



Review of the fossil genus *Largusoperla* (Plecoptera: Perlidae): Annotated checklist, taxonomic identification, and description of a new species

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Abstract

The fossil stonefly genus *Largusoperla* Chen *et al.*, 2018 is reviewed. An annotated checklist including diagnostic characters and an updated identification key to all currently known species of this genus are provided. Generic characters of *Largusoperla* are summarized based on all available specimens and published descriptions. In addition, a new species of this genus, *L. reni* **sp. nov.** is described, illustrated, and compared with similar taxa.

Key words: Plecoptera, *Largusoperla*, review, new species

Introduction

The fossil genus *Largusoperla* Chen *et al.*, 2018 (Plecoptera: Perlidae, Acroneuriinae) was the first known and apparently the dominant stonefly genus from mid-Cretaceous Burmese amber with eleven named species (Chen 2018a, 2018b, 2018c, Chen *et al.* 2018, Sroka *et al.* 2018). Three species based on males, *L. acus* Chen *et al.*, 2018, *L. arcus* Chen *et al.*, 2018 and *L. flata* Chen *et al.*, 2018 were originally described in the newly proposed genus *Largusoperla*. Subsequently Chen (2018a) described *L. difformitatem* Chen, 2018, from a male possessing asymmetrical paraprocts. Later, Chen (2018b) described two additional species based on males, *L. dewalti* Chen, 2018 and *L. borisi* Chen, 2018, and provided for first time an identification key. In the meantime, Sroka *et al.* (2018) proposed four new species, *L. billwymani* Sroka *et al.*, 2018 (holotype male), *L. charliewattsi* Sroka *et al.*, 2018 (holotype male), *L. brianjonesi* Sroka *et al.*, 2018 (holotype female) and *L. micktaylori* Sroka *et al.*, 2018 (holotype female). Recently, Chen (2018c) described another new species, *L. crassus* Chen, 2018, based on a male which also has asymmetrical paraprocts but possessed conspicuous tergal characters firstly reported for *Largusoperla*. Based on these tergal characters of *L. crassus*, *Largusoperlini* was proposed as a new tribe of the Acroneuriinae (Chen 2018c).

As the number of species in *Largusoperla* has increased substantially, a review is required to better understand this fossil genus of Perlidae. In this study, an annotated checklist, review of diagnostic characters, and an updated identification key to all currently known species of the genus is provided. Based on all available specimens and published descriptions, the generic characters of *Largusoperla* are summarized. In addition, another new species of this genus is described and illustrated.

Materials and methods

The ambers studied herein were collected from Kachin, Hukawng Valley (26°20'N, 96°36'E) of northern Myanmar

with an age from the earliest Cenomanian (98.79 ± 0.62 Ma) of mid-Cretaceous (Shi *et al.* 2012). The specimens of *L. acus*, *L. arcus* and *L. flata* are deposited in the Nanjing Institute of Geology and Palaeontology, China (NIGP); other specimens originally in the Chen Amber Collection are transferred and deposited in the Insect Collection of Jiangsu University of Science and Technology, Jiangsu Province, China (ICJUST). All specimens were studied except for those of Sroka *et al.* (2018) deposited in the State Museum of Natural History, Stuttgart, Germany (SMNS), whose published descriptions were used for comparison. Observations and measurements were performed with a Leica SZ45 stereo microscope. Color images were taken using a Canon EOS 6D digital camera with a Canon MP-E 65 mm 5X macro lens and optimized with Adobe Photoshop CS6. Line drawings were made by tracing the photographs and cross checked with specimens under the microscope.

The wing venation homology follows that of Béthoux (2005). Nomenclature and abbreviations are as follows: h: humeral cross vein; ScP, posterior subcosta; RA, anterior radius; RP, posterior radius; M, media; MA, anterior media; MP, posterior media; Cu, cubitus; CuA, anterior cubitus; CuP, posterior cubitus; AA, anterior analis; AA1, first anterior analis; AA2, second anterior analis. In addition, crossveins are indicated according to the veins they connect. For example, the crossvein rp-ma connects RP and MA.

Results

Annotated checklist of *Largusoperla*

Largusoperla acus Chen *et al.*, 2018

Largusoperla acus Chen *et al.* 2018. Zootaxa, 4378(4): 575. Specimen status: male holotype, NIGP167158 (NIGP).

Remarks. *Largusoperla acus* is the type species of *Largusoperla*. It can be easily distinguished from all other congeners by the paraproct strongly sclerotized and subapically constricted to a needle-like spine (Fig. 1; Figs. 5–7 in Chen *et al.* 2018).

Largusoperla flata Chen *et al.*, 2018

Largusoperla flata Chen *et al.* 2018. Zootaxa, 4378(4): 576. Specimen status: male holotype, NIGP167159 (NIGP).

Remarks. *Largusoperla flata* can be easily distinguished from all other congeners by the paraprocts weakly sclerotized, almost flat, subtriangular from ventral aspect, lateral margins thickened and up-folded, apex blunt and darkly pigmented (Fig. 1; Figs. 12–13 in Chen *et al.* 2018).

Largusoperla arcus Chen *et al.*, 2018

Largusoperla arcus Chen *et al.* 2018. Zootaxa, 4378(4): 577. Specimen status: male holotype, NIGP167160 (NIGP).

