Cretaceous Research xxx (2014) 1–19

Contents lists available at ScienceDirect

Cretaceous Research

journal homepage: www.elsevier.com/locate/CretRes

Libanopsinae, new subfamily of the family Sphindidae (Coleoptera, Cucujoidea) from Lower Cretaceous Lebanese amber, with remarks on using confocal microscopy for the study of amber inclusions

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ARTICLE INFO

Article history:
Received 19 January 2014
Accepted in revised form 14 February 2014
Available online xxx

Keywords:
Coleoptera
Sphindidae
New subfamily
New genus and species
Lebanese amber
Lower Cretaceous

ABSTRACT

The present study provides a description of a new subfamily Libanopsinae subfam. nov. with the new genus *Libanopsis* gen. nov. and five new species (*L. poinari* sp. nov., *L. impexa* sp. nov., *L. limosa* sp. nov., *L. straminea* sp. nov., *L. slipinskii* sp. nov.) from Lower Cretaceous Lebanese amber. This extinct subfamily is rather isolated from other subfamilies of the family Sphindidae and shares many external structural features with the extant families Sphindidae and Protocucujidae). The systematic position of the new fossils is discussed and key to species is proposed. It was shown that the confocal laser scanning microscopy (CLSM) is a powerful method for surface reconstruction and studying tiny elements of bio-inclusions in amber (especially arthropod exoskeletons). The quality of CLSM images drastically depends on the medium used for mounting specimens: epoxy resin totally masks the signal from the inclusion under examination, whereas samples prepared Canada Balsam are highly appropriate for CLSM study.

1. Introduction

The superfamily Cucujoidea embraces the greatest number of families within the superfamilies of the order Coleoptera. Bouchard et al. (2011) listed 35 recent and 2 extinct families in this group, however the numbers are disputed with various specialists, having their own interpretations. Also most present-day researchers distinguish many small separate families with comparatively minute diagnostic characters (Leschen et al., 2005 etc.) and the authors of the present paper feel obliged to follow this tradition. Great complexities exist in the interpretation of fossils which, on one hand, are still rather poorly known in general, especially those from the Mesozoic. On the other hand, the most reliable records from this era are mostly from the few Cretaceous amber inclusions. Unfortunately small cucujoids are comparatively rare in compression deposits and are often not well preserved, making them difficult to study and compare with recent representatives. As a result, uncommon extinct species and groups were tentatively placed in taxa proposed for the Recent fauna. Thus the fossil record of this superfamily (especially its Mesozoic part) is unreliable for phylogenetic reconstructions. A more detailed overview of cucujoids in the fossil record is available in the catalogue of Kirejtshuk and Ponomarenko (2013).

This paper describes a new Mesozoic sphindid subfamily (Libanopsinae subfam. nov.) from Lower Cretaceous Lebanese amber. This fossil group has clear connection with members of the Recent fauna and could be very significant in illustrating the Mesozoic diversification of the superfamily Cucujoidea. This new subfamily of the Sphindidae Jacquelin du Val, 1858 is represented by a new genus, *Libanopsis* gen. nov., with five new species: *L. poinari* sp. nov., *L. impexa* sp. nov., *L. limosa* sp. nov., *L. straminea* sp. nov., *L. slipinskii* sp. nov., which constitute the earliest record of the family.

2. Material and methods

The Lebanese amber specimens examined are deposited in the Lebanese University, Faculty of Sciences II, Department of Natural Sciences. For this study, basic optical equipment was used, including a stereomicroscope (Leica MZ 16.0) in the Saint Petersburg Institute and a stereomicroscope (Olympus SCX9) in the Paris

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http://dx.doi.org/10.1016/j.cretres.2014.02.008
0195-6671/

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Museum. In addition, the specimens were studied with a confocal laser scanning microscope in the Saint Petersburg State University (see below). The method of preparation of the specimens for study was described in Azar et al. (2003).

Strata. Lower Cretaceous; ante mid-Barremian (circa 130–145 Myr), after the most recent geological and stratigraphical data (unpublished, in preparation).

