Journal of Insect Biodiversity

ISSN: 2147-7612

RESEARCH ARTICLE

Morphology of two *Larinus* Dejean (Coleoptera: Curculionidae) species introduced into North America for biological control of knapweeds

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Abstract: The morphology of two introduced knapweed feeders *Larinus obtusus* Gyllenhal, 1835 and *Larinus minutus* Gyllenhal, 1835 is investigated. The external and internal morphological characters are described for both sexes. The most promising discriminative characters including male and female terminal segments and genitalia structure are illustrated.

Key words: introduced *Larinus*, morphology, Lixinae, weed biological control agent.

Introduction

Some species of *Larinus* Dejean, 1821 (Coleoptera: Curculionidae) are considered beneficial phytophagous weevils associated with Carduoideae (Asteraceae) important for the biological control of thistles where native (Abela-Hofbauerová *et al.* 2011, Gültekin 2004; 2006; Gültekin *et al.* 2008; Zwölfer *et al.* 1971), and where they have been intentionally introduced as biocontrol agents (Lang *et al.* 1996, McClay 1990, Sobhian & Fornasari 1994, Woodburn & Briese 1996). According to present knowledge, in the Nearctic region five *Larinus* species have been introduced to or have accidentally arrived, of which four are listed by O'Brien & Wibmer (1982), and another, *Larinus turbinatus* Gyllenhal, 1835, has been added by Hoebeke & Spichiger (2016).

Knapweeds (*Centaurea stoebe* L. and *Centaurea diffusa* Lamarck) are invasive plants that have been the target of classical biological control in North America for more than four decades. The seedhead-feeding weevils *Larinus minutus* Gyllenhal, 1835 and *Larinus obtusus* Gyllenhal, 1835 are two of the most-widely released control agents (Harris 2005; Lang *et al.*

1996), and have more recently been introduced into the eastern United States (Carson & Landis 2014).

Recently, a comprehensive taxonomic review has been published by Gültekin & Alonso-Zarazaga (2015) based on examination of the Schoenherr collection which contains considerable numbers of type specimens of *Larinus* described by Gyllenhal (1835). Gültekin & Alonso-Zarazaga (2015) designated several lectotypes, including those of *L. obtusus* and *L. minutus*, with digital photographs of type material. However, for applied entomologists is sometimes difficult to correct name these two very similar species basing on only external morphological features, as highlighted by Harris (2005) with the following sentence "When the species are difficult to distinguish morphologically, considerable confusion can occur over the impacts, distributions and coexistence of each. This is currently the situation with *L. minutus* and *L. obtusus* introduced on knapweeds in North America."

The aim of the current paper is to help discriminate these two species by presenting detailed comparative external and internal morphological characters useful for species recognition.

Material and methods

Measurements were taken using a stereomicroscope Leica MZ7.5 with an ocular micrometer. Body length was measured from the anterior margin of eye to the posterior margin of elytra; rostrum length from apex of rostrum to anterior margin of eye; and prothoracic length from anterior to posterior margin.

For dissections, dry adult samples were placed in lukewarm clean water overnight. All sclerotized body parts of females were dissected completely, whereas only the abdomen of males was removed and dissected. Specimens were placed in 10% KOH overnight, cleaned with distilled water, and rinsed in 70% ethanol. Observations and photographs of internal structures were made in glycerine under a stereomicroscope. All genitalia and cleared structures were glued dry on cards and mounted under the pinned specimen from which they were dissected.

Photographs of morphological characters were taken with a Canon EOS 70D DSLR digital camera attached to a Leica Z16APO macroscope provided with a ring LED light using EOS Utility software. Digital images were then elaborated with Adobe Photoshop 6.0 for stacking, and the program CorelDRAWX7 was used for labelling and plate composition.

Specimens collected in Turkey, Russia, Transcaucasia, Balkans, Italy and North America were used for morphology and photos.

Comparative diagnostic morphological characters are summarized in Table 1.

The abbreviation meaning are F: funicle, T: tarsomere.

Results

Larinus obtusus Gyllenhal, 1835

Body ovate (Figs 1A–B) of black and matt color; scape always, funicle and claws occasionally, apices of tibiae and femora and dorso-apical margin of prothorax chesnut-brownish. Body length 4.10–6.50 mm, width 2.40–3.40 mm. Vestiture of short recumbent grayish narrow hair-like scales, elytra always with distinct mottled pattern in fresh samples. Underside of head and postocular area without bi- or trifurcate scales (Fig. 5A), surface of