Remarks. *Largusoperla arcus* has a rounded hammer, which was incorrectly illustrated as elliptical in Fig. 19 of Chen *et al.* 2018. This species can be distinguished from all other congeners by the paraprocts strongly sclerotized, distantly located and diverging in ventral view, gradually tapered to an unmodified sharp apex (Fig. 1; Fig. 19 in Chen *et al.* 2018).

Largusoperla difformitatem Chen, 2018a

Largusoperla difformitatem Chen 2018a. Zootaxa, 4442(4): 573. Specimen status: male holotype, CZT-PLE-MA2 (ICJUST).

Remarks. *Largusoperla difformitatem* can be distinguished from congeners by the paraprocts asymmetrical, left paraproct subtriangular with sharp apex, widened at mid-length, right paraproct subtriangular with sharp apex, constricted at mid-length (Fig. 1, Fig. 2A). In addition, *L. difformitatem* has subapical angles on anterior margin of all wings, which is absent in other congeners.

Largusoperla dewalti Chen, 2018b

Largusoperla dewalti Chen 2018b. Zootaxa, 4450(4): 496. Specimen status: male holotype, CZT-PLE-MA3 (ICJUST).

Remarks. *Largusoperla dewalti* also has asymmetrical paraprocts (Fig. 1, Fig. 2B), which were incorrectly drawn in Fig. 4 of Chen (2018b). The left paraproct is apparently longer than its right paraproct. It can be distinguished from congeners by the paraprocts very thick, hairy, finger-shaped, unevenly sclerotized with a banded pattern, apex rounded (Fig. 2B).

***Largusoperla borisi* Chen, 2018b**

Largusoperla borisi Chen 2018b. Zootaxa, 4450(4): 496. Specimen status: male holotype, CZT-PLE-MA4 (ICJUST).

Remarks. The hammer of *L. borisi* is not visible in the holotype. But it can be distinguished from other congeners by the paraprocts strongly sclerotized, long triangular, inner margin concave, outer margin convex, apex obtuse and triangular (Fig. 1, Fig. 2C).

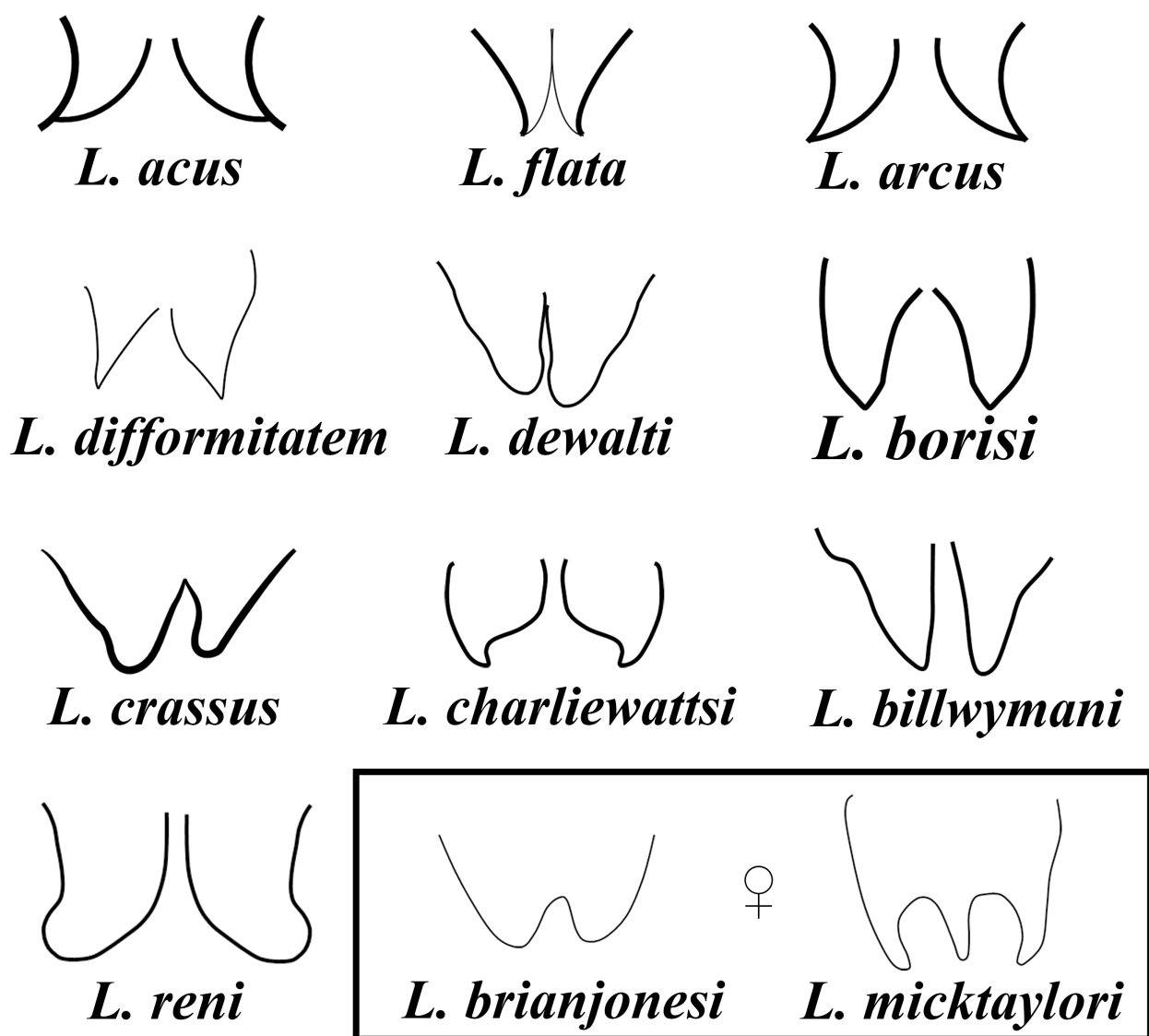


FIGURE 1. Available male paraprocts and female subgenital plates in *Largusoperla*, ventral view.