Localities. Specimens 841 BC and 474 B originated from the Hammana/Mdeyrij outcrop [Caza (Department) Baabda, Mouhafazet Jabal Loubnan (Mount Lebanon Governorate)], Central Lebanon (Fig. 1); specimen J-6F from the Bkassine (Jouar Ess-Souss) outcrop [Caza (Department) Jezzine, Mouhafazet Loubnan El-Janoubi (South Lebanon Governorate)], Southern Lebanon (Fig. 2); specimen TAR-101A from the Bouarej outcrop [Caza (Department) Zahleh, Mouhafazet El Beqaa (Beqaa Governorate)] Central Lebanon (Fig. 1); and specimen NBS-4C from the Nabaa-Ess-Sukkar/Brissa outcrop [Caza (Department) Danniyeh, Mouhafazet Loubnan Esh-Shemali (North Lebanon Governorate) Northern Lebanon (Fig. 3).

Confocal laser scanning microscopy (CLSM)

For CLSM studies, polished blocks of amber with specimens embedded in epoxy resin and Canada Balsam were used. The CLSM procedure was carried out in the Center of Microscopy and Microanalysis at Saint-Petersburg State University using the Spectral confocal & multiphoton system of Leica TCS SP2 with objectives 63× N.A. 1.4-0.60 Oil lBL HCX PL APO and 40× N.A. 1.25-0.75 Oil CS HCX PL APO at an excitation wavelength of 405 nm (blue laser, 50% intensity), and an emission wavelength range of 415–750 nm. The most common acquisition resolution was 2048 × 2048 pixels, level of gain 550–650, frame average 1 or 2, and zoom range of 1.5–3.0 times. Between 30 and 65 optical slices were recorded from each specimen examined. The digital images of the confocal stacks were processed using Fiji Open Source Image Processing Package to obtain the maximum intensity projections (MIP). Three-dimensional representation of the insect surface topography (Isosurface and Volume rendering images) were carried out using Amira® 5.3.2 software according Amira® User's Guides based on the confocal stacks obtained from the single insect specimen that provided the best image. All 3-dimensional images were recorded using "PrintScreen" keyboard function or "Snapshot" command embedded in Amira®. All images were checked for brightness and contrast correction with Photoshop®CS11 version 12.1 (Adobe Systems Inc.).

3. Systematic paleontology

Order Coleoptera Linnaeus, 1758
Infraorder Cucujiformia Crowson, 1960
Superfamily Cucujoidea Latreille, 1802
Family Sphindidae Jacquelin du Val, 1858
Subfamily Libanopsinae Kirejtshuk, subfam nov.

Type genus *Libanopsis* gen. nov.

Diagnosis. Body rather small (length 1.1–1.8 mm); subelliptic, moderately convex dorsally and subflattened ventrally; dorsum dark brownish to blackish; underside brown to blackish; appendages usually lighter; mostly mat; dorsum with sparse, stout and erect hairs; underside with fine and moderately dense pubescence. Dorsum diffuse, coarsely and sparsely punctured, with smooth microsculpture. Underside with fine and moderately dense punctures with apparently smooth sculpture between them. Head large, more or less short, subtriangularly transverse, somewhat convex dorsally, more or less declined ventrally, with large eyes; eyes coarsely facetted (on underside as large as on dorsum); antennal insertions located just before anterior edge of eyes. Labrum unilobed and slightly exposed from under anterior edge of frons. Mandibles apparently moderately developed, gradually curved externally and with bidentate apices. Maxillae apparently with one lobe (?galea) setose at apex and 4-segmented palpi. Labial palpi 3-segmented. Antennae 10-segmented, moderately long, with