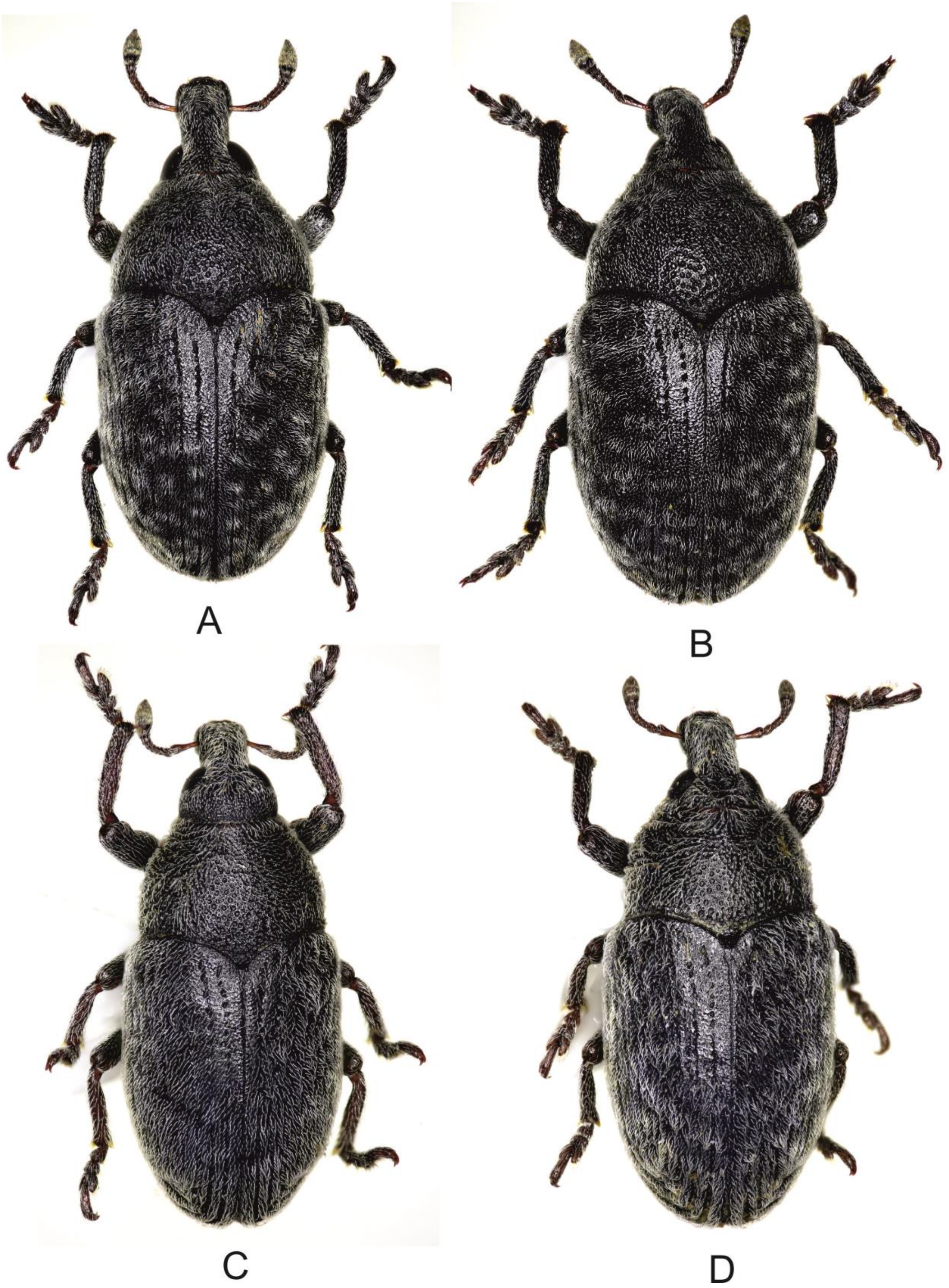

Figure 1. Adults of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal in dorsal view. **A,** *L. obtusus*, male; **B,** *L. obtusus*, female; **C,** *L. minutus*, male; **D,** *L. minutus*, female.

mesosternum and metepisternum with numerous such scales. Legs covered with short recumbent or subrecumbent hair-like scales, bi- or trifurcate scales more dense on coxae, especially on mesocoxae.

Head spherical; forehead slightly to moderately depressed, interocular area narrower than base of rostrum, interocular pit present; eyes broad ovate, 1.70–1.85× as wide as long, large and slightly convex. Rostrum in dorsal view (Figs 2A–B) subrectangular, subparallel sided, 0.55–0.67× as long as pronotum in male and 0.60–0.80× in female, dilated after antennal insertion, apex 1.18–1.22× as wide as its base in male and 1.25–1.30× in female. Epifrons convex with a raised sharp central carina and two obtuse interrupted submarginal carinae; frons transversely and weakly depressed before epistomal area, the three carinae continue to the basal half of frons, frontal pit visible, surface of epifrons and frons finely and densely punctuated (Figs 2A–B). Basal margin of scrobes partly visible dorsally. In lateral view rostrum (Fig. 3A) strongly curved and pregena wider than subgena. Punctures subrounded and coarse on subgena. Scrobes not connected each other at base of rostrum ventrally, submentum strongly swollen toward anterior apex (Fig. 3C). Antenna (Fig. 4A): scape clubbed, gradually tapering anteriorly, suddenly and strongly swollen at anterior apex, 1.20–1.40× as wide as F1, 0.55–0.58× as long as total length of funicle; F1 and F2 subconical, F1 1.15–1.20× as long as F2; F3 almost subequal in length and width, and 0.75–0.80× as long as F2; F4–F7 gradually widened, transverse, F7 the widest, F5–F7 distinctly wider than previous ones; F7 1.76–1.87× as wide as F1. Club elongate with acuminate apex, 1.60–1.75× as long as wide at widest part.

Prothorax (Fig. 5C) subtrapeziodal, transverse, 1.50–1.60 times as wide as long, basal margin strongly prolonged toward elytra at scutellar corner, lateral margin parallel-sided at basal fourth, gradually, strongly and roundly tapering anteriorly, constricted just before apex in the form of short apical collar. Pronotal disc convex, two types of punctuation on surface, large rounded punctures sparse, small micro-punctures dense on their interspaces. Apical margin of prothorax evenly curved toward postocular area, postocular lobes slightly developed, prosternum moderately and roundly emarginate. Intercoxal process prolonged between procoxa. Mesoscutum subtrapeziodal, surface with subround dense punctures, scutellum small with short stalk, apex of scutellum reaches the level of posterior margin of postscutellum, postmedial projection roundly and distinctly expanded, wider than width of prealar projection (Fig. 5E). Metepisternum transverse, anteriorly broadened with sharp antero-ventral projection, posteriorly narrowed. Anterior margin 1.54–1.65× as wide as width of posterior margin.

Elytra subparallel-sided at basal half, gradually narrowed from basal fourth to posterior apex, widest at base, 1.20–1.30× as long as wide, 1.13–1.21× as wide as prothorax. Elytral interstriae flat, of subequal width on elytral disc; striae fine, shallow and formed by separated or partly confluent punctures on disc.

Legs. Procoxa slightly separated each other by anterior sternellar tubercle, protibiae 0.90–0.95× as long as rostrum length, in male outer margin slightly curved apicad of basal third, inner margin strongly emarginate and serrate, premucro reduced, of very obtusely triangular shape before uncus, unci strong, large and sharp, distinctly longer than premucro (Fig. 6A). In female outer margin of protibia nearly straight, inner margin moderately emarginate after basal third and serrate, premucro strongly developed with a sharp and wide triangular plate (Fig. 6B). Unci well developed, slightly longer than premucro. Spines on apical comb dense (9–10 spines present), base of spines partly connected each other. Meso- and metatibia slightly and gradually dilated from base to apex, spines on apical comb longer and denser than protibial ones. Tarsi (Fig. 7A) wide, T1 triangular with asymmetrical base,

Figure 2. Rostrum of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal in front view. **A,** *L. obtusus*, male; **B,** *L. obtusus*, female; **C,** *L. minutus*, male; **D,** *L. minutus*, female.

