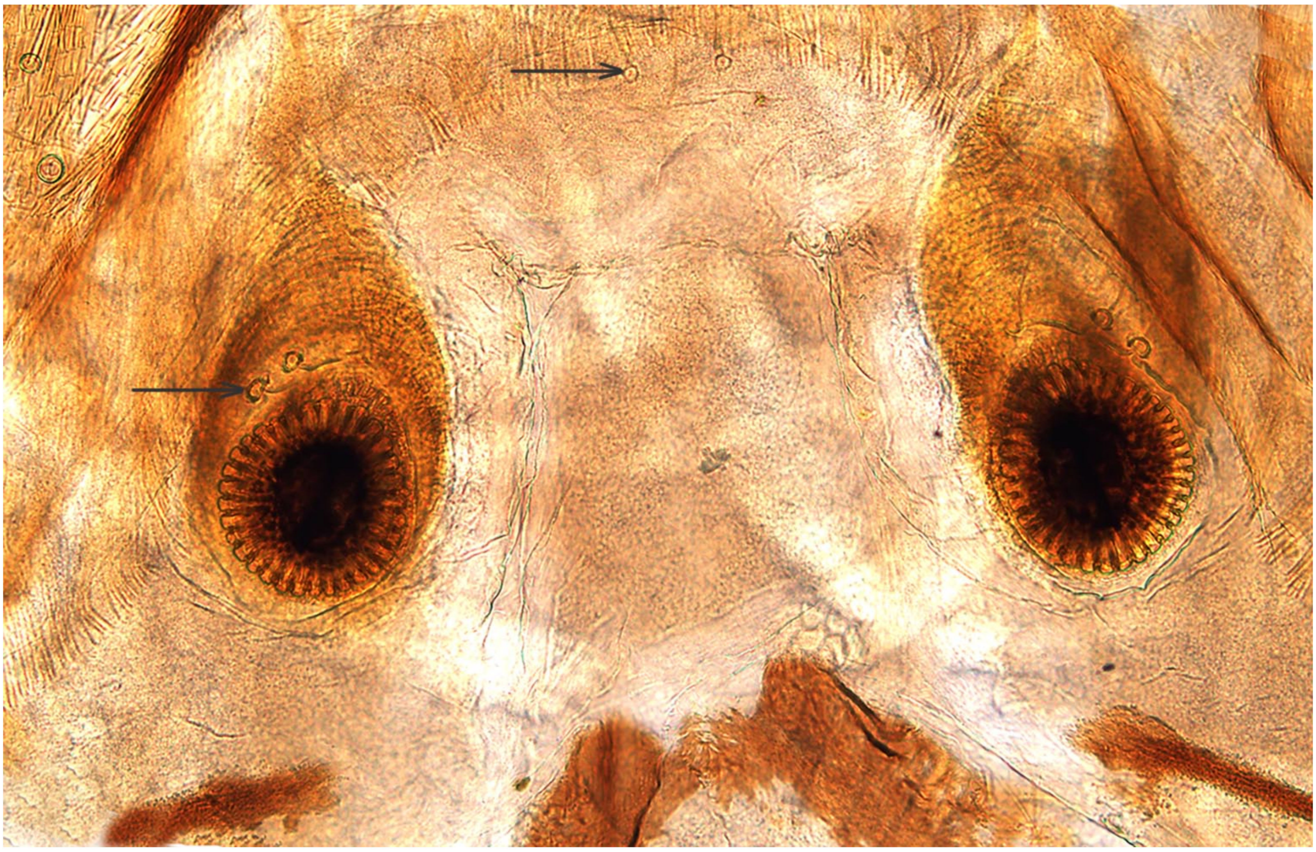

Figure 5. Larva of *Hexatoma* (*Eriocera*) *gifuensis* Alexander, 1933. Sensory structures of spiracular field (arrowed).

Material examined. South Korea: Gyeongsangnam-do, Hapcheo-gun, Bongsan-myeon, Apgok-ri; 3 September 2011; 1 larva; leg. Jeong Mi Hwang; NIBR0000508505; GenBank accession Nr. OQ404918. South Korea: Gyeongsanbuk-do, Andong-si, Imha-myeon, Odae-ri; 23 May 2012; 1 larva; leg. Kim Joong Yeob; NIBR 0000564419. South Korea: Gangwon-do, Pyeongchang-gun, Bongpyeong-myeon; 23 October 2011; 1 larva; leg. Min Jeong Baek; NIBR 0000508514. South Korea: Gangwon-do, Pyeongchang-gun, Bongpyeong-myeon; 23 October 2011; 1 larva; leg Min Jeong Baek; NIBR 0000508513. South Korea: Gyeongi-do, Yangju-si, Jangheung-myeon, Hoguk-ro, 157 m; 37.71058°, 126.98719°; 5 July 2019; 1 larva; leg. V. Podeniene. South Korea: Ganwon-do, Yeongwol-gun, Hanbando-myeon, Ungjeong-ri; 37.224944°, 128.338583°; 30 April 2012; 2 larvae; leg. HJ Park; NIBR. South Korea: Gyeonggi–do, Paju–si, Gunnae–myeon, Jeongja–ri, Warrior Base Training Area, 20 m; 37.918612°, 126.747222°; 18 July 2017; 1 female; leg. T. A. Klein, H.–C. Kim, NJ trap; NIBR; GenBank accession Nr. OQ404919.

Habitat. The larvae of this species develop in the bottom gravel of small and medium-sized rivers.

Hexatoma (Eriocera) pernigrina Alexander, 1938

Figures 6–13

Diagnosis. Larva: the maxilla is short (as long as mandible). The ventral spiracular lobes have slightly extended darker setae at the apex. The lateral spiracular lobes have a dark, narrow, proximally widened sclerite. Ventral spiracular lobes have a thin dark sclerite bifurcating proximally into a "Y"-shape, and the branches do not merge medially. A mature larva is medium sized and 16.0–24.0 mm long. Pupa: the cephalic crest consists of four unequal lobes; the posterior (dorsal) lobe is smaller, and the anterior pair is prominent and horn-shaped. The eye sheath has a prominent tubercle. The antennal sheath is short, extending slightly beyond the base of the wing. Respiratory horns are elongated, slightly wider at the base, and taper toward the end; the apexes are directed outwards. Raw of prominent spines on the pleurite and sternite of the terminal segment in both sexes.

Description. The length of the last instar larva is 16.0–24.0 mm, and the width is 3.0–4.0 mm. The body is covered with yellowish-brown hairs, which give the body a golden color (Figure 6A,B). All thoracic, first, and second abdominal segments are short. The width of abdominal segment III is equal to its length. Abdominal segments IV to VI are 1.5 times as long as wide. Abdominal segment VII is twice as long as wide. The terminal segment is constricted. The penultimate segment is distinctly inflated, covered with short hairs forming long regular transverse rows, and it has four long setae spaced equidistantly from each other on the ventral side (Figure 6C).

The head capsule is 2.4–2.6 mm long and 1.0–1.2 mm wide; it is an elongated oval in shape, depressed dorsoventrally and reduced (Figure 7A). The median part of the labrum is broadly rounded. The lateral lobes of the labrum are fleshy, broad, and sparsely covered with setae (Figure 7A). One sensory papilla has a long seta, another sensory papilla has a short seta, and a prominent tubercle has sensory pegs in the anterior part of the labrum. The long seta is almost three times as long as the short seta. A short seta and a sensory pit are situated near the posterior-lateral side of the labrum (Figure 7B). The clypeus is distinctly separated from the labrum and trapezoidal with the broadly rounded posterior part. The frons consists of two large rectangular lateral plates: the anterior part is sclerotized, and the posterior part is membranous. The basal segment of the antenna is cylindrical, with the apical part directed outward (Figure 7C), four times as long as wide, with short and long setae on the apical part; long seta almost twice as long as short seta. The apical segment is short and sculptured; its length is equal to the diameter of the basal segment. The mandible is sickle shaped, with a sharp, curved apical tooth (Figure 7D) and three small teeth at the base. The first basal tooth is small and sharp; the second tooth is well developed, twice as large as the first tooth; the third tooth is inconspicuous (Figure 7E). The inner part of the maxilla is very short, almost seven times as short as the outer lobe, with the sclerite at the base and the sensory papilla at the apex. The outer lobe of the maxilla is long and directed outward; the basal part is sclerotized. The apical part bears only narrow elongated sclerite and is membranous without short setae. Cardo is reduced. The maxilla is short, almost equal in length to the mandible. The hypopharyngeal part of the head capsule is membranous. The posterior part of the head capsule consists of one pair of rod-shaped internolateralia (dorsal) and one pair of rod-shaped externolateralia (lateral). The externolateralia is bent inwards. The internolateralia is straight and diagonal until midlength, then divergent (Figure 7A).

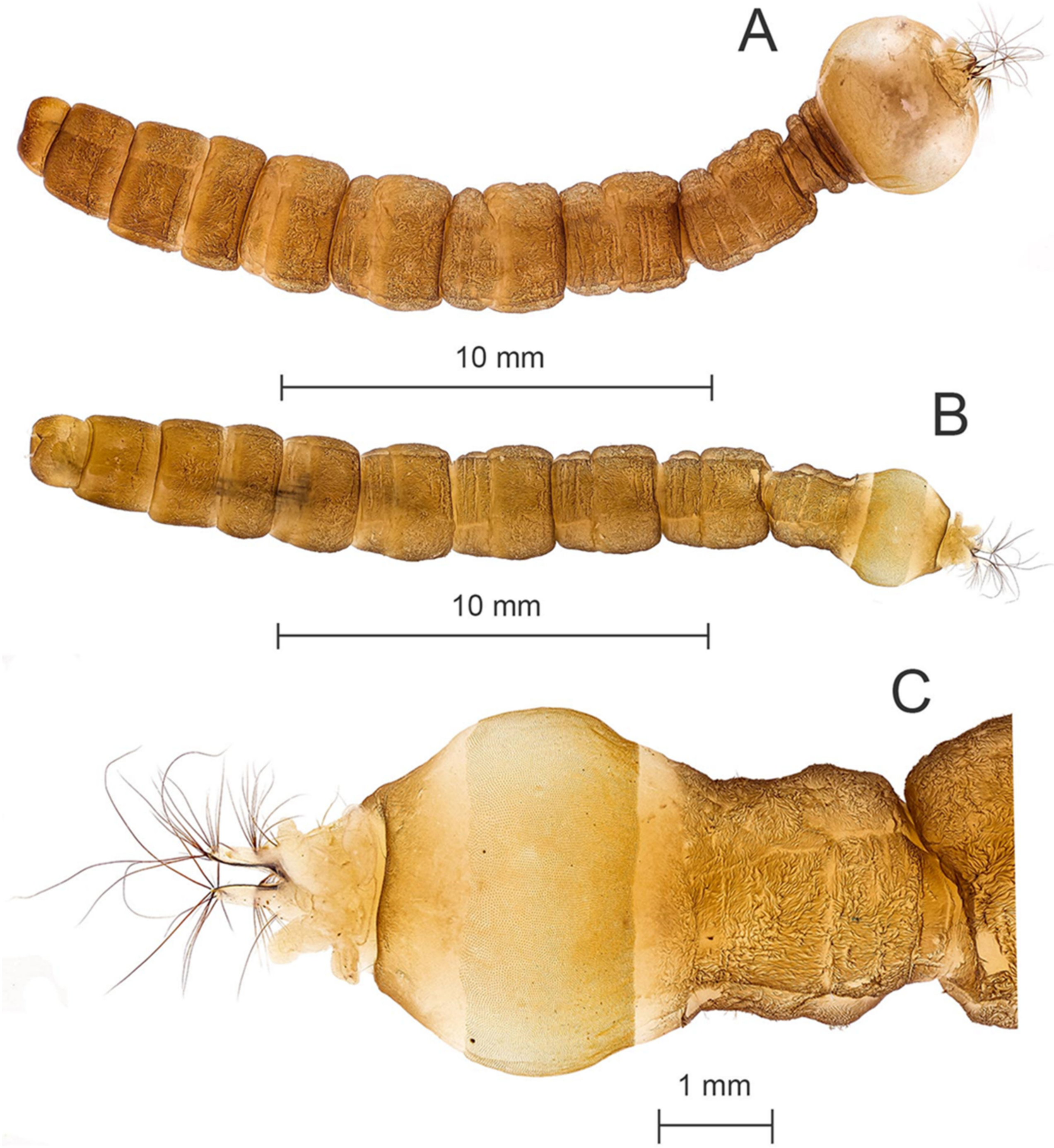

Figure 6. Larva of *Hexatoma* (*Eriocera*) *pernigrina* Alexander, 1938. (**A**) General view, dorsal aspect. (**B**) General view, ventral aspect. (**C**) Terminal segment, ventral view.