***Largusoperla crassus* Chen, 2018c**

Largusoperla crassus Chen 2018c. Zootaxa, 4462(2): 296. Specimen status: male holotype, CZT-PLE-MA5 (ICJUST).

Remarks. *Largusoperla crassus* can be distinguished from other congeners by the paraprocts asymmetrical, subtriangular and slightly upcurved, apparently mostly sclerotized except for the paler bases; left paraproct narrow,

right paraprot expanded, apex blunt, subapically with several creases (Fig. 1, Figs. 2D-E). This species is the only currently described *Largusoperla* species with well-visible tergal structures.

***Largusoperla billwymani* Sroka et al., 2018**

Largusoperla billwymani Sroka et al. 2018. PeerJ, 6 (e5354): 16. Specimen status: male holotype, SMNS BU-229 (SMNS).

Remarks. *Largusoperla billwymani* appears similar to *L. borisi*. However, the apical segment of maxillary palp is shortened in *L. billwymani* but elongated in *L. borisi*; RP of forewing has four branches in *L. billwymani* but has three branches in *L. borisi*; paraprot is thick, marginally sclerotized with blunt apex in *L. billwymani*, but is thin, completely sclerotized with triangular apex in *L. borisi* (Table 1).

***Largusoperla charliewattsi* Sroka et al., 2018**

Largusoperla charliewattsi Sroka et al. 2018. PeerJ, 6 (e5354): 13. Specimen status: male holotype, SMNS BU-10 (SMNS).

Remarks. *Largusoperla charliewattsi* can be distinguished from other congeners by the paraprots strongly sclerotized, width subequal to length, apices hook-like and curved to each other (Fig. 1; Figs. 7D, 8B in Sroka et al. 2018).