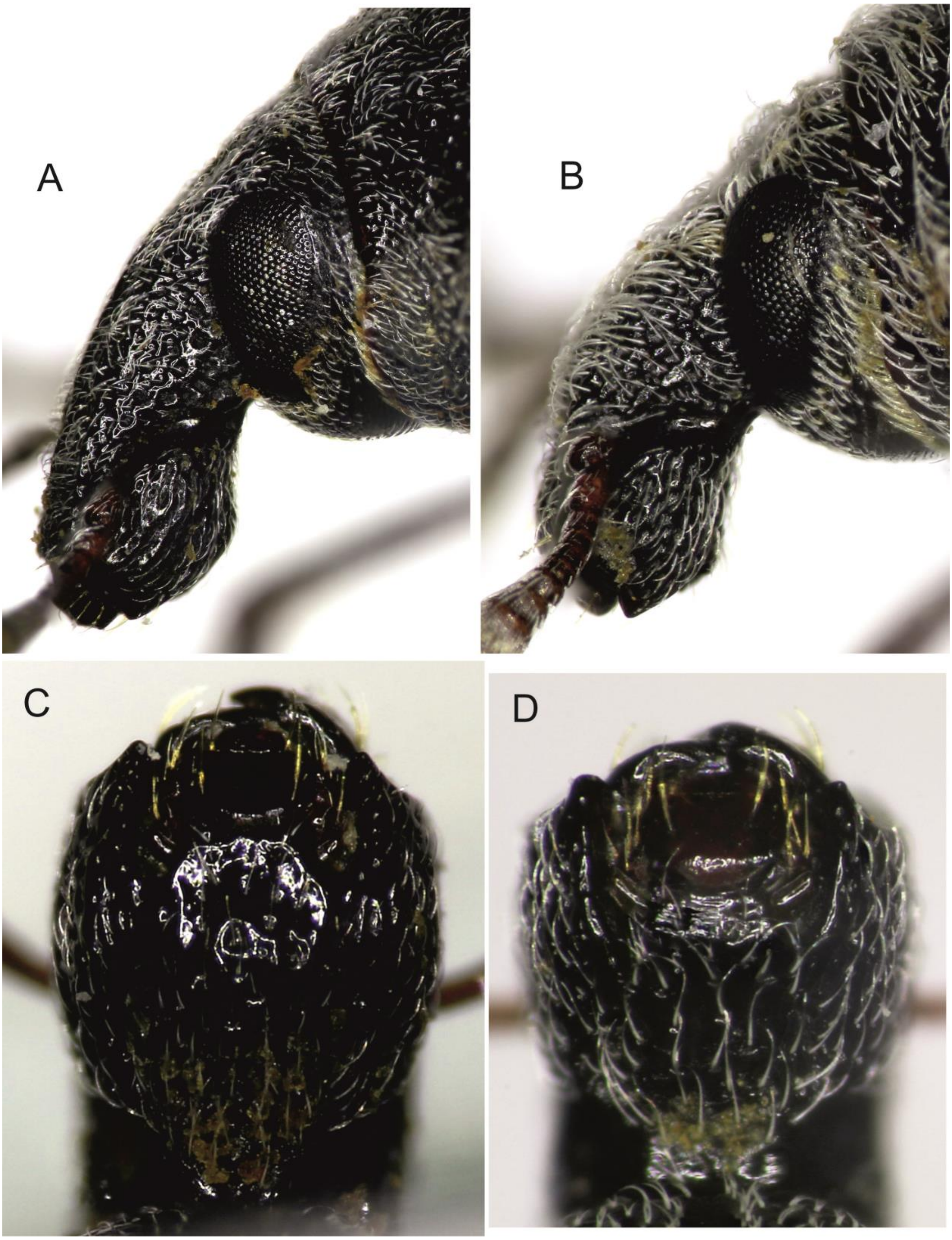

Figure 3. Rostrum of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal in lateral and ventral view, female. **A,** *L. obtusus*; **B,** *L. minutus*; **C,** *L. obtusus*; **D,** *L. minutus*.

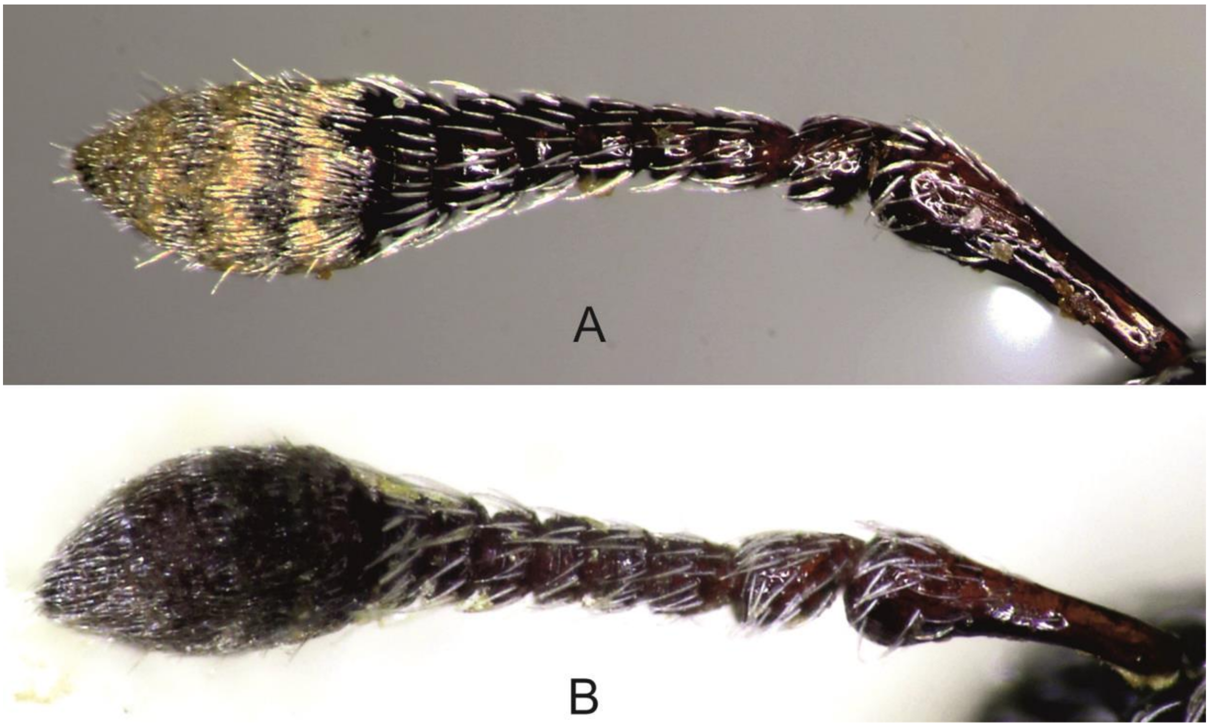

Figure 4. Antenna of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal. **A,** *L. obtusus*, female; **B,** *L. minutus*, female.