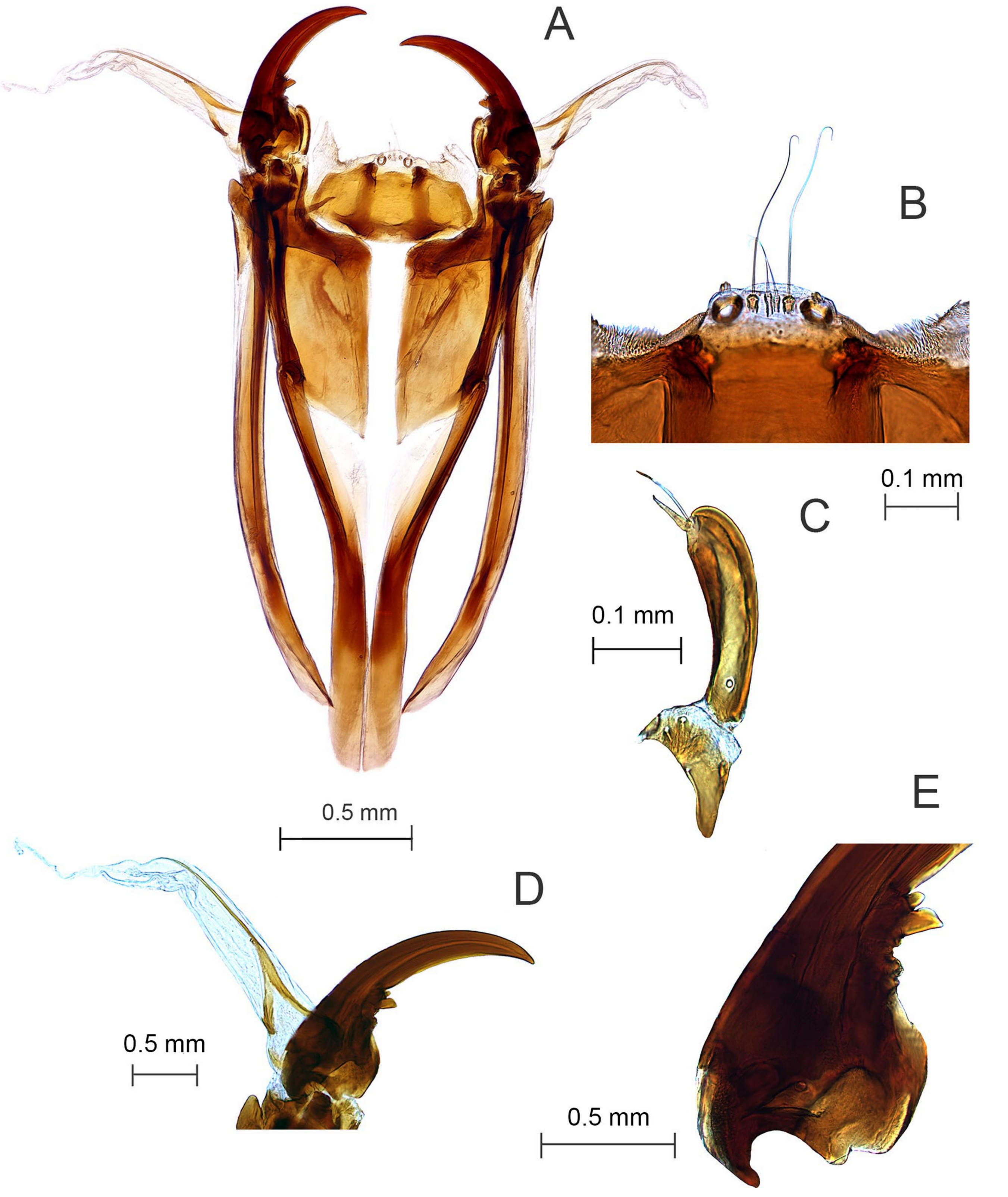

Figure 7. Larva of *Hexatoma* (*Eriocera*) *pernigrina* Alexander, 1938. (**A**) General view of head capsule, dorsal aspect. (**B**) Labrum. (**C**) Right antenna, dorsal view. (**D**) Right mandible and maxilla, dorsal view. (**E**) Basal part of mandible.

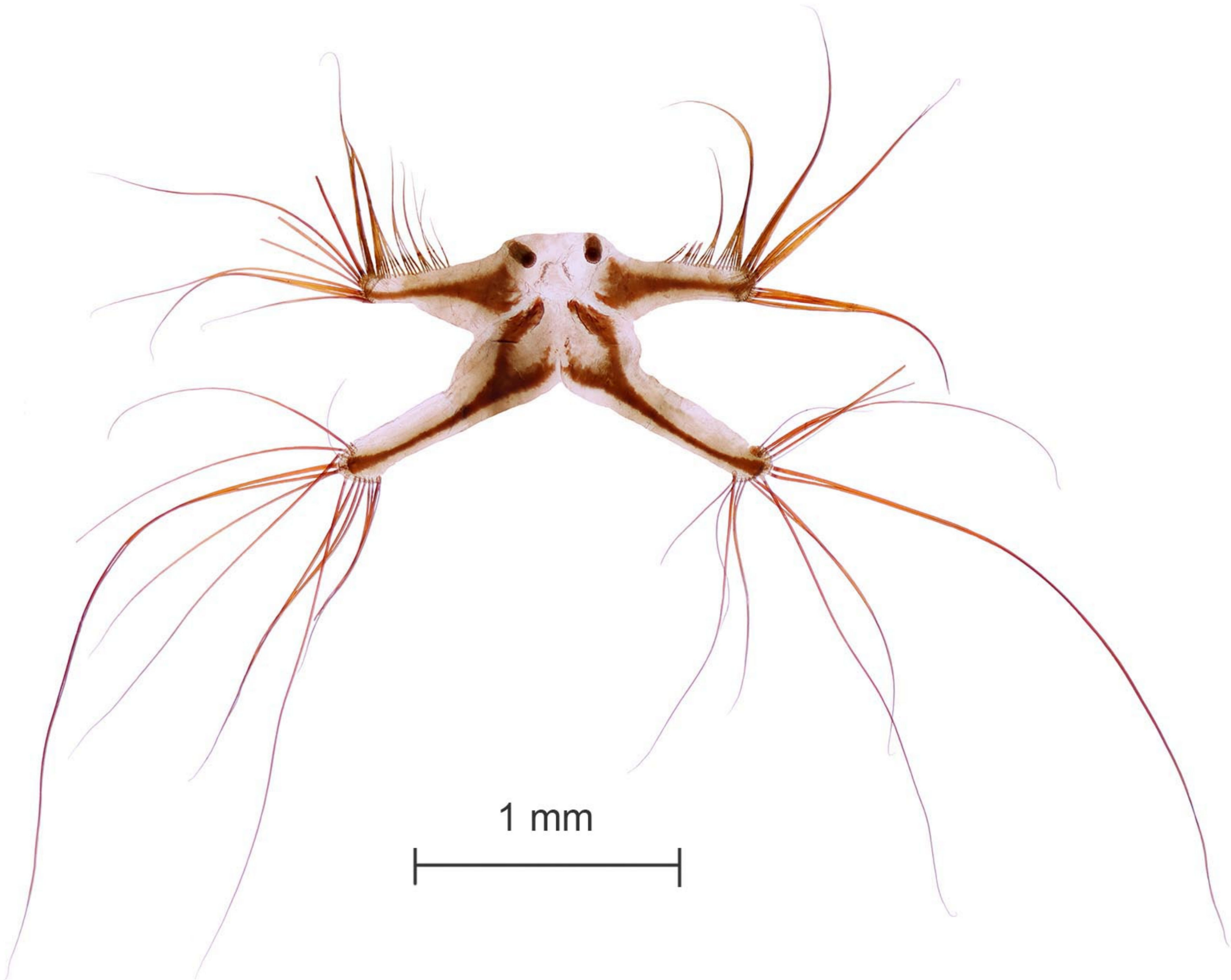

Figure 8. Larva of *Hexatoma* (*Eriocera*) *pernigrina* Alexander, 1938. General view of spiracular field.

Anal division. A spiracular field is surrounded by four (lateral and ventral) flattened elongated lobes (Figure 8). The ventral lobe is 1.5 times as long as the lateral lobe. The lateral lobe is more than twice as long as the width of the base. The ventral lobe is more than three times as long as the width of the base. The apical part of each ventral lobe has dark brown hairs of different lengths, the longest hairs being more than three times as long as the lobe. The outer edge of the lateral lobe is fringed with short hairs; the apical part has hairs twice the length of the lobe. The dorsal margin without hairs. The lateral lobe has a narrow, dark sclerite which widens considerably at the base. The ventral lobe has a dark narrow sclerite bifurcating at the base ("Y"-shaped sclerite); its outer branch is much longer than the inner branch. Spiracles are small, round, and widely separated from each other, the distance between them more than three times the diameter of a spiracle.

The anus is surrounded by four long, white, fleshy anal papillae. The lobes are almost conical and uniform in size (Figure 6C).

Pupa. A male pupa is 14.0–17.0 mm long and 2.5–3.6 mm wide. A female pupa is 17.0–21.0 mm long and 3.0–4.0 mm wide. Mature pupae of males and females are light brown (Figures 9A–C and 10A–C).

Head: the cephalic crest consists of four unequal lobes with wrinkled surfaces. The posterior (dorsal) lobe is smaller and rounded with two long setae apically (Figure 11A,B). The anterior pair is prominent, horn-shaped, with one long seta at the base. The eye sheath has a prominent tubercle (Figure 11A). The antennal sheath is short, extending slightly beyond the base of the wing (Figures 9B and 10B). Tubercles on the antennal scape and pedicel are prominent, with both segments enlarged.

Figure 9. Male pupa of *Hexatoma* (*Eriocera*) *pernigrina* Alexander, 1938. (**A**) General view, ventral aspect. (**B**) General view, lateral aspect. (**C**) General view, dorsal aspect.

The labrum is an elongated oval with two small tubercles near the middle. The labial lobe is rhombic. The maxillary palp is broad and transverse, with small tubercles. *Thorax*: respiratory horns are almost half the width of the thorax, with small annulations along the entire length of the horn; respiratory horns are slightly broader at the base and taper towards the end, with the apices pointing outwards (Figure 11B); horns are dark brown with a light brown base. The thoracic dorsum is smooth. The apex of the wing sheath reaches the middle of the second abdominal segment (Figures 9A, 10A and 11B). The sheath of the legs reaches more than two-thirds of the length of the third abdominal segment; the hind pair is slightly longer than the other pairs (Figures 9A and 10A).