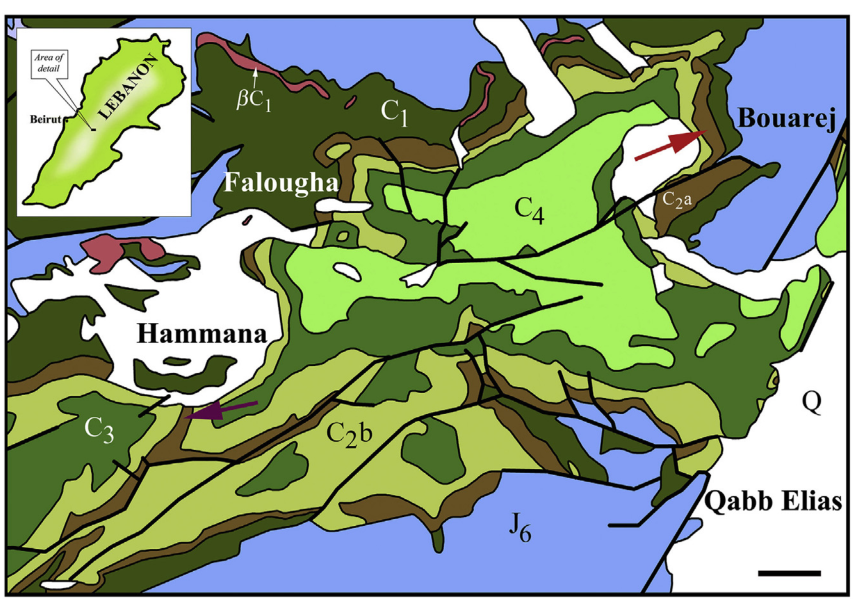

Fig. 1. Geological map of Hammana, and Bouarej amber outcrops. Abbreviations: J6 = Kimmeridgian; βJ6 = Volcanic Kimmeridgian; C1 = Neocomian – Ante Barremian; βC1 = Volcanic Neocomian; C2a = Barremian – ante mid Barremian; C2b = End Barremian – Aptian; C3 = Albian; C4 = Cenomanian; Q = Quaternary; thickened lines represent faults; scale bar = 1 km. Purple arrow indicates the Hammana outcrop; red arrow indicates the Bouarej outcrop. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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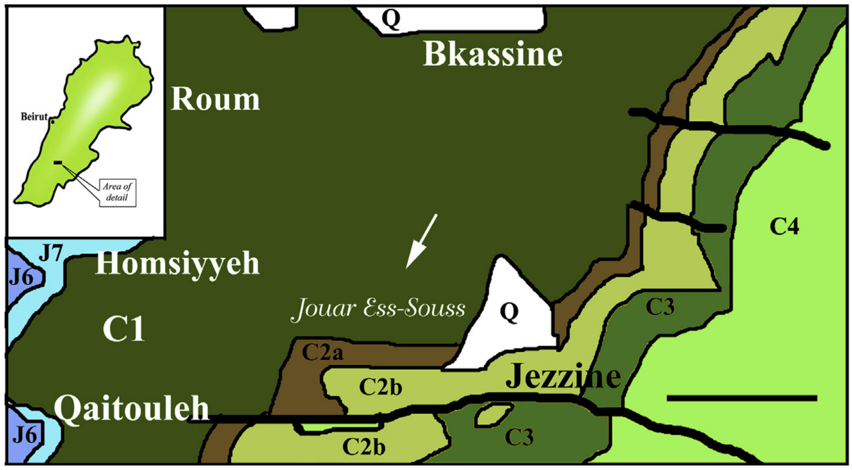

Fig. 2. Geological map of Bkassine outcrop. Abbreviations: J6 = Kimmeridgian; J7 = Tithonian; C1 = Neocomian – Ante Barremian; C2a = Barremian – ante mid Barremian; C2b = End Barremian – Aptian; C3 = Albian; C4 = Cenomanian; Q = Quaternary; thickened lines represent faults; scale bar = 1 km. White arrow indicates the amber outcrop.