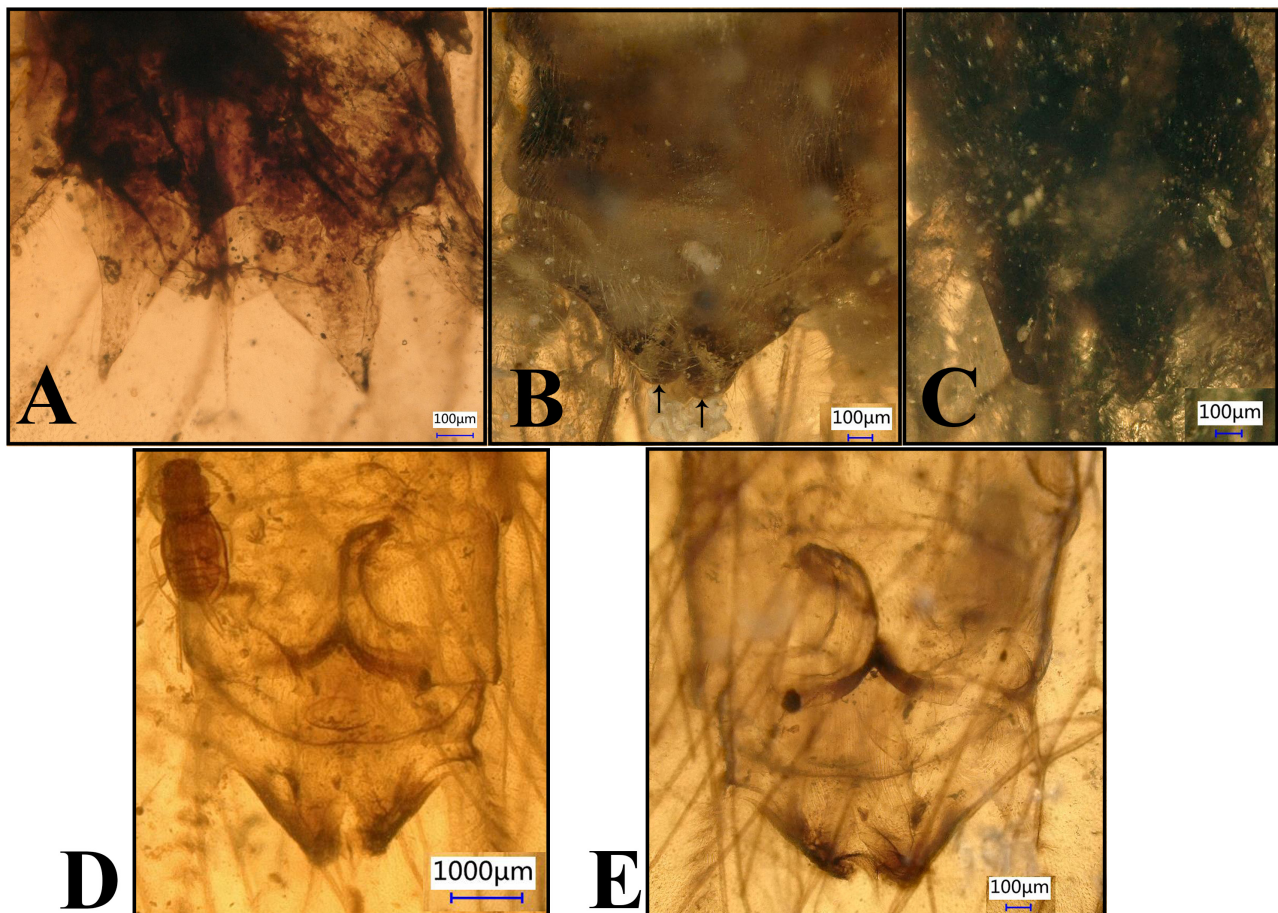


FIGURE 2. Photograph of terminalia. A. *Largusoperla difformitatem*, ventral view; B. *Largusoperla dewalti*, ventral view; C. *Largusoperla borisi*, ventral view; D. *Largusoperla crassus*, ventral view; E. *Largusoperla crassus*, dorsal view.

***Largusoperla brianjonesi* Sroka et al., 2018**

Largusoperla charliewattsi Sroka et al. 2018. PeerJ, 6 (e5354): 24. Specimen status: female holotype, SMNS BU-311 (SMNS).

Remarks. The holotype and only known specimen of *L. brianjonesi* is a female. This specimen is characterized by the posterior margin of subgenital plate with two broadly rounded lobes (Fig. 1; Figs. 15D, 16G in Sroka et al. 2018).

***Largusoperla micktaylori* Sroka et al., 2018**

Largusoperla micktaylori Sroka et al. 2018. PeerJ, 6 (e5354): 19. Specimen status: female holotype, SMNS BU-227 (SMNS); female paratype, SMNS BU-312 (SMNS).

Remarks. The holotype and only known specimen of *L. micktaylori* is also a female. This specimen is characterized by the posterior margin of subgenital plate with three long, narrow, apically pointed processes (Fig. 1; Fig. 11D in Sroka et al. 2018).

***Largusoperla* sp. Sroka et al., 2018**

Largusoperla sp. Sroka et al. 2018. PeerJ, 6 (e5354): 27. Specimen status: male holotype, SMNS BU-228 (SMNS).

Remarks. The specimen of *L. sp.* is not well preserved, without the hammer and paraprocts visible to allow a detailed comparison with other described taxa (Sroka et al. 2018)., This species is not included in the following key.

***Largusoperla reni* Chen & Wang, sp. nov.**

Figs. 3–5.

Description. Macropterous; body length (excluding antennae and cerci) ca. 12.5 mm, slender, generally brown (Fig. 3).

Head (Fig. 3): Head elliptical, with a dark stigma covering the three ocelli and the anterior half of head, compound eyes dark. Antennae dark brown, with over 50 segments. Maxillary palp slender and five-segmented, with a shortened apical segment, longer than the three-segmented labial palp.

Pronotum (Fig. 3): Pronotum narrower than head, oval and mostly brown. Mesothorax and metathorax strongly sclerotized, nearly as wide as pronotum. Legs mostly brown; tibia dark, two tibia spurs present; first two tarsal segments shortest with conspicuous euplantulae.

Left forewing (Fig. 4): Wings hyaline, veins dark brown. Forewing length ca. 12.0 mm; ScP reaches RA beyond ra-rp; 11 crossveins and the single h vein present in the costal area; two crossveins present in the apical area; RP originates from basal $\frac{1}{3}$ of RA and with four branches; M forked at near apical $\frac{1}{3}$ of the wing length, MA partially fused with RP, MP partially fused with the four-branched CuA; CuP and AA1 simple, AA2 with three branches; area between M and CuA with eight crossveins; area between CuA and CuP with five crossveins.

Abdomen (Figs. 3, 5): Abdomen slender, near half the length of the body, with dark lateral and ventral marks; posterolateral margins of each segment not extended. Sternum 9 extended backwards, posteromedially with a rounded hammer; an oval membranous area presents on posterior half of sternum 8 and anterior half of sternum 9; a pair of elliptical membranous area present beside the hammer. Paraprocts completely sclerotized, elongated and kidney-shaped with obtuse apices, length twice the width; each paraproct with a convex inner margin and a concave outer margin; basolateral part of right paraproct with a notch, which may be the damage caused by fossilization. Cercus hairy, with about 8 segments.

Type material. Holotype male, No. NIGP169678. This specimen is well preserved. Wings are overlapped so only the left forewing is visible.

Etymology. The new species is named for Professor Ren Dong (Capital Normal University, China) for his great contributions to the knowledge of fossil insects.

Syninclusions. Unidentified Psocodea, Hymenoptera, and Diptera.

Remarks. The body color, wing venation and terminalia structures, especially the ventral hammer and large paraprocts of the new species indicate its taxonomic inclusion in genus *Largusoperla*. The shortened apical segment of maxillary palp, the rounded hammer, and the long kidney-shaped paraproct of *L. reni* appears similar to those of *L. billwymani*. However, *L. reni* can be distinguished from *L. billwymani* by CuA and AA2 with more branches in forewing, M fused with RP and CuA, and by the completely sclerotized paraprocts.