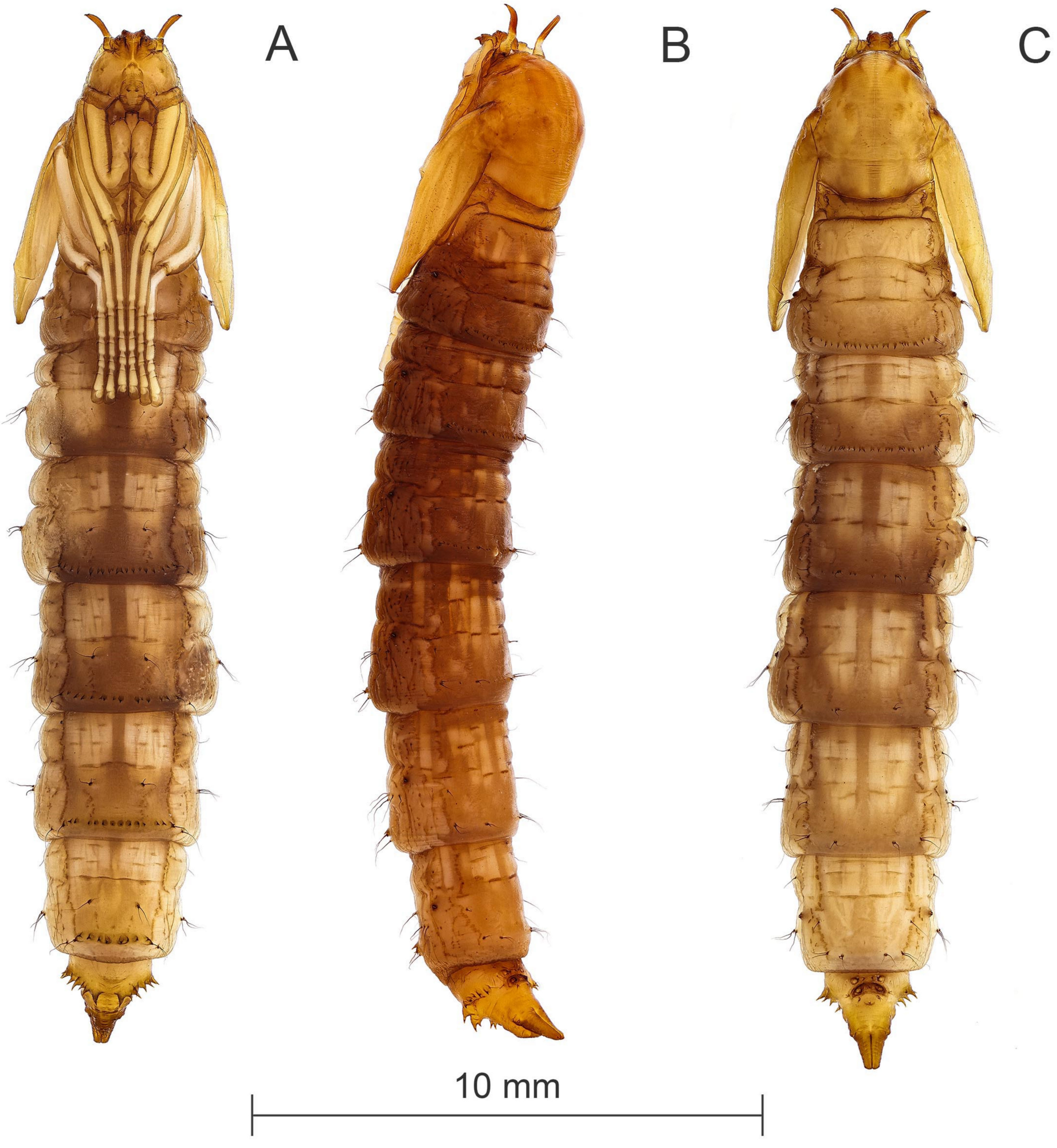

Figure 10. Female pupa of *Hexatoma* (*Eriocera*) *pernigrina* Alexander, 1938. (**A**) General view, ventral aspect. (**B**) General view, lateral aspect. (**C**) General view, dorsal aspect.

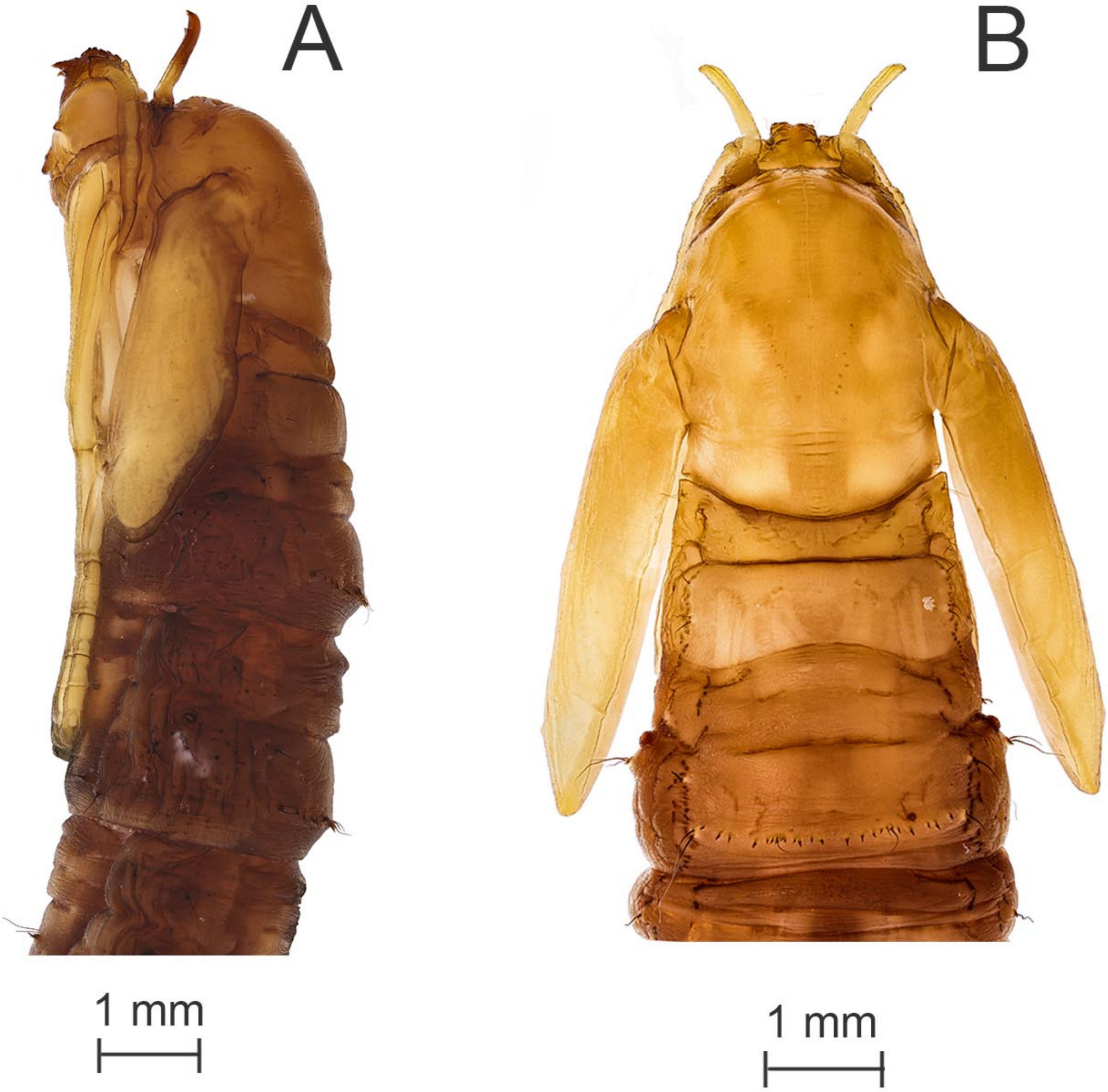

Figure 11. Pupa of *Hexatoma* (*Eriocera*) *pernigrina* Alexander, 1938. (**A**) Anterior part of body, lateral aspect. (**B**) Anterior part of body, dorsal aspect.

Abdomen: segments II–VII have annuli dividing each abdominal segment into anterior and posterior parts. The length and width of the two parts are similar. The posterior part of abdominal segments II–VII have dorsal and ventral transverse rows of spines and small tubercles with long stout setae. The number of spines and tubercles varies from one tergite to another as follows: segments II–III have about 30, segment IV about 20, segment V about 14, and segments VI–VII have only 8 tubercles with a long seta each. The number of spines and tubercles on sternites varies from one sternite to another as follows: segment IV has about 20, segment V from 14 to 16, segment VI from 12 to 14, and segment VII has from 6 to 8 tubercles (some with long setae). Sternites of segments II–VII have two additional tubercles with seta on each side, located almost in the middle of the posterior part. Prominent spiracles almost in the middle of pleurites of abdominal segments III–VII. Three setae are located close to the dorsal margin of spiracles. Segments III–VII each have a small tubercle with a seta in the middle of the anterior part of the pleurites. The terminal segment of the male is blunt and narrow. Ventral lobes (anal spines) are small, with rounded, upward-pointing ends; the tips of ventral lobes reach the base of posterotergal spines (Figure 12A,B). Posterotergal spines are very large, sharply tapering, and directed upwards. Anterodorsal and mediodorsal spines are small, similar in shape and size, with a few setae on the tips, situated almost in the middle of the tergum of the terminal segment. Fourteen distinct spines are situated on the pleurite and sternite of the terminal segment (Figure 12A,B). The terminal segment of the female is elongated, with very long and sharp sheaths of cerci and valves; both sheaths are very close to each other (Figure 13A,B). The sheaths of cerci are much longer than the sheaths of valves, directed upwards with a few setae dorsally. There is only one posterotergal spine much smaller than the paired mediodorsal and anterodorsal spines. The small tubercle has a long seta near the anteriodorsal spine. Sixteen prominent spines are situated on the pleurite and sternite of the terminal segment (Figure 13A,B).

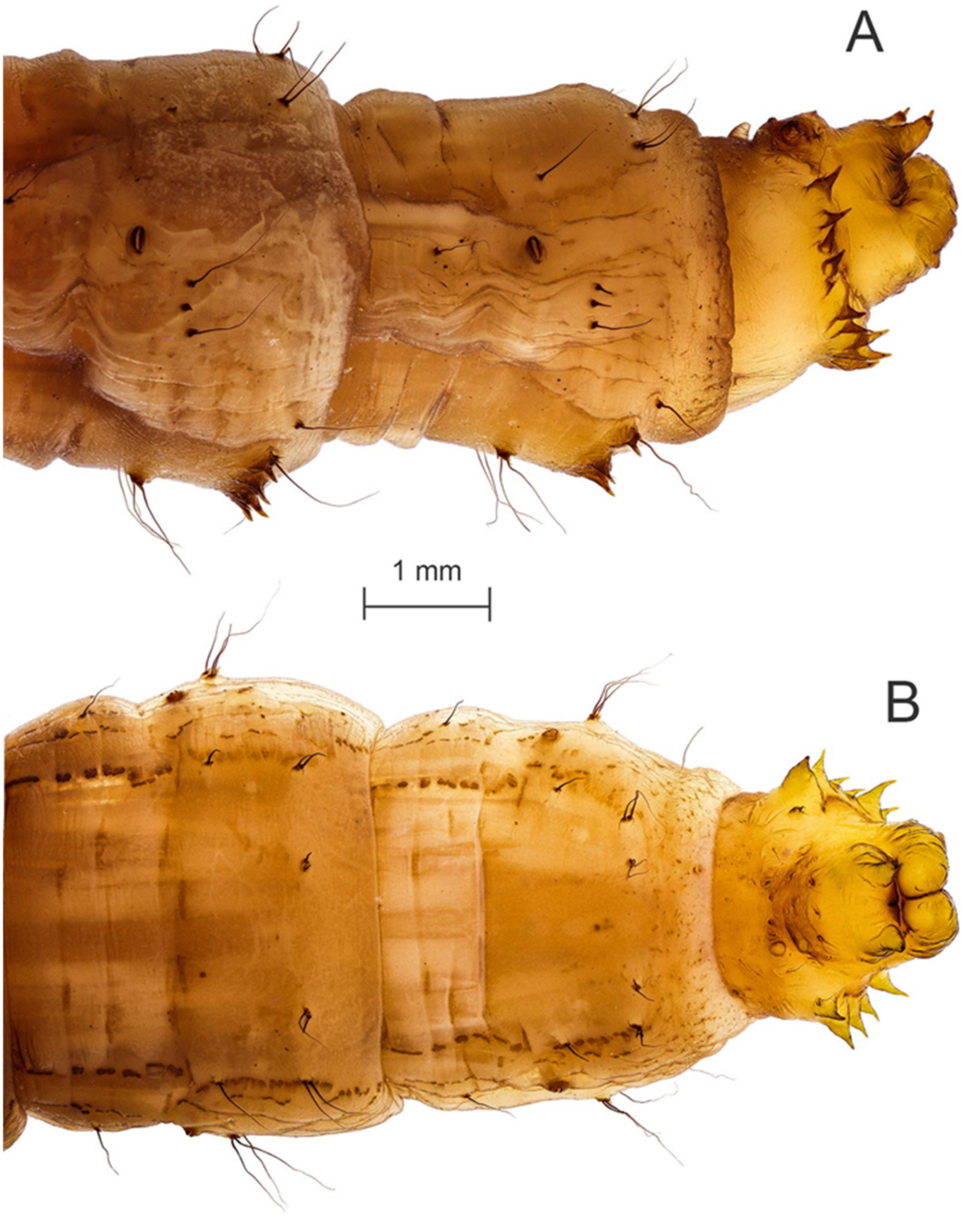

Figure 12. Terminal segments of male pupa of *Hexatoma* (*Eriocera*) *pernigrina* Alexander, 1938. (**A**) Lateral aspect. (**B**) Dorsal aspect.