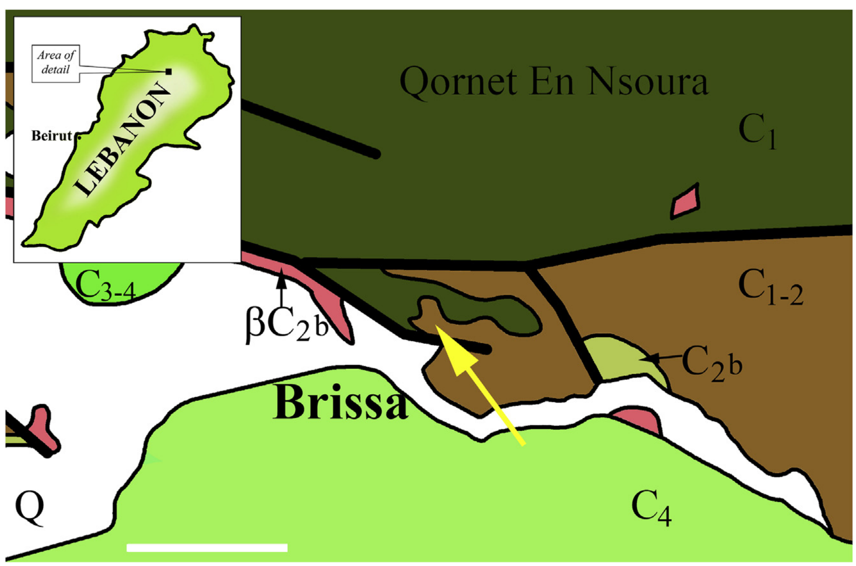

Fig. 3. Geological map of Nabaa Ess-Sukkar/Brissa outcrop. Abbreviations: C1 = Neocomian – Ante Barremian; C1-2 = Neocomian – ante mid Barremian; C2b = End Barremian – Aptian; *β*C2b = Volcanic Aptian; C3-4 = Albian – Cenomanian; C4 = Cenomanian; Q = Quaternary; thickened lines represent faults; scale bar = 1 km. Yellow arrow indicates the amber outcrop. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enlarged antennomeres 1 and 2, and also with 3 apical antennomeres forming a loose club. Pronotum transverse, gently vaulted and with arcuate sides undulated (to crenulated) at edges. Scutellum subtransverse, widened and subtruncate at apex. Elytra complete, somewhat convex along middle and steeply sloping at narrowly subexplanate sides, adsutural lines well expressed at least along distal half of suture. Abdomen invisible dorsally. Fifth maxillary palpomere slightly subconical and somewhat narrowed at base, elongate third labial palpomeres subconical and subtruncate at narrow apex. Antennal grooves developed. Prosternum with process moderately wide (markedly narrower than procoxal cavities and moderately far projecting beyond posterior edge of procoxae). Procoxae transversely oval and open posteriorly. Distance between oval mesocoxae comparable to that between procoxae. Mesoventrite about half as long as prosternum and apparently not depressed in respect to plane prosternum and metaventrite. Metaventrite slightly convex along midline. Metepisterna narrow. Metacoxae narrowly separated or conjointed and not reaching elytral epipleura. Abdomen with five ventrites. Epipleura of elytra moderately narrow and gradually narrowing apically, distinctly slopping externally. Legs narrow and long. Trochanters of elongate to heteromeroid type and moderately short. Tibiae rather thin, without spurs. Femora of usual configuration. Tarsi 5-5-5, with tarsomeres 1–4 more or less lobed and ultimate tarsomere longest, claws simple or subdentate at base.

Comparison.

This new subfamily was mentioned in Kirejtshuk and Azar (2013) as family "somewhat resembling *Calitys* Thomson, 1859 but with oval and open posteriorly procoxae and long erect setae on dorsum". In addition to the peculiar structure of procoxae, the adults are characterised by the 5-segmented tarsi of all legs in combination with the 5-segmented abdomen, complete elytra and unmodified mouthparts. These peculiarities provide an opportunity to compare this group among the superfamilies Cleroidea and

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Fig. 4. *Libanopsis poinari* sp. nov., holotype "841 BC": A – body, dorsal view; B – idem, ventral view; C – anterior part of body, ventral view. Body length 1.4 mm.