FIGURE 3. *Largusoperla reni*, sp. nov. Adult habitus, dorsal view.

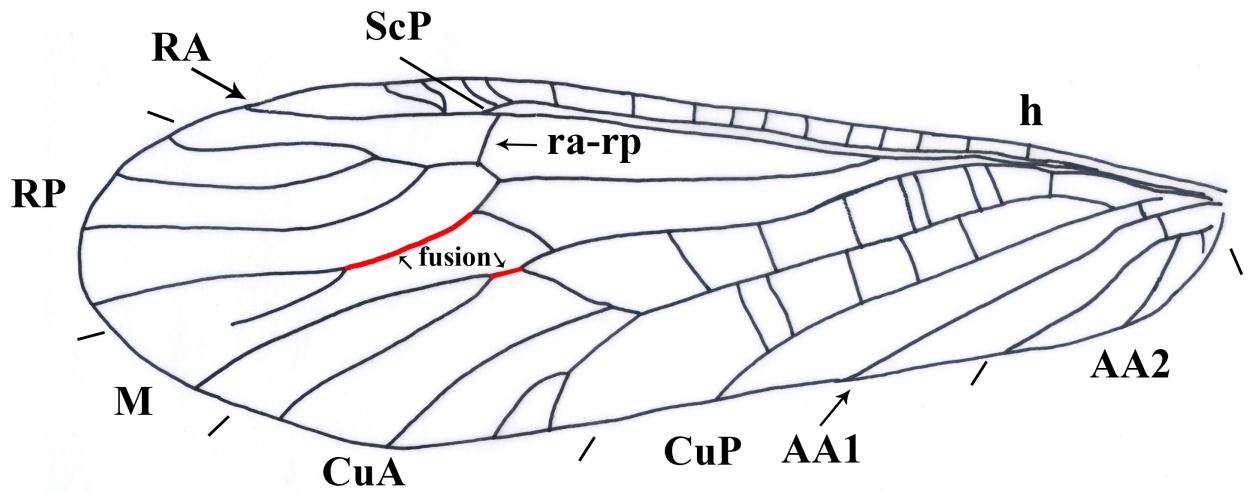


FIGURE 4. *Largusoperla reni*, sp. nov. Left forewing, dorsal view; fusion between veins indicated by red lines.