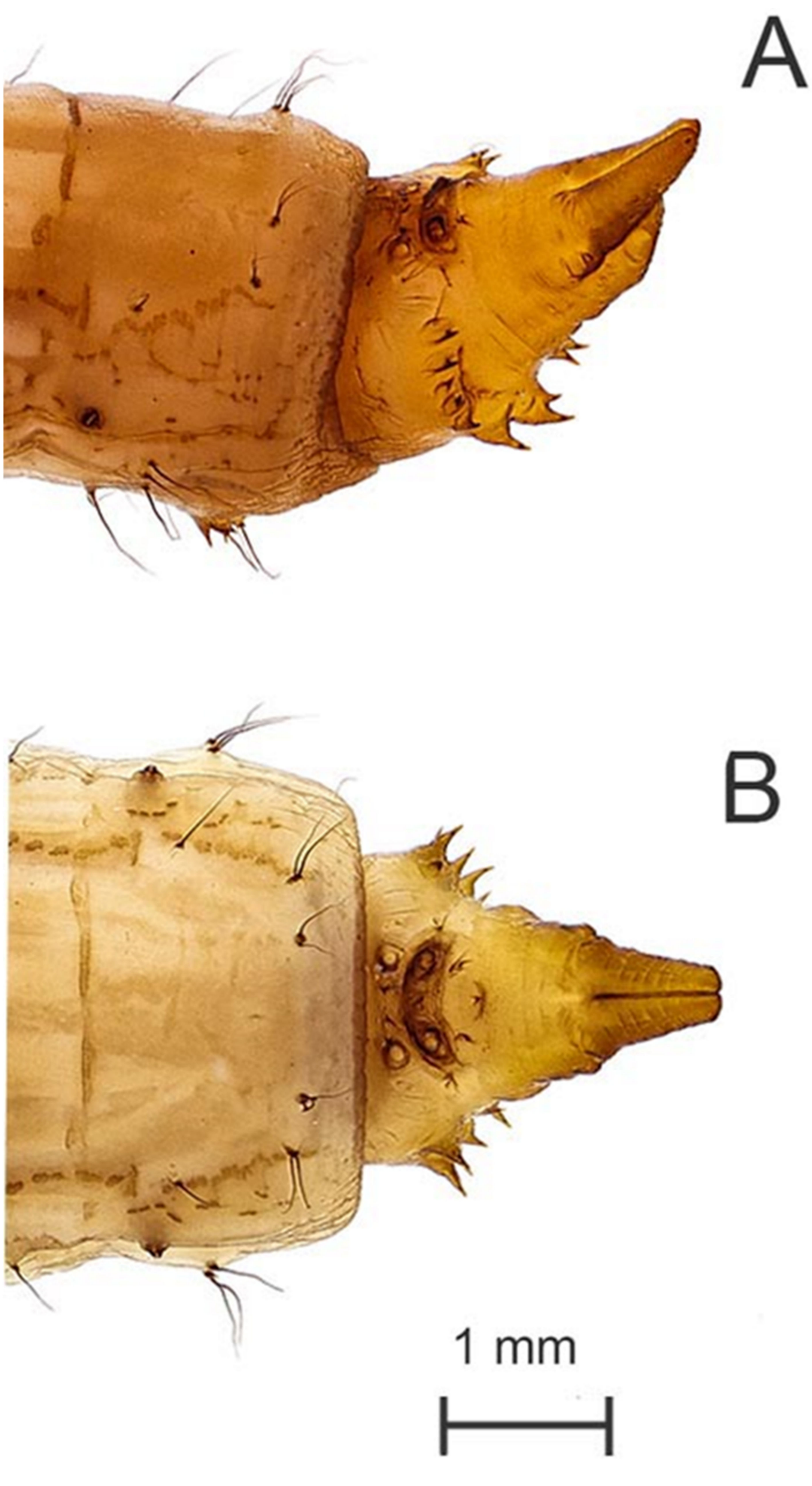

Figure 13. Terminal segments of female pupa of *Hexatoma* (*Eriocera*) *pernigrina* Alexander, 1938. (**A**) Lateral aspect. (**B**) Dorsal aspect.

Material examined. South Korea: Gyeongi-do, Yangju-si, Jangheung-myeon, Hoguk-ro, 157 m; 37.71058°, 126.98719°; 5 July 2019; 3 larvae; leg. V. Podeniene. South Korea: Gangwon-do, Goseong-gun, Ganseong-eup, Jinbu-ri; 250 m; 38.29404°, 128.36287°; 8 July 2015 (2); 3 larvae; leg. V. Podeniene. South Korea: Gyeongsangnam-do, Hadong-gun, Hwagae-myeon, Beomwang-ri; 369 m; 35.27360°, 127.61121°; 8 May 2013; 1 larva; leg. V. Podeniene; GenBank access No. OQ404921. South Korea: Jeollabuk-do, Namwon, Unbong-eup, Hwasu-ri; 509 m; 35.45345°, 127.57759°; 5 larvae; leg. V. Podeniene; GenBank access No. OQ404923. South Korea: Gyeonggi-do, Yangpyeong, Cheongun-myeon, Dowon-ri; 224 m; 37.54507°, 127.79483°; 28 May 2017 (1); 4 larvae; leg. V. Podeniene; GenBank access No. OQ404922. South Korea: Jeollanam-do, Gurve, Masan-myeon, Hwangjeon-ri; 101 m; 35.24366°, 127.48964°; 8 May 2013; 41 larvae, 4 male pupae, 6 female pupae; leg. V. Podeniene. South Korea: Jeollanam-do, Gurye-gun, Toji-myeon, Naeseo-ri, Piagol valley; 490 m; 35.27177°, 127.57146°; 3 June 2016; 1 larva; leg. V. Podeniene. South Korea: Jeollabuk-do, Namwon, Sannae-myeon, Deokdong-ri; 521 m; 35.36458°, 127.56743°; 7 May 2013 (4); 1 larva; leg. V. Podeniene. South Korea: Jeollabuk-do, Namwon, Jucheon-myeon, Gogi-ri; 771 m; 35.36654°,127.50798°; 7 May 2013; 1 larva; leg. V. Podeniene. South Korea: Gyeongsangbuk-do, Gyeongju, Yangbuk-myeon, Janghang-ri; 333 m; 35.76236°, 129.36407°; 28 May 2016 (1); 2 larvae; leg. V. Podeniene. South Korea: Gyeongsangnam-do, Hadong-gun, Hwagae-myeon, Beomwang-ri; 364 m; 35.27655°, 127.61796°; 8 May 2013 (3); 1 larva; leg. V. Podeniene. South Korea: Gyeonggi-do, Gapyeong-gun, Oeseo-myeon, Samhoe-ri, Mt. Hwayasan; 149 m; 37.69183°, 127.41014°; 9 July 2019 (01); 2 larvae; leg. V. Podeniene. South Korea: Jeollanam-do, Gurye-gun, Toji-myeon, Naeseo-ri, Piagol valley; 310 m; 35.25825°, 127.58208°; 29 June 2015; 20 larvae; leg. V. Podeniene; GenBank access No. OQ404924. South Korea: Jeollanam-do, Gurye-gun, Toji-myeon, Naeseo-ri, Piagol valley; 448 m; 35.26586°, 127.58090°; 2 July 2015; 1 larva; leg. V. Podeniene. South Korea: Gyeonggi-do, Suwin-si, Jangan-gu, Sanggwanggyo-dong, Mt. Gwanggyo; 29 May 2012; leg. Jaelck Jo; NIBR0000510152. South Korea: Gyeongsanbuk-do, Munggyeong-eup, Mungyeongsaejae Provincial Park, St.1; 36.767806°, 128.074472°; 14 April 2010; 1 larva; leg. J.M. Hur, W.J. Choi, S.Y. Song; NIBR 0000487782. South Korea: Gangwon-do, Inje-gun, Girin-myeon, Jindong-ri, Bangtaecheon; 18 April 2012; 3 larvae; leg. Jaeck Jo; NIBR0000509835; NIBR0000510035; NIBR0000509935. South Korea: Gyeonggi-do, Pocheon-si, Ildong-myeon, Hwadae-ri, Undangyo; 23 June 2006; 1 larva; leg. YC Jeon; NIBR 0000126528; GenBank access No. OQ404923. South Korea: Jeollanam–do, Gurve, Masan–myeon, Hwangjeon-ri; 100 m; 35.243667°, 127.489667°, 8 May 2013; 1 male; leg. S. Podenas, H.-W. Byun; NIBR; GenBank access No. OQ404925.

Habitat. The larvae of this species develop in the bottom gravel of small and medium-sized rivers.

Hexatoma (Eriocera) pianigra Podenas, 2022

Figures 14–16

Diagnosis. Larva: the maxilla is short (as long as mandible). All marginal hairs on the lateral and ventral spiracular lobes are of similar length. Lateral spiracular lobes each have a narrow dark sclerite, bifurcating into a "Y"-shaped pattern proximally, the inner branch considerably longer. Ventral spiracular lobes each have a narrow dark sclerite, bifurcating into a "Y"-shaped pattern proximally, the outer branch considerably longer; sclerites do not fuse medially. A mature larva is large, with a length of 22.0–42.0 mm. Pupa is unknown.

Description. The length of the last instar larvae is 22.0–42.0 mm, and the width is 3.5–4.5 mm. The body is covered with very short yellowish hairs, which give the body a whitish color (Figure 14A,B). All thoracic, first, and second abdominal segments are short. Abdominal segments III to VI are 1.5 times as long as wide. Abdominal segment VII is twice as long as wide. The terminal segment is constricted. The penultimate segment is distinctly inflated, covered with short hairs forming long regular transverse rows. It has four long setae spaced equidistantly from each other on the ventral side.

The head capsule is 2.7–2.8 mm long and 1.35–1.4 mm wide. In general, it is very similar to that of *H*. (*E*.) *pernigrina* (Figure 15A,B). Differences were observed in the length of setae on the anterior part of the labrum, the number and shape of basal teeth of mandible, and the length of the basal segment of the antenna. The long seta on the anterior part of the labrum is twice as long as the short seta (Figure 15C). The basal segment of the antenna is five times as long as wide (Figure 15D,E). The mandible has three small teeth at the base (Figure 15B). The first and second basal teeth are small, triangular, blunt, and similar in size; the third tooth is wide and blunt (Figure 15E).

Figure 14. Larva of *Hexatoma* (*Eriocera*) *pianigra* Podenas, 2022. (**A**) General view, dorsal aspect. (**B**) General view, ventral aspect.

Anal division. A spiracular field is surrounded by four (lateral and ventral) flat elongated lobes (Figure 16). The ventral lobe is only slightly longer than the lateral lobe. The lateral lobe is twice as long as the width of the base. The ventral lobe is 2.5 times as long as the width of the base. The apical part of the ventral lobe has dark brown hairs of different lengths, the longest hairs slightly longer than the lobe itself. The outer margin of the lateral lobe is bordered with short hairs; the apical part of the lobe has hairs of the same length as the lobe itself. The dorsal margin of the spiracular field bears two tufts of four setae separated by a wide bare area. The lateral lobe has a narrow, dark sclerite bifurcating at the base of the lobe ("Y"-shaped sclerite); its outer branch is much shorter than the inner branch. The ventral lobe has a dark, narrow sclerite bifurcating at the base of the lobe ("Y"-shaped sclerite); its outer branch is much longer than the inner branch. There is a pair of sensory structures at the edge of the spiracular field above each spiracle. There is also a pair of sensory structures at the dorsal margin of the spiracular field. Spiracles are small and round, with a distance between them less than twice the diameter of a spiracle (Figure 16).