Cucujoidea of the infraorder Cucujiformia because the groups of the superfamily Tenebrionoidea have not imaginal tarsi 5-5-5 and those of Lymexyloidea demonstrate more or less modified mouthparts (particularly maxillary palpi). Instead of showing some external similarity to some cleroids such as Trogossitidae Latreille, 1802, sensu lato (including Peltidae Latreille, 1807 and Lophocateridae Crowson, 1964); Phloiophilidae Kisenwetter, 1863, the adults of this new subfamily have no remains of tarsal empodia and their metacoxae do not reach the elytral epipleura, therefore, they can be scarcely placed with the cleroids. At the same time, adults with 10-segmented clubbed antennae, procoxae widely open posteriorly, five abdominal ventrites and tarsi 5-5-5 in both sexes occur in Lithophilini Imhoff, 1856 (Coccidulinae Mulsant, 1850: Coccinellidae Latreille, 1807), Hypocoprini (Atomariinae LeConte, 1861: Cryptophagidae Kirby, 1837) and Sphindinae (Sphindidae). However, the new species are quite distinct from the members of both genera *Tetrabrachys* Kapur, 1948 (Lithophilini) and *Hypocoprus* Motschulsky, 1839 (Hypocoprini) due to their large and coarsely faceted eyes, frontal elevations over the antennal insertions, rather different structure of the antennae, pronotum with undulate edges of lateral carinas and clear furrow along the base, transverse scutellum widening apically, (sub) contiguous metacoxae, epipleura slopping externally and other peculiarities. They are distinct also from members of *Tetrabrachys* in the unmodified palpi.

At the same time, the new species demonstrate a considerable similarity with many groups of Sphindidae regarding the sparse sculpture of dorsal integument, large coarsely faceted eyes, antennae (number of antennomeres, large scape and pedicel, loose club), undulate lateral pronotal carinas and small serration in tarsal claws, but they differ from the recent groups in the 1) wide frontal elevations over the antennal insertions covering antennal bases and forming wide lateral lobes, 2) epipleura steeply sloping externally, 3) lack of clear "fronto-clypeal" suture, 4) comparatively narrow prosternal process, 5) procoxae widely opened posteriorly, 6) strongly transverse scutellum widening posteriorly with subtruncate apex, 7) (sub) contiguous metacoxae and 8) long and sparse erect setae on dorsal integument. The heteromeroid rather than elongate trochanters (particularly in posterior legs) and tarsi with formula 5-5-5 of *Libanopsis* species are also somewhat similar to those in Sphindidae.

Nevertheless, some recent members of the subfamily Sphindinae with 10-segmented antennae are also characterised by the