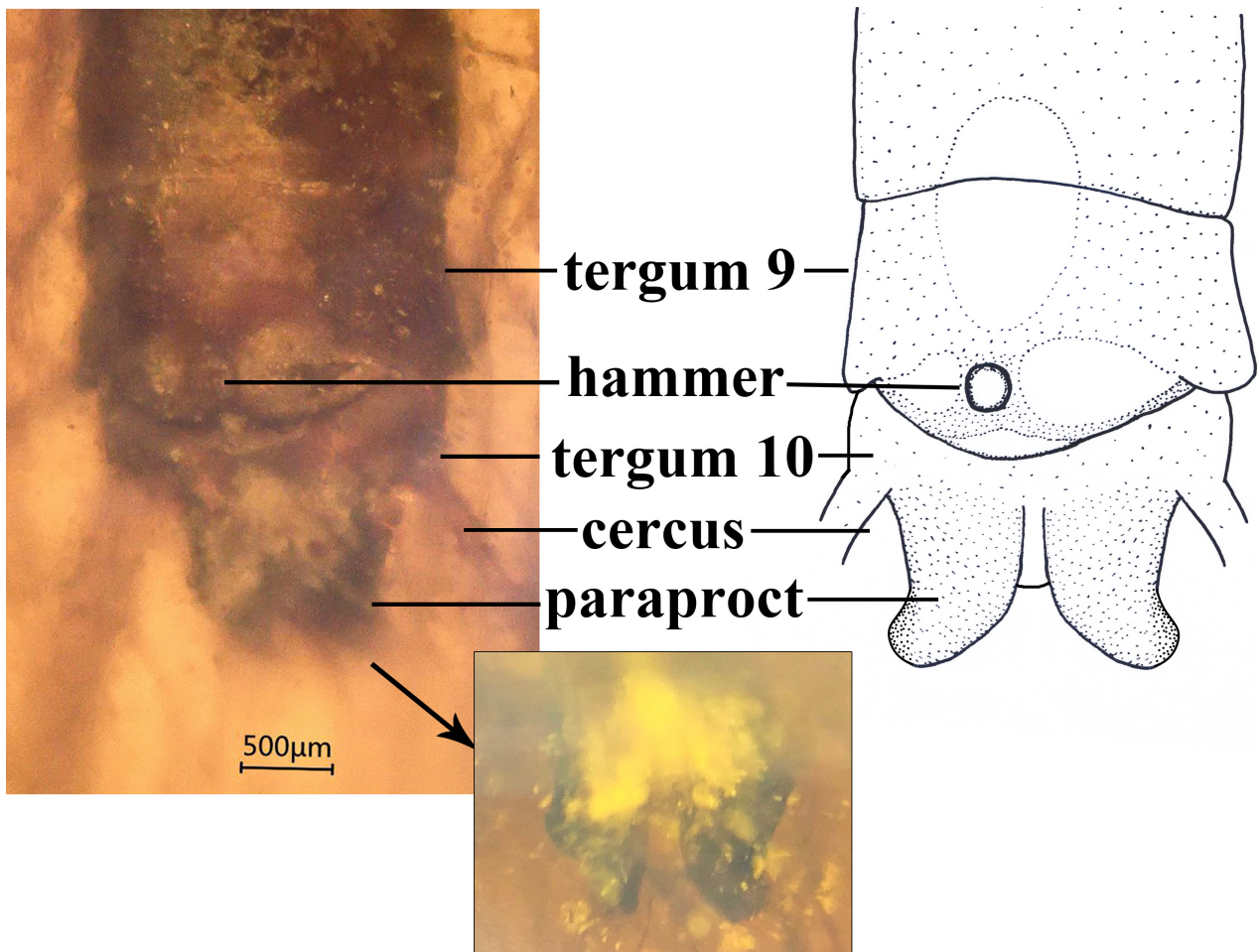


FIGURE 5. *Largusoperla reni*, sp. nov. Photograph and reconstruction of terminalia, ventral view.

Generic characters of *Largusoperla*

Currently, the most observable characters for species determination in genus *Largusoperla* appear limited to the

paraproct and hammer shape, which are relatively well-preserved in the limited material available. With the availability of additional material, other characters may become available. Based on the currently available single known specimens, the holotypes, the generic characters of this genus are summarized below (Table 1; Figs. 1, 6–7).

General habitus. Body usually slender (except for *L. crassus*, which is tumid), length ranged from 7.5 to 12.5 mm in males, 8.6 to 10.5 mm in females. Macropterous, wings obviously exceeding the abdomen.

Head (Fig. 6). Head usually oblate, with a dark stigma covering part of the head, including the three ocelli; posterior ocelli usually larger than the anterior one. Compound eyes dark and rounded. Antennae brown and hairy, length approximately $0.6\text{--}0.8 \times$ body length, with up to 50 segments or less. Maxillary palps slender, usually with five segments or less, apical segment thinner and sometimes elongated or shortened; labial palps slender and three-segmented, apical segment sometimes shortened.

Thorax. Pronotum rugose, narrower or about as wide as head, mostly brown except for the pale median band; shape near trapezoidal, anterior margin wider than posterior margin, corners obtuse. Meso- and metathorax dark and sclerotized, with similar length to prothorax; meso- and metathoracic gill remnants visible.