The anus is surrounded by four long, white, fleshy anal papillae. Lobes are almost conical, the inner pair being twice as long as the outer pair (Figure 14B).

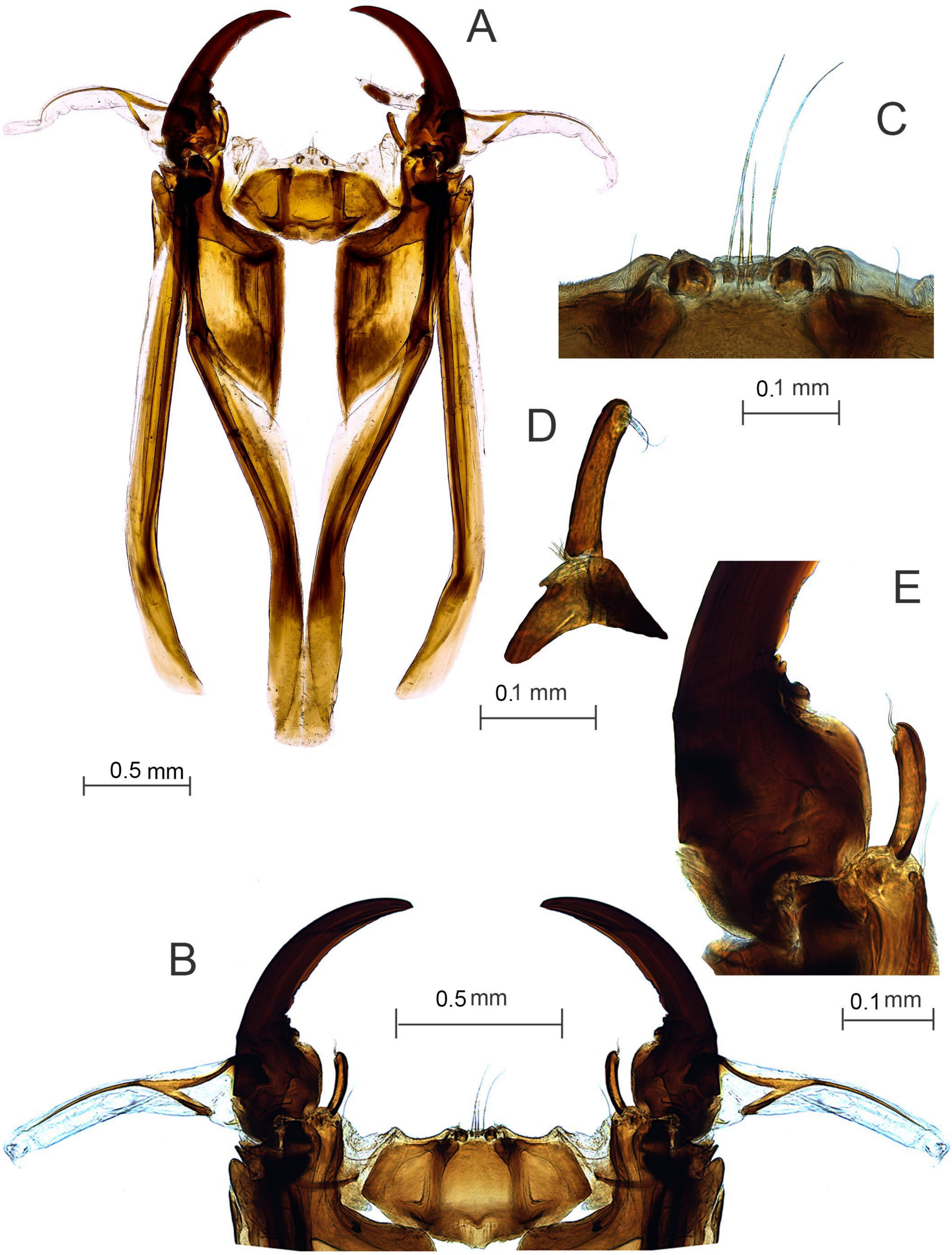

Figure 15. Larva of *Hexatoma* (*Eriocera*) *pianigra* Podenas, 2022. (**A**) General view of head capsule, dorsal aspect. (**B**) Anterior part of head capsule, dorsal aspect. (**C**) Labrum. (**D**) Left antenna, dorsal view. (**E**) Basal part of mandible and right antenna.

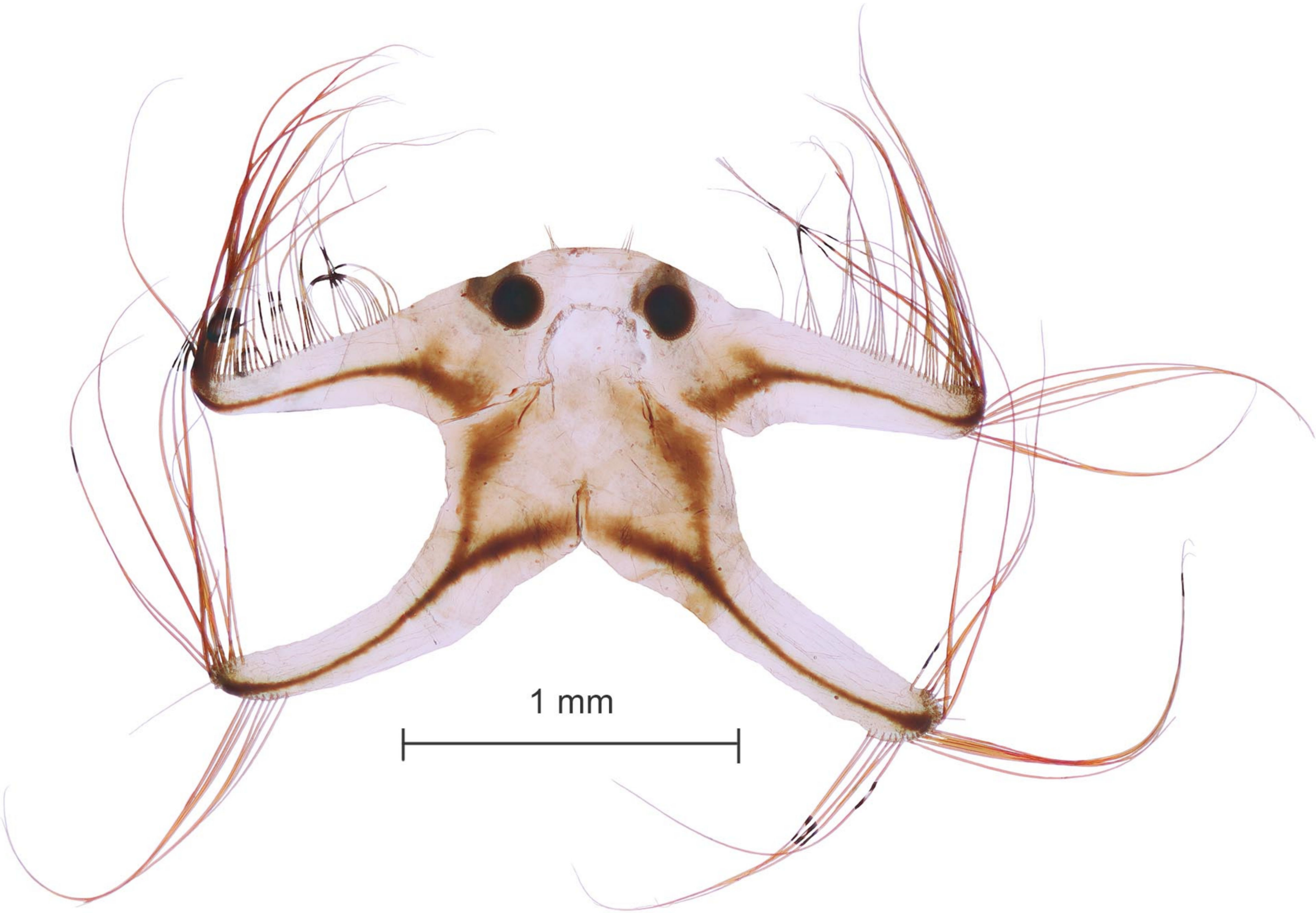

Figure 16. Larva of *Hexatoma* (*Eriocera*) *pianigra* Podenas, 2022. General view of spiracular field.

Material examined. South Korea: Jeollanam-do, Gurve, Masan-myeon, Hwangjeon-ri; 101 m; 35.24366°, 127.48964°, 8 May 2013 (1); 1 larva; leg. V. Podeniene; GenBank accession No. OQ404926. South Korea: Jeollanam-do, Gurye-gun, Toji-myeon, Naeseo-ri, Piagol valley; 593 m; 35.27448°, 127.56378°; 1 July 2015 (2); 1 larva; leg. V. Podeniene. South Korea: Jeollanam-do, Gurye-gun, Toji-myeon, Naeseo-ri, Piagol valley; 310 m; 35.25825°, 127.58208°; 29 June 2015 (2); 6 larvae; leg. V. Podeniene; GenBank accession No. OQ404926 and OQ404929. South Korea: Jeollanam-do, Gurye-gun, Toji-myeon, Naeseo-ri, Piagol valley; 593 m; 35.27448°, 127.56378°; 1 May 2015 (1); 2 larvae; leg. V. Podeniene. South Korea: Gangwon-do, Chuncheon-si, Dongsan-myeon, Bongmyeong-ri, KNU Experimental Forest; 197 m; 37.78194°, 127.81973°; 9 July 2015 (1); 5 larvae; leg. V. Podeniene. South Korea: Gangwon-do, Pyeonchang-gun, Jinbu-myeon, Dongsan-ri, Odaesan NP; 730 m; 37.73767°, 128.59166°; 6 July 2015 (1); 14 larvae; leg. V. Podeniene; GenBank accession No. OQ404927.

Habitat. The larvae of this species develop in the bottom gravel of small and medium-sized rivers.

Pupa. Unknown.

Hexatoma (Eriocera) sp. 1

Figures 17–19

Diagnosis. Larva: the maxilla is short (as long as mandible). Marginal hairs on the lateral and ventral spiracular lobes are similar in length, without significantly extended darker setae. The lateral spiracular lobes each have a dark, narrow sclerite slightly expanding proximally. The ventral spiracular lobes each have a dark, narrow sclerite bifurcating into a "Y"-shaped pattern proximally. The branches are not fused medially. A mature larva is medium sized, with a length of 21.0–32.0 mm.

Figure 17. Larva of *Hexatoma* (*Eriocera*) sp. 1 (**A**) General view, dorsal aspect. (**B**) General view, ventral aspect.

Description. The length of the last instar larvae is 21.0–32.0 mm, and the width is 3.5–4.2 mm. The body is covered with short yellowish-brown hairs, which give the body a golden color (Figure 17A,B). The anterior part of segments has very short appressed hairs, while the posterior part is covered, bearing longer hairs. All thoracic, first, and second abdominal segments are short. The width of abdominal segments III–IV is equal to their lengths. Abdominal segments V–VI are 1.5 times as long as wide. Abdominal segment VII is twice as long as wide. The terminal segment is constricted. The penultimate segment is distinctly inflated, covered with short hairs forming long regular transverse rows; it has four long setae spaced equidistantly from each other on the ventral side.

The head capsule is 2.5 mm long and 1.25 mm wide. In general, it is very similar to that of *H*. (*E*.) *pernigrina* and *H*. (*E*.) *pianigra*. Differences were observed in the length of setae on anterior part of the labrum, the number and shape of basal teeth of mandible, and the length of the basal segment of the antenna (Figure 18A–E). The long seta on the anterior part of the labrum is twice as long as the short seta (Figure 18B,C). The basal segment of the antenna is five times as long as wide (Figure 18D). The mandible has four teeth at the base (Figure 18B,E); the first and third basal teeth are very small; the second and fourth teeth are large and sharp (Figure 18E). The inner part of the maxilla is almost four times as short as the outer lobe. It bears a sclerite at the base and a sensory papilla at the apex. The outer lobe of the maxilla is long and curved outward; the basal part is sclerotized, the apical part bearing only a narrow elongated sclerite; the apical part is membranous, without short setae (Figure 18F). Cardo was reduced to a very small sclerite.