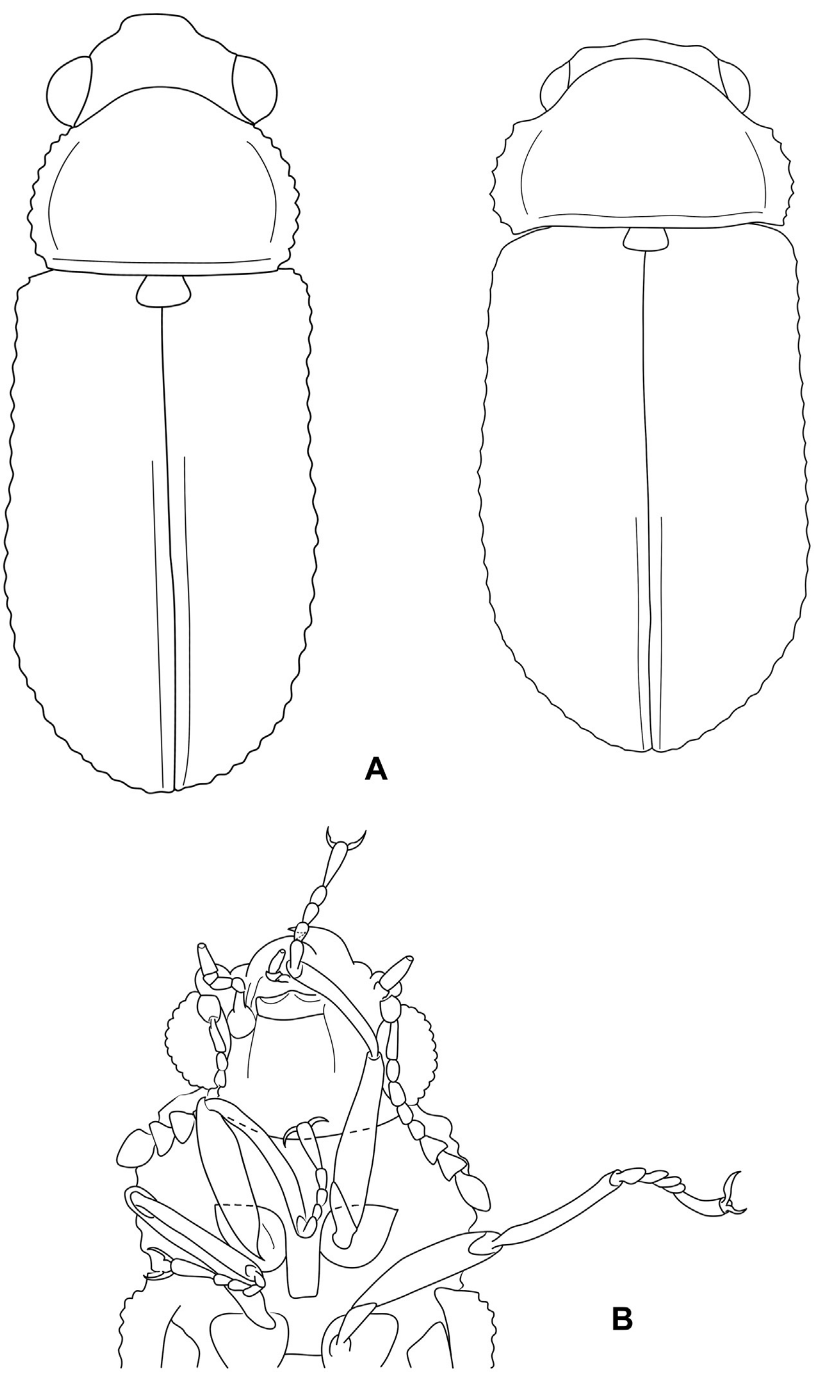

Fig. 5. Species of *Libanopsis* gen. nov.: A – body of holotype of *L. poinari* sp. nov., "841 BC", dorsal view; B – anterior part of body of the same specimen, ventral view; C – body of holotype of *L. impexa* sp. nov., "474 B", dorsal view. Body length of holotype of *L. poinari* sp. nov. 1.4 mm. Body length of holotype of *L. impexa* sp. nov. 1.3 mm.

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somewhat similar body shape and undulate lateral edges of the pronotum (*Sphindus* Dejean, 1821), subquadrangular scutellum and rather sparse and coarse punctation of the dorsum (*Notosphindus* McHugh and Wheeler, 1991 but with another shape), and tarsi 5-5-5 in both sexes (Forrester, 2003: different species of *Aspidiphorus* Sturm, 1826 have males with both tarsal formula 5-5-4 and 5-5-5). The species of the genus *Eurysphindus* LeConte, 1878 shows the epipleura subhorizontal with outer parts slightly declined downwards (somewhat similar to those in species of the new genus), subexplanate pronotal sides with undulate edges, diffuse punctation of elytra, thick suberect pubescence on dorsum and more or less narrowly separated pairs of coxae. The sphindid groups with 11-segmented antennae also show some similarities with species of the new genus. Particularly, the subfamily Protosphindinae, which have according to Sen Gupta and Crowson (1979) the "sides of frons markedly raised, partly hiding antennal insertions", although, in contrast to *Libanopsis* species, they bear other different diagnostic features (listed above), especially very different sculpture and punctation of the elytra (not sparse and diffuse coarse puntures) and lack of thick setae on the dorsum.

The new sphindid subfamily differs from the apparently closest cucujoid group, the Protocucujidae Crowson, 1954, in the absence of well developed bisetose empodia, non flattened antennal club, the frons completely covering the base of the antennae and forming wide lateral lobes (in species of the genus *Ericmodes* Reitter, 1878 the frons only slightly cover the antennal bases), 10-segmented antennae, anterior part of prothorax strongly extended anteriorly, procoxae widely open posteriorly, subcontiguous metacoxae, epipleura sloping externally, male tarsi 5-5-5.