T2 trapeziform, T3 bilobed and 1.30–1.40× as wide as T2 in widest part; T5 cylindrical, curved, gradually widened toward apex, 1.45–1.55× as long as T3. Claws connate at base, slightly unequal length (Fig. 7C). Spongy pads complete and dense on T1–T3 except for a bare central line.

Abdomen. Ventrites broadly trapeziform, 1.05–1.13× as wide as long at widest part, visible first ventrite depressed medially in male, flat in female.

Male terminal segments and genitalia. Tergite 8 (Fig. 8A) subtrapeziodal and well sclerotized; sternite 8 (Fig. 8E) contiguous. Penis in dorsal view (Figs 9A–C) nearly parallel-sided, slightly constricted at basal and apical third, weakly swollen at middle, dorsal surface membranous, dorso-lateral margins sclerotized, median orifice wide, ventral plate angularly narrowed, apex obtuse. In lateral view penis strongly curved, lateral margins completely sclerotized. Spiculum gastrale stick-like, thick and curved (Fig. 9D).

Female terminal segments and genitalia. Tergite 8 (Fig. 8C) subtrapeziodal, central part membranous. Sternite 8 nearly Y-shaped with short apodeme (Fig. 10A), lateral arms three times longer than apodeme, vertical arm short, narrow angularly and abruptly bent inward, posterior margin bearing a series of short hairs. Gonocoxites moderately sclerotized (Fig. 10B), hemisternite 1.50–1.65× as long as wide at widest part, base of stylus short, conical and slanted inward posteriorly, outer margins bearing 2–3 short hairs; stylus subconical, slightly longer than base of stylus, slightly bent inward, bearing 3–4 very short hairs on tip. Spermatheca C-shaped, ramus slightly wider than nodulus, apex of cornu obtuse (Fig. 10C).

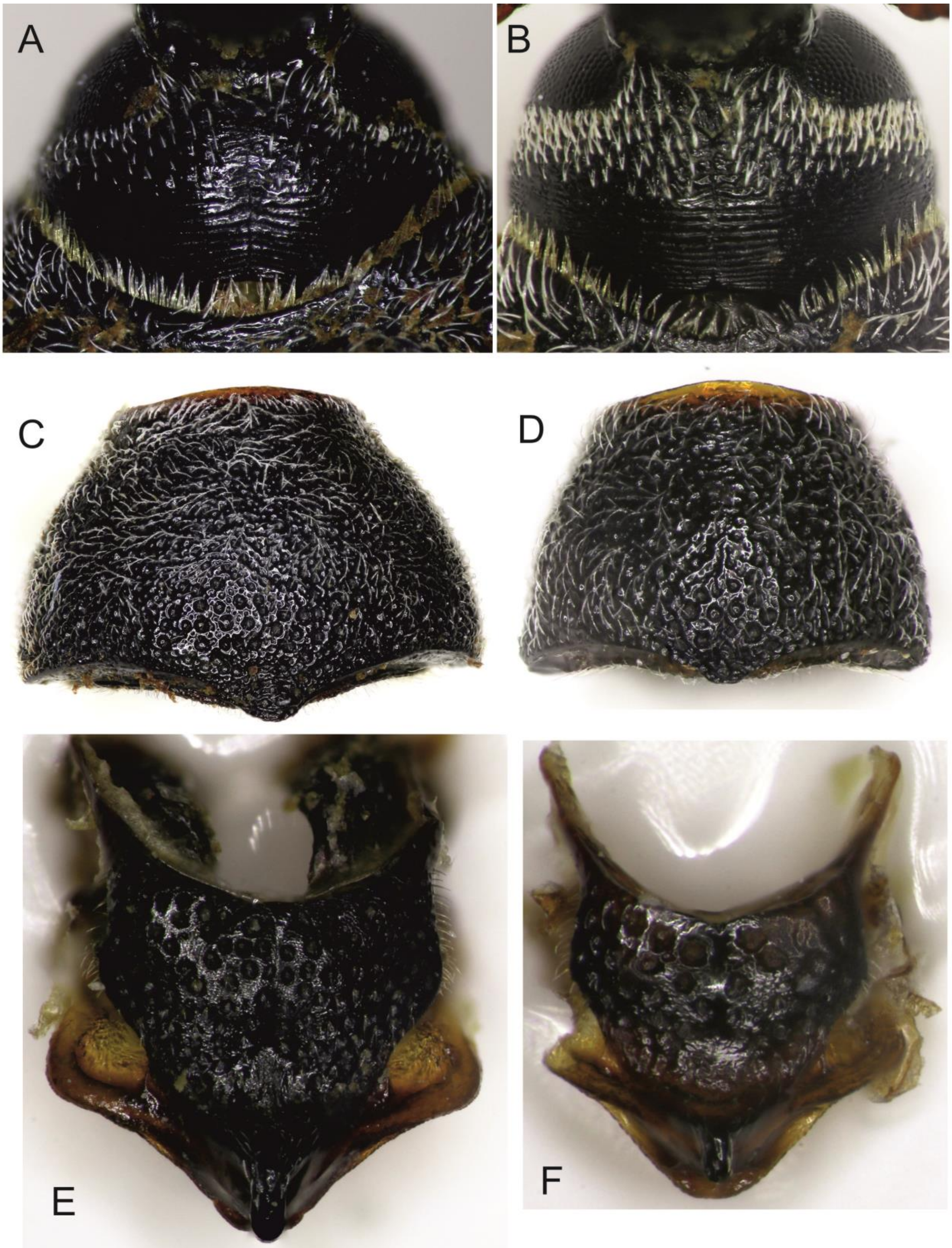

Figure 5. Head, prothorax and mesothorax of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal. **A,** head in ventral view of *L. obtusus*; **B,** head in ventral view of *L. minutus*; **C,** prothorax in dorsal view of *L. obtusus*; **D,** prothorax in dorsal view of *L. minutus*; **E,** mesothorax in dorsal view of *L. obtusus*; **F,** mesothorax in dorsal view of *L. minutus*.

Larinus minutus **Gyllenhal, 1835**

Body subovate (Figs 1C–D), piceous; tibiae, tarsi, antenna and dorso-apical margin of prothorax always reddish brown. Body length 3.50–6.50 mm, width 1.70–3.10 mm. Vestiture of longer, more strongly raised and uniform grayish hair-like pubescence, usually lacking distinct macules and mottled pattern on elytra. Underside of head (Fig. 5B) and postocular area with bi- and trifurcate scales, surface of mesosternum and metepisternum with very sparse and tiny such scales. Legs covered with long semi-erect hair-like scales or hairs, bi- or trifurcate scales more dense on coxae, especially on mesocoxae.