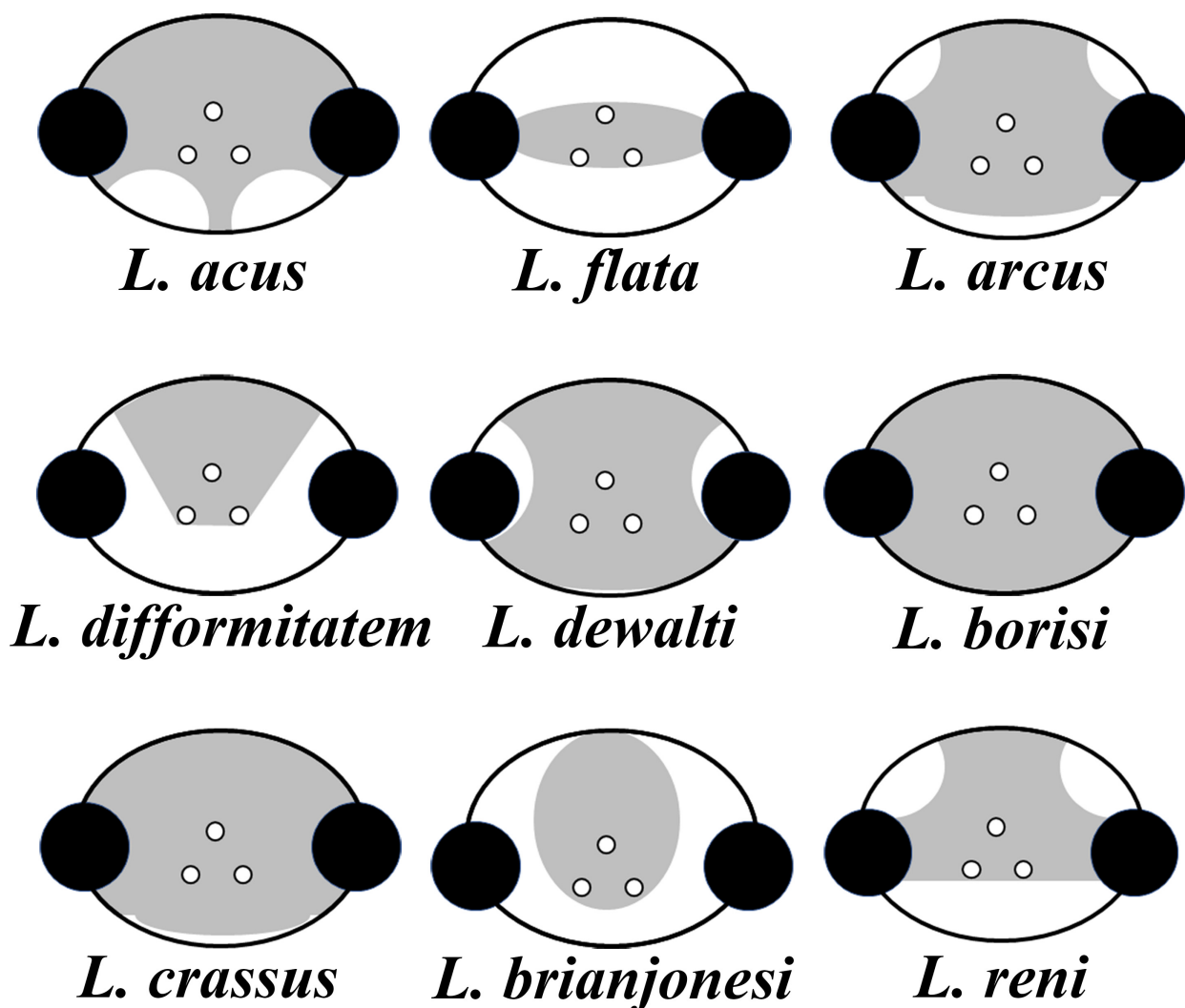


FIGURE 6. Available head patterns of *Largusoperla*, dorsal view.

Forewings. ScP reaches RA beyond ra-rp; RP with 2–4 branches; M with two branches; CuA with 3–4 branches; AA2 with 2–3 branches. A subapical angle present in anterior margin of all wings of *L. difformitatem*.

Hindwings. Hindwings wider than forewings, but usually covered by forewings and partially visible; apical venations generally similar to forewings.

TABLE 1. Diagnostic characters in species of *Largusoperla*. Bs: body size, including habitus and approximate length; Php: percentage of head pattern; Amp: apical segment of maxillary palp; Sa: segmentation of antenna; Ws: wing shape; RP: number of branches of RP in forewing; CuA: number of branches of CuA in forewing; AA2: number of branches of AA2 in forewing; Am: abdominal mark; Hs: hammer shape; Ps: paraproct thickness; Pt: paraproct sclerotization; Pm: paraproct modification; Sc: segmentation of cercus; Sp: subgenital plate of female.

	Bs	Php	Amp	Sa	Ws	RP	CuA	AA2	Am	Hs	Ps	Pt	Pm	Sc	Sp
<i>L. acus</i>	slender, 9 mm,	≈80%	shortened	30–38	normal	3	3	2	lateral	elliptical	completely	thin	apical spine	9–10	-
<i>L. flata</i>	slender, 8.2 mm	≈25%	elongated	23–38	normal	3	3	≥1	lateral	elliptical	weakly	thin	lateral fold	10–11	-
<i>L. arcus</i>	slender, 7.5 mm	≈80%	subequal	41	normal	2	3	2	lateral	rounded	completely	thin	claw-shaped	9–11	-
<i>L. difformitatem</i>	slender, 9.5 mm	≈33%	shortened	> 10	angled	3	3	?	lateral	elliptical	weakly	thin	asymmetrical, subtriangular	?	-
<i>L. dewalti</i>	slender, 9.1 mm	≈90%	?	> 29	normal	3	3	2	lateral & ventral	elliptical	partially	thick	asymmetrical, finger-shaped, banded	10	-
<i>L. borisi</i>	slender, 8.0 mm	100%	elongated	46	normal	3	3	2	lateral	?	completely	thin	long triangular, inner concave, outer convex	10	-
<i>L. crassus</i>	plump, 11 mm	≈90%	shortened	≥39	normal	3	3	3	lateral	elliptical	partially	thick	asymmetrical, sclerotized margins	11	-
<i>L. billwymani</i>	slender, 10.2 mm	?	shortened	?	normal	4	3	2	lateral	rounded	partially	thick	sclerotized margins	13	-
<i>L. charlieiwattsi</i>	slender, 8.1 mm	?	subequal	?	normal	4	4	2	lateral	elliptical	completely	thin	apical hook	?	-
<i>L. brianjonesi</i>	10.3 mm	≈50%	subequal	?	normal	3	3	2	?	-	-	-	-	?	bilobed
<i>L. micktaylori</i>	8.6–10.5 mm	?	shortened	?	normal	3	4	3	?	-	-	-	-	?	trilobed
<i>L. sp.</i>	slender, 10.5 mm	?	shortened	?	normal	3	4	3	?	elliptical	completely	thin	?	?	-
<i>L. reni</i>	slender, 12.5 mm	≈70%	shortened	> 50	normal	4	4	3	lateral & ventral	rounded	completely	thick	inner convex, outer concave	≈8	-

Abdomen (Figs. 1, 7). Male abdomen usually with dark lateral marks and sometimes ventral marks (e.g. *L. dewalti*); abdominal segments, especially tergum 9 often with extended posterolateral margins; sternum 9 extended backwards, posteromedially with a distinctly elevated, rounded or elliptical hammer; tergum 9 with hair patches (currently only found in *L. crassus*, which has well-preserved tergal characters); tergum 10 cleft at least in posterior half; epiproct sclerite present and dark in color, with specific shapes (currently only found in *L. crassus*); paraprocts broad and elongated, sometimes asymmetrical, from weakly sclerotized to completely sclerotized, with specifically modified shapes and color patterns. Cerci hairy and with about 10 segments, each segment usually with several long apical bristles.