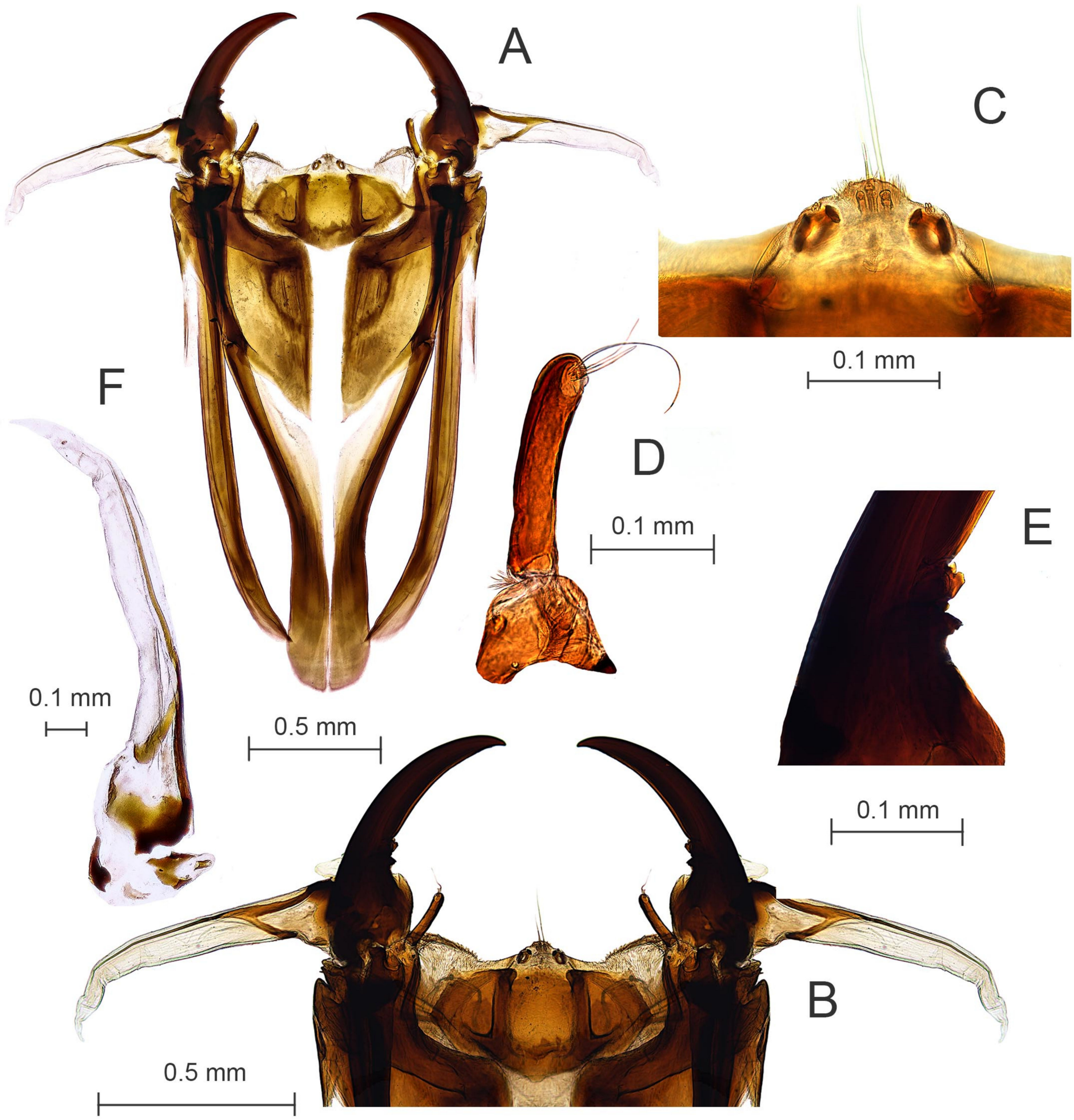

Figure 18. Larva of *Hexatoma* (*Eriocera*) sp. 1. (**A**) General view of head capsule, dorsal aspect. (**B**) Anterior part of head capsule, dorsal aspect. (**C**) Labrum. (**D**) Left antenna, dorsal view. (**E**) Basal part of mandible. (**F**) Right maxilla, dorsal view.

Anal division. A spiracular field is surrounded by four (lateral and ventral) flat elongated lobes (Figure 19). The dorsal lobe is vestigial. The ventral lobe is only slightly longer than the lateral lobe. The lateral lobe is twice as long as the width of the base. The ventral lobe is 2.5 times as long and wide as the base. The apical part of each ventral lobe has dark brown hairs of different lengths, the longest hairs twice as long as the lobe itself. The outer margin of the lateral lobe is completely bordered with hairs of different lengths (hairs reaching the base of spiracles); the apical part of the lobe has hairs slightly longer than the lobe itself. The dorsal margin bears two tufts of long hairs separated by a narrow bare area. The lateral lobe has a narrow, dark sclerite extending into a rhomb proximally. The ventral lobe has a dark, narrow sclerite bifurcating proximally ("Y" shaped sclerite); its outer branch is much longer than the inner. There is a pair of sensory structures at the edge of the spiracular field above each spiracle. There is also a pair of sensory structures located at the dorsal margin of the spiracular field. Spiracles are large, round, with a distance between them less than twice the diameter of a spiracle.

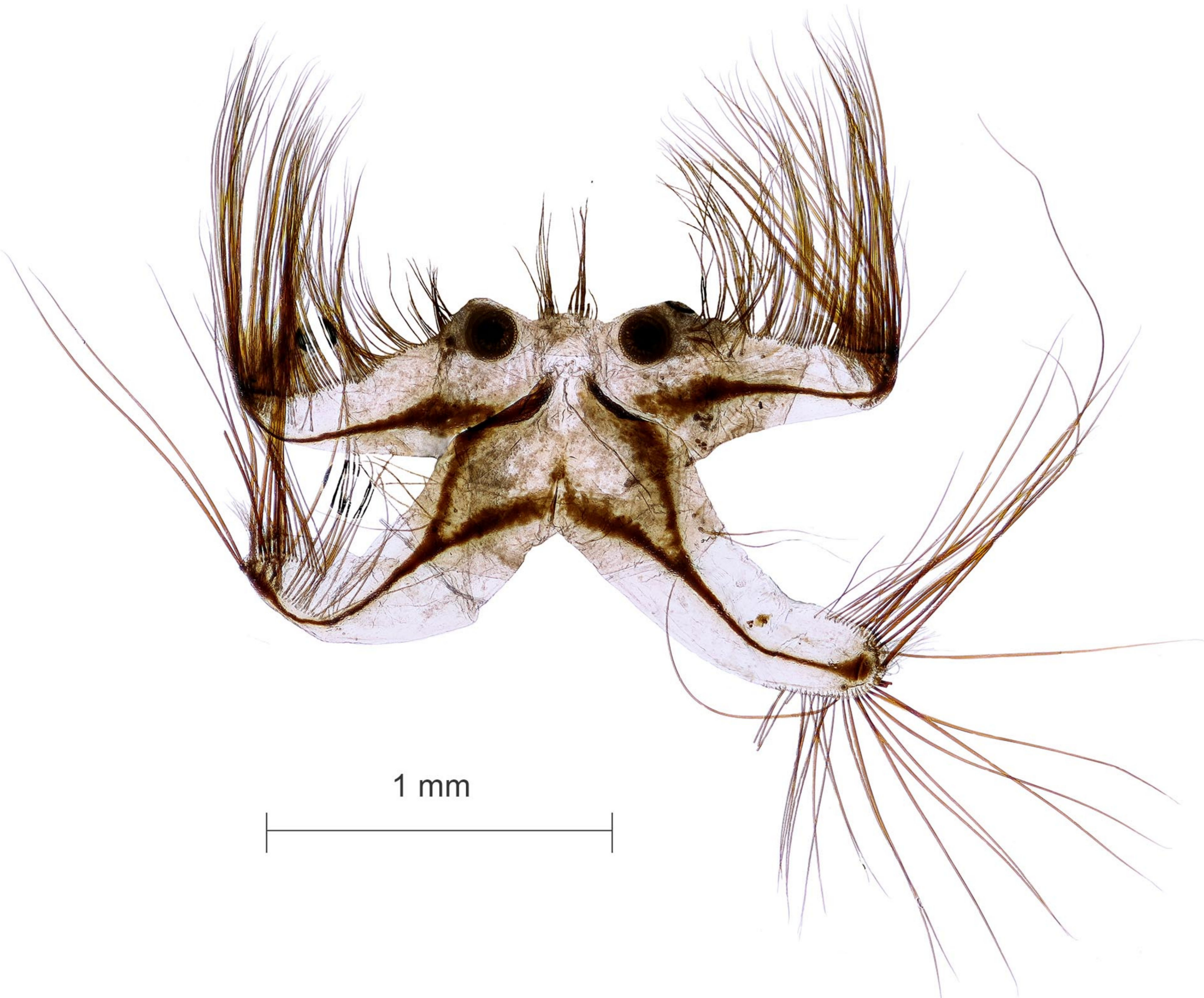

Figure 19. Larva of *Hexatoma* (*Eriocera*) sp. 1. General view of spiracular field.

The anus is surrounded by four long, white, fleshy anal papillae. The lobes are almost conical, the inner pair twice as long as the outer pair (Figure 17B).

Material examined. South Korea: Gangwon-do, Pyoengchang-gun, Jinbu-myeon, Cheokcheon-ri; 19 June 2011; 1 larva; leg. Jeon Jae Bae; NIBR 0000508501; GenBank access No. OQ404933. South Korea: Gyeongsanbuk-do, Nam-gu, Pohang-si, Jeonil-eup, Hakjeon-ri; 24 September 2011; leg. Tae Jung Yoon; NIBR 0000508473. South Korea: Gyeongsanbuk-do, Nam-gu, Pohang-si, Jeonil-eup, Hakjeon-ri; 24 September 2011; leg. Tae Jung Yoon; NIBR 0000508553; GenBank access No. OQ404931. South Korea: Jeollabuk-do, Buan-gun, Wido-myeon, Chido-ri; 28 May 2011; 1 larva; leg. Don Gun Kim; NIBR 0000508472. South Korea: Gyeongsangbuk-do, Gimcheon-si, Mt. Hwangak; 20/April/1996; 3 larvae; NIBR.

Habitat. The larvae of this species develop in the bottom gravel of small and medium-sized rivers.

Hexatoma (Eriocera) sp. 2

Figures 20–22

Diagnosis. Larva: the maxilla is short (as long as mandible). All marginal hairs on the lateral and ventral lobes of the spiracular field are of similar length, without significantly extended darker setae. The lateral spiracular lobes each have a dark, narrow sclerite, significantly expanding proximally. The ventral spiracular lobes each have a thin dark sclerite, bifurcating into a "Y"-shaped pattern proximally, with its lines not fused medially. A mature larva is medium sized, with a length of 19.0–29.0 mm.

Description. The length of the last instar larvae is 19.0–29.0 mm, and the width is 3.0–5.0 mm. The body is covered with very short yellowish hairs, which give the body a whitish color (Figure 20A,B). All thoracic and abdominal segments I–III are short. The width of abdominal segment III is equal to its length. Abdominal segments IV to VI are slightly longer than wide. Abdominal segment VII is twice as long as wide. The terminal (anal) segment is constricted. The penultimate segment is distinctly inflated, covered with short hairs forming long regular transverse rows. It has four long hairs spaced equidistantly from each other on the ventral side.