Head spherical; forehead nearly flat, weakly depressed around interocular pit; interocular area narrower than base of rostrum, eyes broad ovate, 1.65–1.75× as wide as long, large and slightly convex. Rostrum in dorsal view (Figs 2C–D) subrectangular, subparallel-sided, 0.60–0.70× as long as pronotum in male, 0.65–0.77× in female, widened apicad of antennal insertion, apex 1.10–1.13× as wide as its base in male, 1.15–1.18× in female. Epifrons convex with an obtuse central carina and two weak interrupted submarginal carinae; frons transversely and weakly depressed before epistomal area, the three carinae continue to the basal half of frons, frontal pit slightly visible, surface of epifrons and frons finely and densely punctured. Basal margin of scrobes partly visible dorsally. In lateral view rostrum strongly curved, pregena slightly wider than subgena. Punctures fine and dense on epifrons, partly concealed by hairy pubescence. In lateral view rostrum strongly curved, pregena slightly wider than subgena. Punctures on subgena larger than those on pregena. Scrobes not connected each other at base of rostrum ventrally (Fig. 3B), submentum weakly swollen toward anterior apex (Fig. 3D). Antenna (Fig. 4B): scape clubbed, gradually widened from base to apex, suddenly and strongly swollen at apex, 1.35–1.62× as wide as F1, 0.65–069× as long as total length of funicle; F1 subconical, 1.35–1.40× as long as F2; F3–F5 very slightly and gradually widened apically, F4 and F5 transverse and subequal in length and width each other, F6–F7 distinctly and gradually widened, F7 the widest; F7 1.60–1.65× as wide as F1. Club elongate with acuminate apex, 1.50–1.63× as long as wide at widest part.

Prothorax subtrapezoidal (Fig. 5D), transverse, 1.35–1.50× as wide as long, basal margin moderately prolonged toward elytra at scutellar corner, lateral margin parallel-sided at basal third, gradually, slightly and roundly tapering anteriorly, slightly constricted just before apex in the form of short apical collar. Pronotal disc nearly flat, rounded punctures of moderate to small size dense on disc. Apical margin of prothorax evenly curved toward postocular area, postocular lobes slightly developed, prosternum moderately and roundly emarginate. Intercoxal process prolonged between procoxae. Mesoscutum subtrapeziodal, surface subroundly and coarsely punctuated, scutellum small with short stalk, apex of scutellum not reaching the level of posterior margin of postscutellum, postmedial projection moderately expanded, narrower than prealar projection width (Fig. 5F). Metepisternum transverse, anteriorly slightly broadened with sharp projection antero-ventrally, posteriorly narrowed. Anterior margin 1.35–1.40× as wide as width of posterior margin.

Elytra usually subparallel-sided on basal half, narrowed from middle to apex, widest at base, 1.35–1.40× as long as wide, 1.05–1.10× as wide as prothorax. Elytral interstriae flat, subequal in width on elytral disc; striae fine, shallow and formed by separate punctures on disc.

Legs. Procoxa slightly separated each other by anterior sternellar tubercle, protibiae subequal in length to rostrum, in both sexes outer margins of protibia nearly straight, inner margin moderately emarginate and very obtusely serrate, male premucro strongly reduced (Fig. 6C), female with weakly serrate margin and a sharp denticle on premucro (Fig. 6D). Unci well developed, distinctly longer than premucro in both sexes. Spines on apical comb

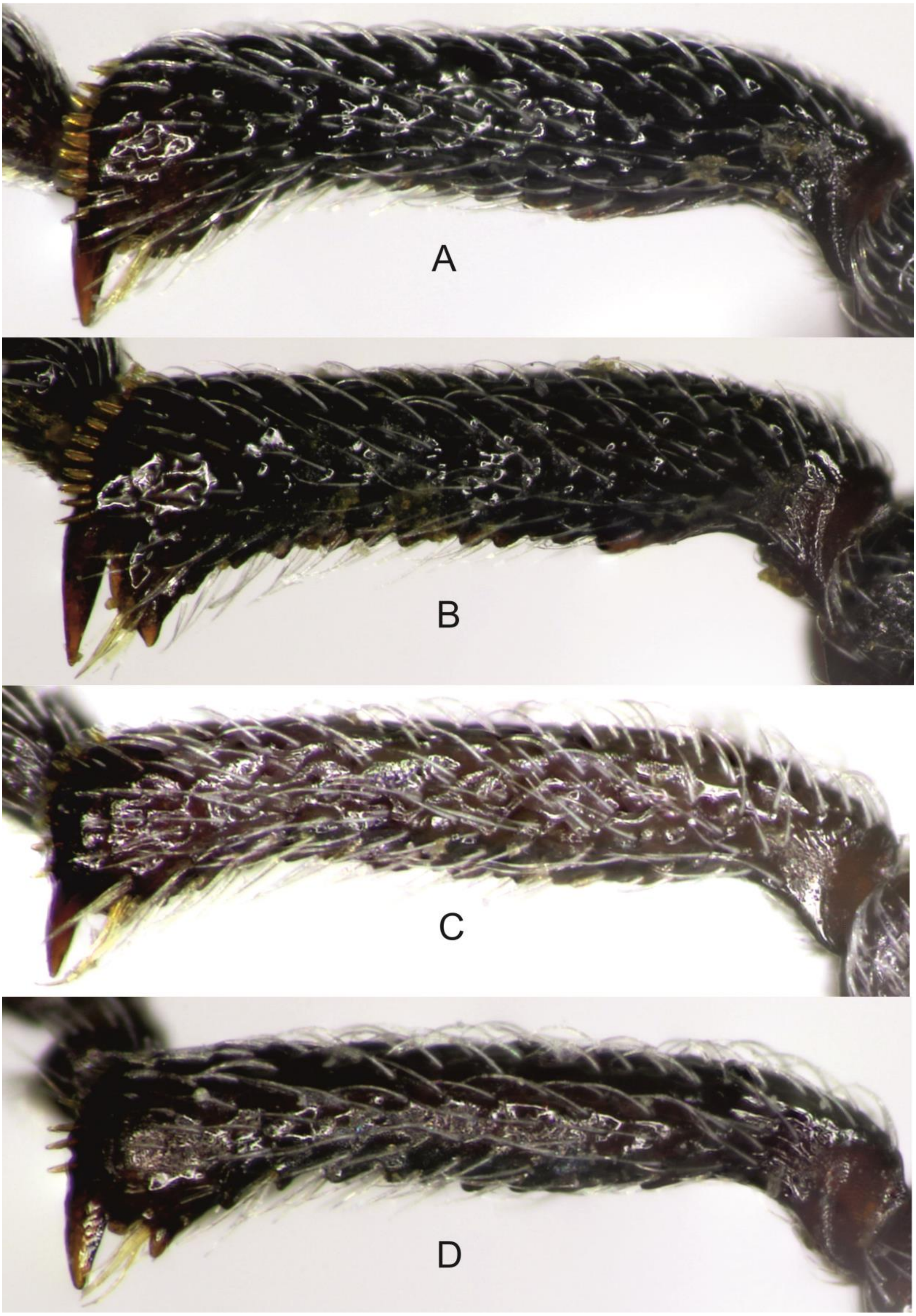

Figure 6. Protibia of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal in dorsal view. **A,** *L. obtusus*, male; **B,** *L. obtusus*, female; **C,** *L. minutus*, male; **D,** *L. minutus*, female.