Female abdomen with strongly elongated subgenital plate, which has two or three lobes (currently only found in the three female specimens described in Sroka *et al.* 2018).

Remarks. Regarding the specimens currently available, the fossil genus *Largusoperla* exhibits high species-level biodiversity in Burmese amber. Additional species of this genus are expected to be found and described in the future.

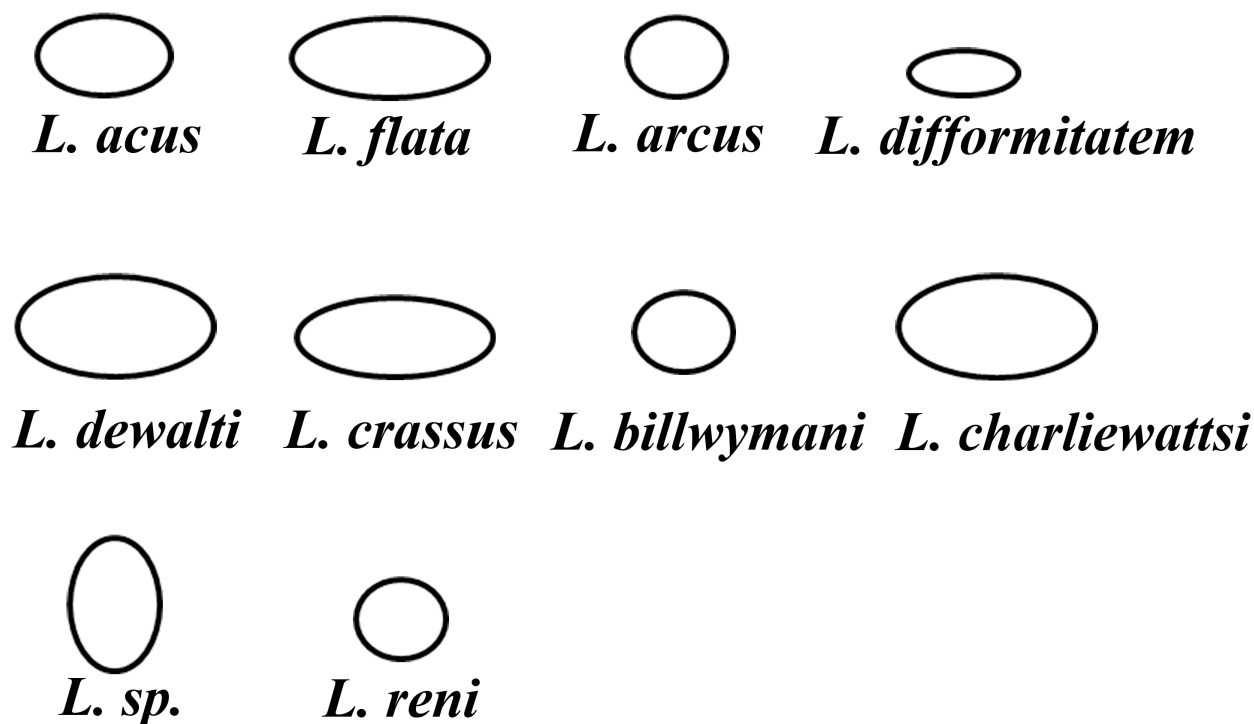


FIGURE 7. Available hammer shapes of *Largusoperla*, ventral view.

Key to males of *Largusoperla* species (*L. sp.* is excluded)

1. Paraprocts distinctly asymmetrical, one paraproct larger, other smaller. 2
- Paraprocts symmetrical, shape of paraprocts similar 4
2. Paraprocts weakly sclerotized, subtriangular with sharp apex; left paraproct widened at mid-length, right paraproct constricted at mid-length *L. difformitatem*
- Paraprocts partially sclerotized, apex blunt. 3
3. Paraproct finger-shaped and thick, with banded color pattern; left paraproct longer than the right paraproct. *L. dewalti*
- Paraproct subtriangular, without banded pattern, subapically with creases; left paraproct smaller than the right paraproct *L. crassus*
4. Paraprocts weakly sclerotized, almost hyaline, laterally thickened *L. flata*
- Paraproct partially or completely sclerotized, not hyaline 5
5. Paraproct subapically constricted to a needle-like spine. *L. acus*
- Paraproct without needle-like apical spine 6
6. Paraproct with an incurved apical hook, width almost equals the length *L. charliewattsi*
- Paraproct without an incurved apical hook, width usually shorter than the length 7

7. Apical segment of maxillary palp elongated; RP of forewing with three branches; paraproct with concave inner margin and convex outer margin, apex obtuse with a small triangle. *L. borisi*
- Apical segment of maxillary palp apparently shortened or subequal to basal segments; RP of forewing with two or four branches. 8
8. CuA of forewing with four branches; M partially fused with RP and CuA; apex of paraproct obtuse *L. reni* **sp. nov.**
- CuA of forewing with three branches; M not fused with RP or CuA. 9
9. Apical segment of maxillary palp subequal; RP of forewing with two branches; paraproct strongly out-curved, with convex inner margin and concave outer margin, apex sharp. *L. arcus*
- Apical segment of maxillary palp shortened; RP of forewing with four branches; paraproct points backward, apex blunt *L. billwymi*

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