The head capsule is 2.4 mm long and 1.25 mm wide. In general, it is very similar to that of *H*. (*E*.) *pernigrina*, *H*. (*E*.) *pianigra*, and *H*. (*E*.) sp. 1. Differences were observed in the length of setae on the anterior part of the labrum, number and shape of basal teeth of the mandible, and the length of the basal segment of the antenna (Figure 21A–D). The long seta on the anterior part of the labrum is almost three times as long as the short seta. The basal segment of the antenna is four times as long as the width of the base. The mandible has three teeth at the base (Figure 21B). The first basal tooth is needle-shaped; the second tooth is small and blunt; the third tooth is blunt and twice as large as the second (Figure 21D).

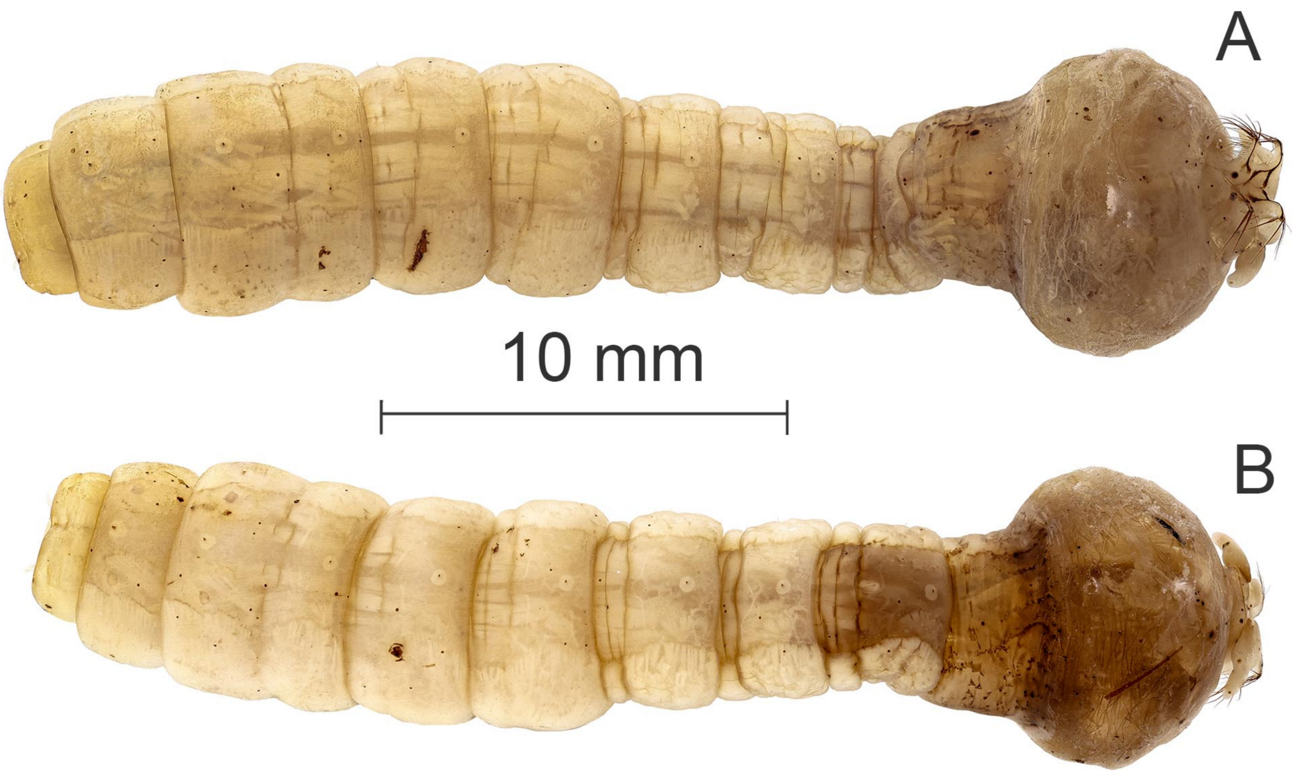

Figure 20. Larva of *Hexatoma* (*Eriocera*) sp. 2 (**A**) General view, dorsal aspect. (**B**) General view, ventral aspect.

Anal division. The spiracular field is surrounded by four (lateral and ventral) flat elongated lobes (Figure 22). The dorsal lobe is vestigial. The ventral lobe is almost twice as long as the lateral lobe. The lateral lobe is twice as long as the width at the base. The ventral lobe is 2.5 times longer than the width of the base. The apical part of each ventral lobe has dark brown hairs of different lengths, the longest hairs being of the same length as the lobe itself; hairs are present only at the apex of the lobe. The outer margin of the lateral lobe is fringed with hairs of different lengths; hairs at the apex are twice as long as the length of the lobe itself. The dorsal margin is not covered with marginal hairs. The lateral lobe has a narrow, dark sclerite, which widened considerably at the base. The ventral lobe has a dark, narrow sclerite bifurcating at the base ("Y"-shaped sclerite), the outer branch much longer than the inner. There is a pair of sensory structures at the edge of the spiracular field above each spiracle. There is also a pair of sensory structures located at the dorsal margin of the spiracular field. Spiracles are very small and round, the distance between them being more than five times the diameter of a spiracle.

The anus is surrounded by four long, white, fleshy anal papillae. The lobes are almost conical, the inner pair twice as long as the outer pair (Figure 20B).

Material examined. South Korea: Jeollabuk-do, Namwon, Jucheon-myeon, Gogi-ri; 450 m; 35.38131°, 127.48412°; 7 May 2013; 3 larvae; leg. V. Podeniene; GenBank access No. OQ404932. South Korea: Chungcheongbuk-do, Boeun-gun, Naesongni-myeon, Daemok-ri, valley; 36.516667°, 127.85°; 16 March 2001; 1 larva; leg. Gab Man Park; NIBR 0000135028.

Habitat. The larvae of this species develop in the bottom gravel of small and medium-sized rivers.

Hexatoma (Eriocera) sachalinensis (Alexander, 1924)

Figure 23

Material examined. North Korea: Kankyo Nando, Puksu Pyaksan; 1828 m; 15/Jun/1939; 1 male (antenna, wing, and middle leg slide mounted); leg. A. Yankovsky; USNM. Russia: Saghalien, Maoka "[Sakhalin Island, Kholmsk]"; paratype; 28/Jul/1922; 1 male (wing slide mounted); leg. T. Esaki; USNM. Mongolia: Uvs Aimag, Davst Soum, Tokhilog Gol, near Mongolian military border outpost; 1003 m; 50.69165°, 092.59283°; 15–15 July 2010; leg. S. Chuluunbat, S. Podenas; MAIS2010071403; ANSP.

This is a new record of species for the Korean Peninsula.

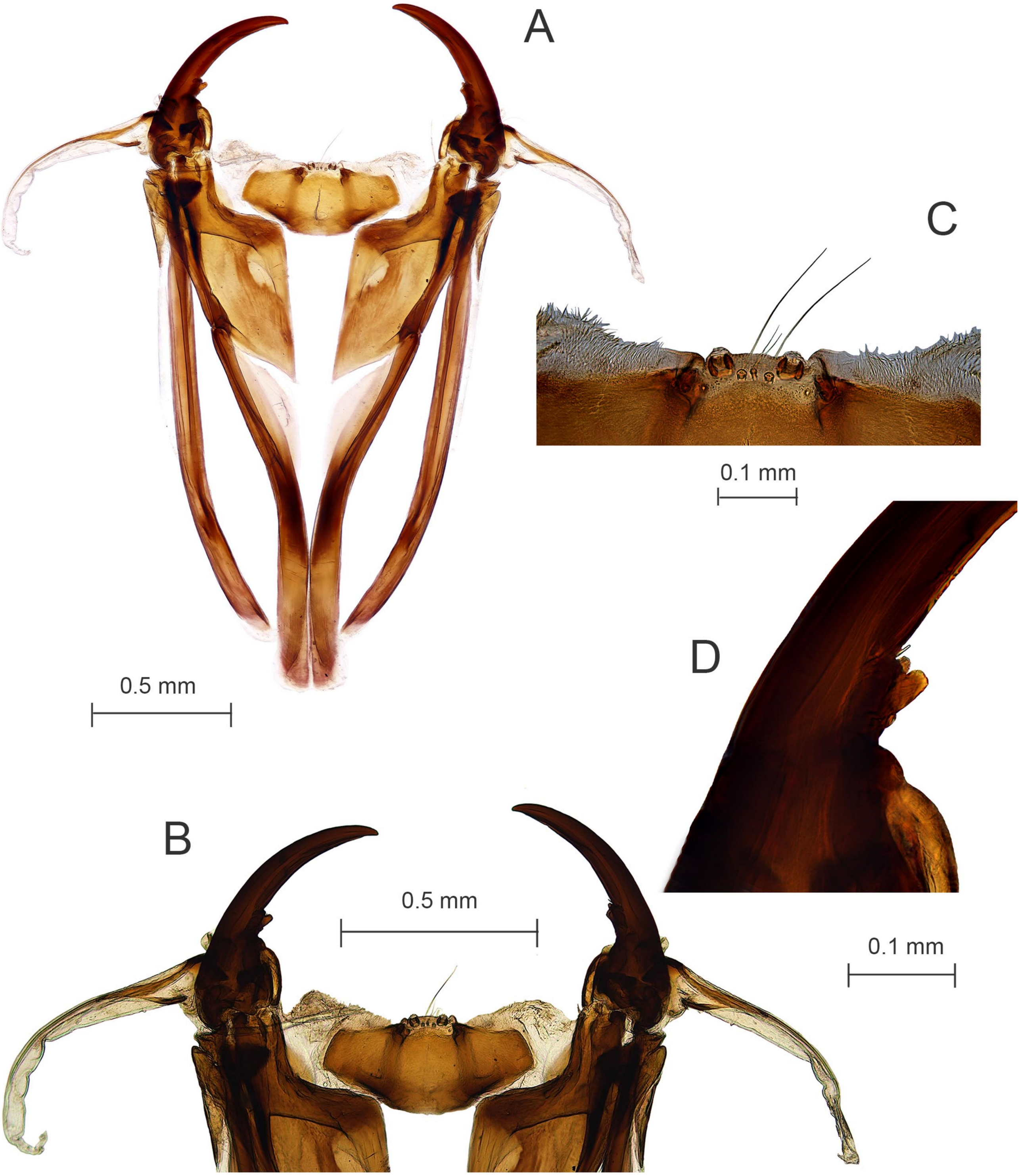

Figure 21. Larva of *Hexatoma* (*Eriocera*) sp. 2. (**A**) General view of head capsule, dorsal aspect. (**B**) Anterior part of head capsule, dorsal aspect. (**C**) Labrum. (**D**) Basal part of mandible.