sparse (5-7 spines present), base of spines not connected each other. Meso- and metatibia very slightly and gradually dilated from base to apex, spines on apical comb longer and denser than those on protibia. Tarsi (Fig. 7B) narrow, T1 triangular with asymmetrical base, T2 trapeziform, T3 bilobed and 1.20–1.33× as wide as T2 at widest part; T5 cylindrical, curved, gradually widened, 1.40–1.65× as long as T3. Claws connate at base, of distinct unequal length (Fig. 7D). Spongy pads moderately dense under T1–T3 except for a bare central line.

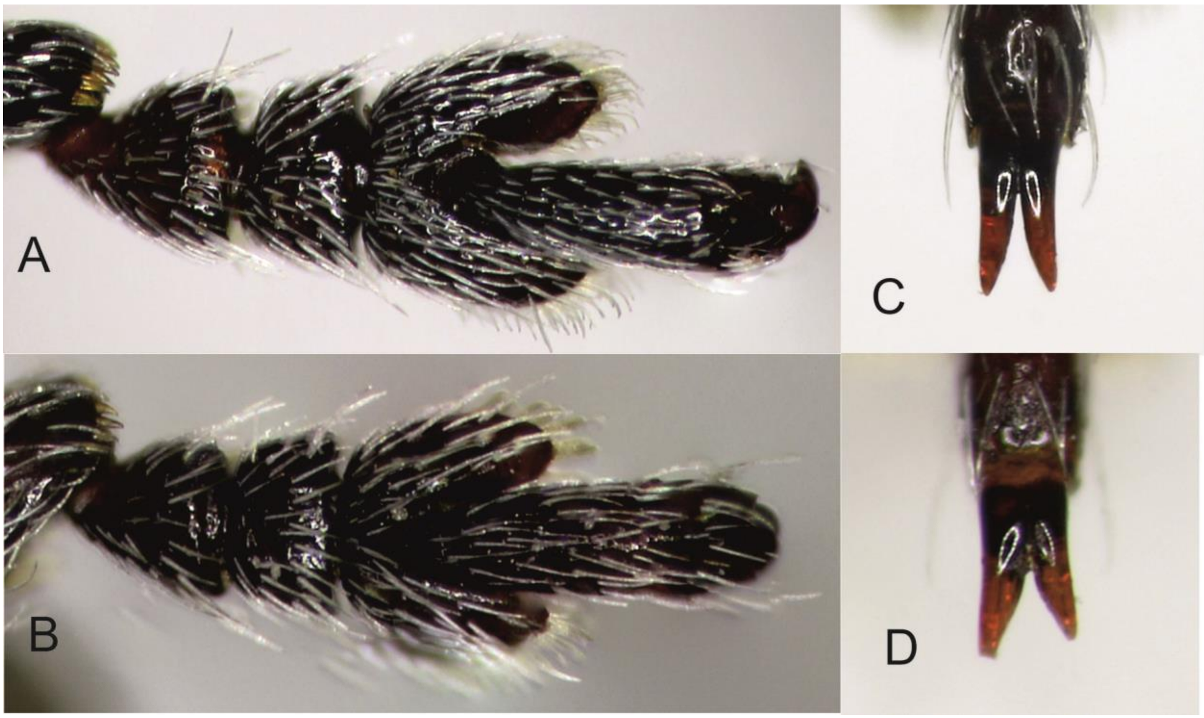

Figure 7. Protarsi and claws of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal in dorsal and front view. **A,** *L. obtusus*, tarsus; **B,** *L. minutus*, tarsus; **C,** *L. obtusus*, claws; **D,** *L. minutus*, claws.

Abdomen. Ventrites narrowly subtrapeziform, 0.85–0.90× as wide as long at widest part, visible first ventrite depressed medially in male, flat in female.

Male terminal segments and genitalia. Tergite 8 subtrapeziodal (Fig. 8B) with basal half membranous; sternite 8 contiguous (Fig. 8F). Penis in dorsal view (Figs 9E–G) gradually narrowing from base to the upper corner of median orifice, from this part the apex of ventral plate strongly and triangularly narrowed, apex sharp, dorsal surface membranous, dorso-lateral margins sclerotized. In lateral view penis strongly curved, lateral margins completely sclerotized. Spiculum gastrale stick-like, thin and slightly curved (Fig. 9I).

Female terminal segments and genitalia. Tergite 8 trapeziodal (Fig. 8D), basal half membranous. Sternite 8 (Fig. 10D) Y-shaped, apodeme long, subequal in length to lateral arm, vertical arm wide, angularly and slightly bent inward, posterior margin bearing a few very short hairs. Gonocoxites (Fig. 10E) moderately sclerotized, elongate, hemisternite 2.50–3.10× as long as wide at widest part, base of stylus very short, conical and strongly slanted outward posteriorly, stylus conical, very short and slanted outward. Spermatheca C-shaped, ramus distinctly wider than nodulus, apex of cornu obtuse (Fig. 10F).

Table 1. Summarizing comparative diagnostic morphological characters.