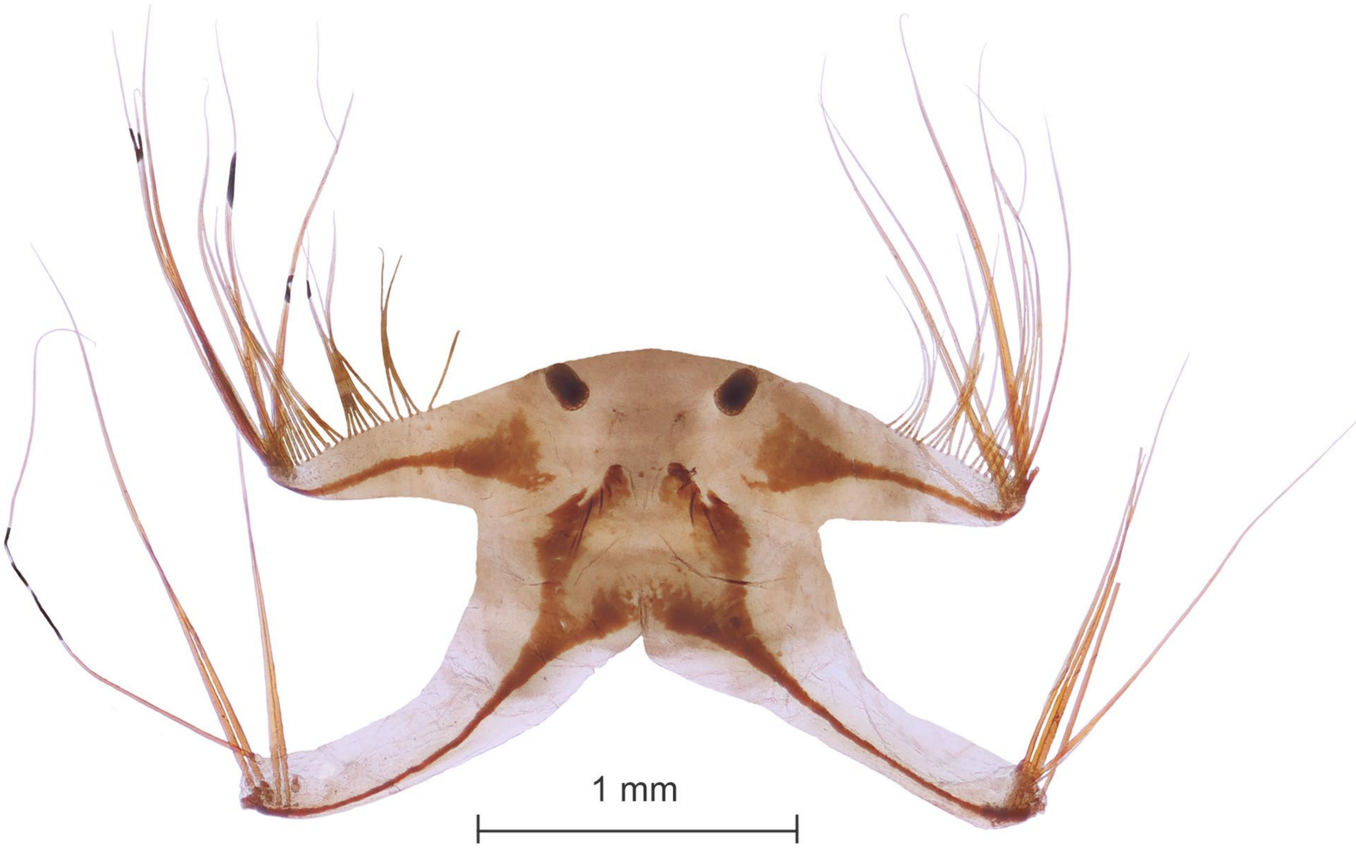

Figure 22. Larva of *Hexatoma* (*Eriocera*) sp. 2. General view of spiracular field.

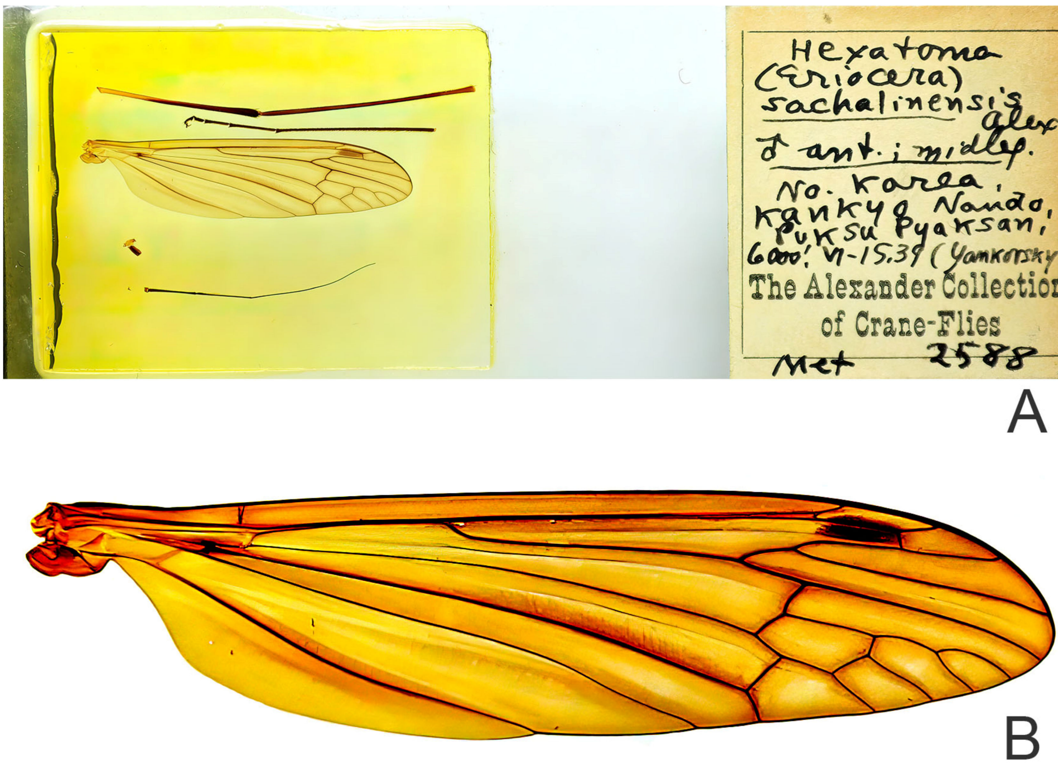

Figure 23. *Hexatoma* (*Eriocera*) *sachalinensis* (Alexander, 1924). (**A**) Slide of Korean specimen in USNM. (**B**) Wing.

4. Discussion

Previous studies have shown that there is no last instar larval characteristic to differentiate between the subgenera *Eriocera* and *Hexatoma* and that differences between the two subgenera have only been found at the pupal stage [4]. Immature stages of subgenera *Cladolipes* Loew, 1865; *Euhexatoma* Alexander, 1936; *Coreozelia* Enderlein, 1936; and *Parahexatoma* Alexander, 1951 are not yet known. The larvae of the subgenus *Eriocera* have been found to be morphologically distinct, despite the fact that only 15 larvae of 563 species are known so far [4]. Based on the morphological characteristics, the larvae of *Eriocera* can be divided into several groups [4]. The Korean species *H.* (*E.*) *gifuensis* belongs to a group consisting of a mixture of species comprising the subgenera *Eriocera* and *Hexatoma*, such as *H.* (*E.*) *ussuriensis* Alexander, 1934; *H.* (*E.*) *gifuensis*; *H.* (*E.*) *fuliginosa* (Osten Sacken, 1860); *H.* (*E.*) *fultonensis* (Alexander, 1912); *H.* (*E.*) *longicornis* (Walker, 1848); *H.* (*E.*) *stackelbergi* Alexander, 1933; *H.* (*H.*) *bicolor* (Meigen, 1818); *H.* (*H.*) *fuscipennis* (Curtis, 1836); *H.* (*H.*) *megacera* (Osten Sacken, 1860); *H.* (*H.*) *vittata* (Meigen, 1830); and *H.* (*H.*) *nubeculosa* (Burmeister, 1829). This group is characterized by the shape of the labrum and clypeus, the length of the antennae and mandibles, the extended setae on the apices of the ventral lobes of the spiracular field, and the arrangement of the setae on the penultimate abdominal segment. The lateral lobes of the labrum are long and narrow, sickle-shaped, directed outwards, and densely covered with long hairs. The larvae of this group are characterized by a posteriorly tapering clypeus, long antenna, and maxilla more than twice as long as the mandible. They have four long setae on the ventral side of the penultimate segment, with setae 1–2 and 3–4 very close to each other. The shape and sclerotization of spiracular lobes, the color of the marginal setae of the spiracular field, the length and arrangement of the body hairs (especially on the terminal segment), and the number and shape of the basal teeth of the mandible are the characteristics by which species of this group can be distinguished.

Hexatoma (*E.*) *pernigrina* and *H.* (*E.*) *pianigra*, and two not associated with adult species, *H.* (*E.*) sp. 1 and *H.* (*E.*) sp. 2, belong to the subgroup *spinosa*, which comprises the abovementioned species, the North American species *H.* (*E.*) *spinosa* (Osten Sacken, 1860), and the East Asian species *H.* (*E.*) *sachalinensis* (Alexander, 1924). In this group, the lateral lobes of the labrum are short and broad at the base, with the lobes membranous and sparsely covered with setae. The larvae of this group are characterized by a posteriorly broadly rounded clypeus, short antennae, and maxillae less than twice as long as the mandible. The larvae of this group have four long setae on the ventral side of the penultimate segment, with all setae equidistant from each other. The shape and sclerotization of the spiracular lobes, the length of the marginal setae of the spiracular field (especially at the apex of the spiracular lobes), the length and arrangement of body hairs, the number and shape of the basal teeth of the mandible, and the distance between the spiracles are the characteristics by which this group can be distinguished. Some of the characteristics of the pupa of *H.* (*E.*) *pernigrina* are similar to those of the pupa of *H.* (*E.*) *spinosa*, both having a highly reduced cephalic crest and the respiratory horns at the apex of the pupa of this group is sharply pointed. However, characteristics of the posterior segment seem to be similar to those of *H.* (*E.*) *californica* (Osten Sacken, 1877), both of which typically have a ring of spines in the anterior part of the terminal segment, which is not characteristic of other species of the subgenus *Eriocera*.

Phylogenetic analysis based on molecular markers not only helps to solve taxonomic problems and facilitates the association of larvae to putative adults but also reveals important adaptive radiations represented by unique sets of morphological characteristics [16,17]. To date, sequences of seven *Hexatoma* species, *H.* (*H.*) *fuscipennis*; *H.* (*E.*) *gressittiana* Alexander, 1943; *H.* (*E.*) *longicornis*; *H.* (*H.*) *nigra* Latreille, 1809; *H.* (*E.*) *nudivena* Alexander, 1933; *H.* (*H.*) *obscura* (Meigen, 1818); and *H.* (*E.*) *spinosa*, are deposited in GenBank and BOLD systems as well as sequences of unidentified *Hexatoma* specimens. In previous studies, *Hexatoma* sequences were analyzed as a part of barcoding projects [18–22] or phylogenetic analyses of the order Diptera [23,24] or superfamily Tipuloidea [25]. Our study is the first contribution to the *Hexatoma* larvae taxonomy using phylogenetic analysis based on mitochondrial COI fragment (DNA barcode) and one of the first attempts to reveal phylogenetic relationships between *Hexatoma* species using molecular markers. Previous studies, based on adult morphological characteristics [8] or larval and pupal morphological traits [14], revealed that the genus *Hexatoma* is not monophyletic. Our analysis shows that *Eriocera*, the largest subgenus of the genus *Hexatoma*, is not monophyletic, a hypothesis that is supported both by larval morphological and genetic characteristics. Two highly divergent lineages can be observed in our ML phylogenetic tree, and this is supported by larval morphological characteristics. One monophyletic clade includes larvae of *H.* (*E.*) *pernigrina*, *H.* (*E.*) *pianigra*, and two not associated with adult species: *H.* (*E.*) sp. 1 and *H.* (*E.*) sp. 2. They share similarities in the head capsule (shape of labrum and clypeus and length of antenna and maxillae), the presence of extended setae on the apices of ventral lobes of the spiracular field, and the location of the setae on the penultimate abdominal segment. Their ventral lobes of spiracular disc bear "Y"-shaped sclerite, separated from its opposing sclerite. *H.* (*E.*) *gifuensis* is morphologically distinct from the previous group and forms a separate clade, which is supported by high bootstrap values and significant genetic distance as well as morphological characteristics, such as being long and narrow, sickle-shaped, directed outwards, and densely covered with long hairs lateral lobes of the labrum and having posteriorly tapering clypeus, long antenna, and maxilla more than twice as long as mandible. It has four long setae on the ventral side of the penultimate segment, with setae 1–2 and 3–4 very close to each other. The ventral lobes of the spiracular disc of *H.* (*E.*) *gifuensis* bear "Y"-shaped sclerite, the inner branch of which is fused with its opposing sclerite. More molecular as well as morphological data on immature and adult characteristics are needed to better understand the taxonomy of this huge and diverse taxonomical group. It is very likely that morphologically and genetically distinct clades could represent different subgenera.

All larvae used in this research were collected from small to medium-sized rivers with gravel or sandy bottoms. We did not observe differences in ecological requirements (water body type) of morphologically different larvae.

Author Contributions: Conceptualization, V.P. and S.P.; methodology, V.P. and M.-J.B.; formal analysis, V.P. and J.H.; investigation, V.P.; data curation, V.P.; writing—original draft preparation, V.P.; writing—review and editing, V.P.; visualization, V.P., S.P. and J.H.; funding acquisition, S.-J.P. and C.-H.B. All authors have read and agreed to the published version of the manuscript.

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