Larinus obtusus	*Larinus minutus*
body length / width ratio: 1.70–1.90 mm	body length / width ratio: 1.90–2.05 mm
underside of head and postocular area with hair-like scales (Fig. 5A)	underside of head and postocular area with bi- and trifurcate scales (Fig. 5B)
eyes 1.70–1.85× as wide as long	eyes 1.65–1.75× as wide as long
rostrum 0.55–0.67× as long as pronotum in male and 0.60–0.80× in female	rostrum 0.60–0.69× as long as pronotum in male, 0.65–0.77× in female
apex of rostrum 1.18–1.22× as wide as its base in male and 1.25–1.30× in female	apex of rostrum 1.10–1.13× as wide as its base in male, 1.15–1.18× in female
central carina on epifrons raised and sharp (Figs 2A–B)	central carina on epifrons obtuse (Figs 2C–D)
submentum strongly swollen anteriorly (Figs 3A–C)	submentum weakly swollen anteriorly (Figs 3B– D)
widest part of scape 1.20–1.40× as wide as F1	widest part of scape 1.35–1.62× as wide as F1
F1 1.15–1.20× as long as F2	F1 1.35–1.40× as long as F2
F7 1.76–1.87× as wide as F1	F7 1.60–1.65× as wide as F1
antennal club 1.60–1.75× as long as wide at widest part	antennal club 1.50–1.63× as long as wide at widest part
prothorax 1.50–1.60× as wide as long (Fig. 5C)	prothorax 1.35–1.50× as wide as long (Fig. 5D)
apex of scutellum reaching level of posterior margin of postscutellum (Fig. 5E)	apex of scutellum does not reach level of posterior margin of postscutellum (Fig. 5F)
surface of mesoscutum with dense punctures (Fig. 5E)	surface of mesoscutum with sparse and coarsely rounded punctures (Fig. 5F)
postmedial projection roundly and distinctly expanded, wider than prealar projection width (Fig. 5E)	postmedial projection moderately expanded, narrower than prealar projection width (Fig. 5F)
anterior margin of metepisternum 1.54–1.65× as wide as width of posterior margin	anterior margin of metepisternum 1.35–1.40× as wide as width of posterior margin
elytra 1.20–1.30× as long as wide, 1.13–1.21× as wide as prothorax	elytra 1.35–1.40× as long as wide, 1.05–1.10× as wide as prothorax
premucro strongly developed with a sharp and wide triangular plate on female protibiae (Fig. 6B)	premucro moderately developed with a sharp denticle on female protibiae (Fig. 6D)
tarsomere 3 1.30–1.40× as wide as tarsomere 2 in widest part, claws slightly unequal in length (Fig. 7C)	tarsomere 3 1.20–1.33× as wide as tarsomere 2 in widest part; claws distinctly unequal in length (Fig. 7D)
penis nearly parallel-sided, slightly constricted at basal and apical third, weakly swollen at middle, ventral plate wide and angularly narrowed, with obtuse apex (Figs 9A–C)	penis gradually narrowing from base to the upper corner of median orifice, from this part apex of ventral plate strongly and triangularly narrowed, with sharp apex, dorsal surface membranous (Figs 9E–G)
female tergite 8 subtrapeziodal (Fig. 8C), sternite 8 nearly Y-shaped with short apodeme (Fig. 10A)	female tergite 8 trapeziodal, sternite 8 Y-shaped with long apodeme (Fig. 10D)
gonocoxite 1.50–1.65× longer than wide, base of	gonocoxite 2.50–3.10× longer than wide, base of

stylus slanted inward posteriorly, stylus subconical and slanted inward (Fig. 10B)	stylus strongly slanted outward posteriorly, stylus conical and slanted outward (Fig. 10E)
ramus of spermatheca slightly wider than nodulus (Fig. 10C)	ramus of spermatheca distinctly wider than nodulus (Fig. 10F)

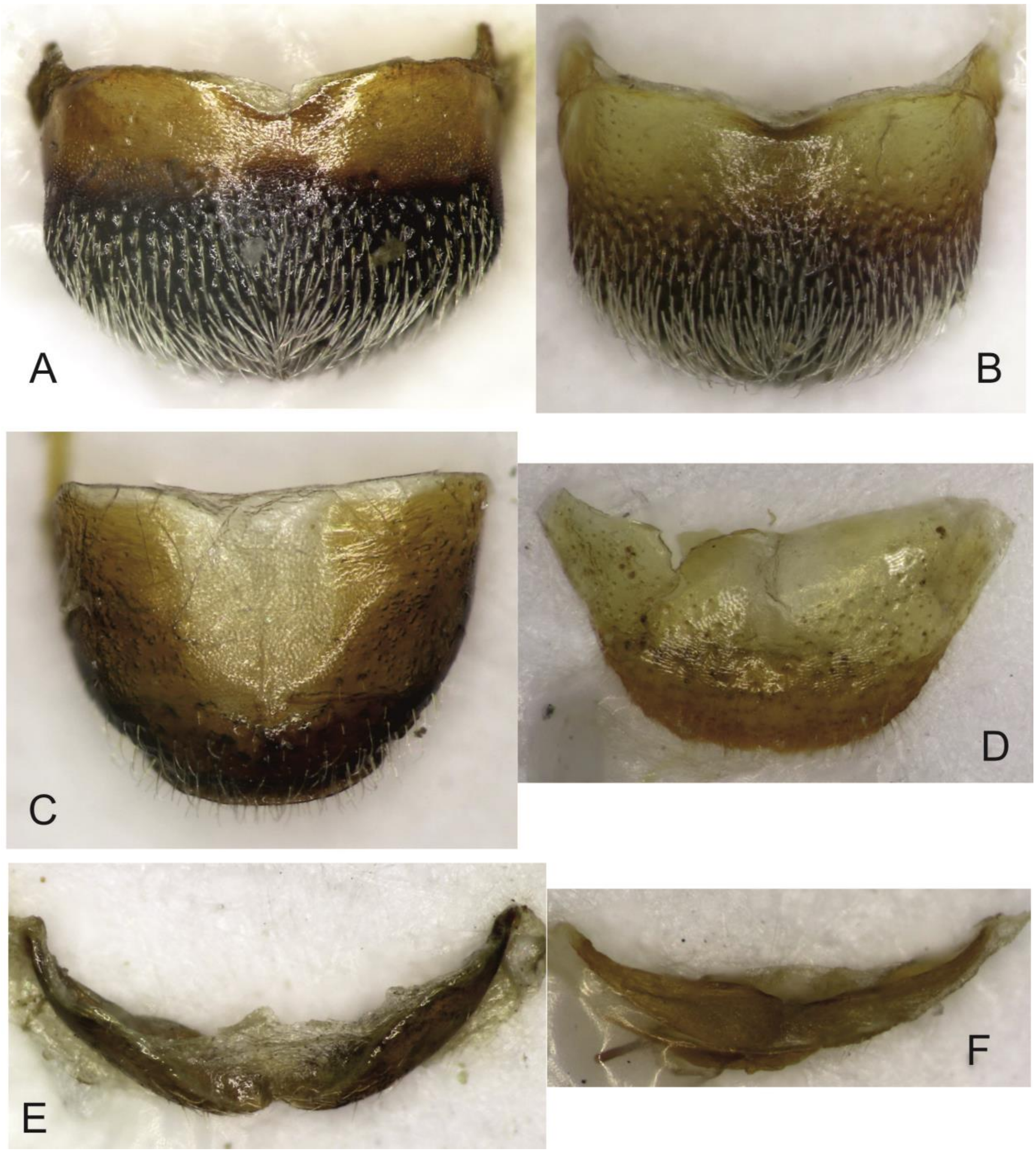

Figure 8. Terminal tergite and sternite of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal in dorsal view. **A,** tergite 8 of *L. obtusus*, male; **B,** tergite 8 of *L. minutus*, male; **C,** tergite 8 of *L. obtusus*, female; **D,** tergite 8 of *L. minutus*, female; **E,** sternite 8 of *L. obtusus*, male; **F,** sternite 8 of *L. minutus*, male.

Figure 9. Male genitalia of *Larinus obtusus* Gyllenhal and *Larinus minutus* Gyllenhal, dorsal view. **A-C,** penis of *L. obtusus*; **D,** spiculum gastrale of *L. obtusus*; **E-G,** penis of *L. minutus*; **I,** spiculum gastrale of *L. minutus*.

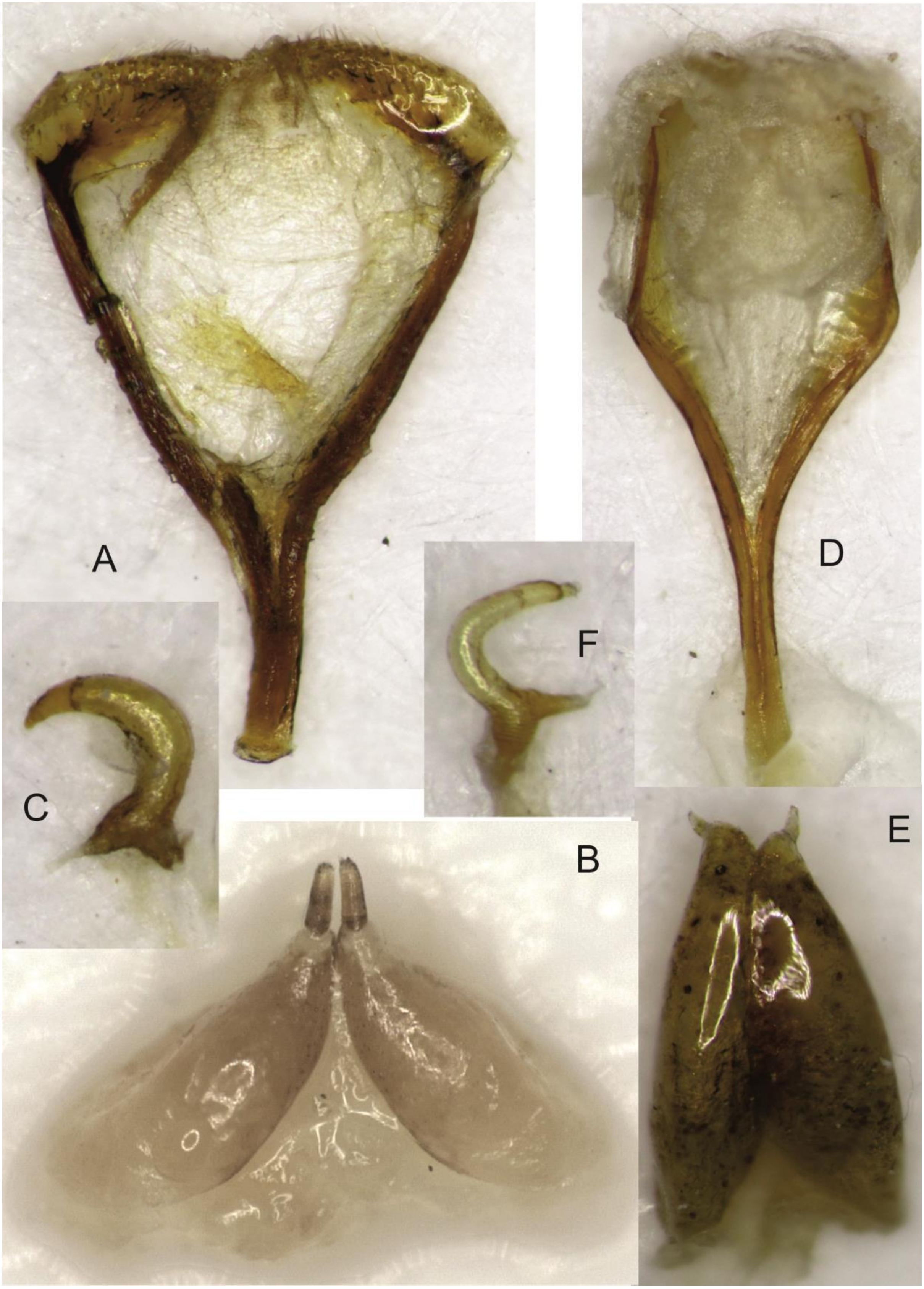

Figure 10. Female genitalia of *Larinus obtusus* Gyllenhal (**A-C**) and *Larinus minutus* Gyllenhal (**D-F**), dorsal view. **A,** sternite 8; **B,** gonocoxite; **C,** spermatheca; **D,** sternite 8; **E,** gonocoxite; **F,** spermatheca.

Acknowledgements

We would like to express our sincere thanks Enzo Colonnelli (Rome) for his valuable review of manuscript, Boris A. Korotyaev (Zoological Institute, Russian Academy of Sciences, St. Petersburg), Mark G. Volkovitsh (Zoological Institute, Russian Academy of Sciences, St. Petersburg), Genrik Davidian (All Russian Institute of Plant Protection, St. Petersburg), Kjell Arne Johanson (Swedish Museum of Natural History, Stockholm), Roman Borovec (Sloupno, Czech Republic) and Carson C. Keever (Department of Biological Sciences, Simon Fraser University, Canada) for their support providing materials and loan. This study was supported by a SYNTHESYS (SE-TAF-477) grant.

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Received: 30.11.2016 **Accepted:** 21.06.2017 **Published:** 29.06.2017
Cite paper: Gültekin L. & Anderson R. S. 2017. Morphology of two *Larinus* Dejean (Coleoptera: Curculionidae) species introduced into North America for biological control of knapweeds. *Journal of Insect Biodiversity* 5(11): 1–17.
http://dx.doi.org/10.12976/jib/2017.5.11 http://www.insectbiodiversity.org