The confocal microscopy gave opportunity to study the mouthparts of the specimens under description in more detail than it is possible due to usage of common optics for study of amber inclusions. They are more or less similar to those in recent members of Sphindidae and Protocucujidae, however, as it is seen on Fig. 6C the maxilla of *Libanopsis poinari* sp. nov. looking like bearing only one lobe, but not two ones as known in the recent groups of the mentioned families (McHugh and Wheeler, 1991; Ślipiński, 1999; Forrester and McHugh, 2007). If this difference between the fossil Libanopsinae subfam. nov. and recent Sphindidae is really stable it could be regarded as an additional trace of rather ancient divergence of these groups (see the below discussion).

Genus *Libanopsis* Kirejtshuk, gen. nov.

Type species. *Libanopsis poinari* sp. nov.

Derivation of name. The name of this new genus is derived from the Greek name of country of origin "*Liban*" and from the Greek "*opsis*" meaning "resembling a (specified) thing".

Composition. Besides the type species, the new genus includes *L. impexa* sp. nov., *L. limosa* sp. nov., *L. slipinskii* sp. nov. and *L. straminea* sp. nov.

Diagnosis. The same as for the new subfamily.

Libanopsis poinari Kirejtshuk, sp. nov.

Figs. 4; 5 A, B; 6; 7 A, B

Derivation of name. The epithet of this new species is dedicated to George Poinar Jr. who cooperated with the senior author of this paper during many years and assisted him with scientific and linguistic matters.

Holotype. "841 BC", probable female; complete specimen; inclusion in a thin subquadrangular piece of more or less homogeneous light amber (3.1 mm long and 2.0 mm wide) with some pieces of brownish organic matter in front of the specimen and at its distal part. The piece of amber was prepared and embedded between two round microscope cover slips in a Canada Balsam medium. The dorsum and underside of the specimen are covered with a thin layer of air making it difficult to observe outlines of scleritesa and the sculpture of the integument.

Diagnosis. This new species differs from the congeners in the characters mentioned in the key to species below. In addition, this new species has a more slender body, the head somewhat longer before the eyes and more flattened dorsally in comparison with *L. impexa* sp. nov., also the eyes are larger, some ratios of the antennomeres differ, the top anterior angles of the pronotum are less distinct, the elytra apices are more widely rounded and it has a longer prosternum. *Libanopsis poinari* sp. nov. also differs from *L. limosa* sp. nov. in having dark setae on the dorsum, apparently less elevated interspaces between punctures on the elytra, a thinner base of tarsomere 1 and some proportions of the antennomeres.

Description of holotype. Body length 1.4 mm, width 0.5 mm, elongate, rather convex dorsally and subflattened ventrally; dorsum dark brown to black; underside dark brown; appendages reddish with lighter tarsi and dark tarsal claws; dorsum with uniform, rather long and sparse, thick erect brownish hairs (setae) somewhat longer than distance between their insertions; underside apparently with fine, short and moderately dense hairs.

Dorsum with subuniform, rather large, diffuse and rather sparse suboval punctures with diameter about twice as great as that of facets; interspaces between them more than two puncture diameters, probably more or less smoothed; interspaces between punctures on elytra rather elevated, forming irregular smooth ridges. Underside apparently with subuniform very fine and sparse punctation and somewhat smoothed interspaces.

Head subflattened dorsally and apparently with transverse impression at the level of eye anterior edges and with slight elevations over antennal insertions; anterior edge of frons transverse and from under it a moderately short transverse and heavily sclerotised labrum is apparently exposed. Eyes rather large, coarsely facetted and globular, distance between them about 4 times as great as eye transverse diameter. Antennae moderately long and not reaching the posterior edge of pronotum; antennomere 1 (scape) rather large and subglobular; antennomere 2 large and sublobular, somewhat smaller than scape and somewhat longer than wide; antennomere 3 narrow and longest in flagellum, apparently comparable in length with scape and slightly shorter than ultimate antennomere; both antennomeres 4 and 6 slightly shorter than antennomere 5 which in turn is shorter than antennomere 3; antennomere 7 shortest and thicker than antennomeres 3–6 at apex (subconical), antennomeres 8–10 forming a loose and slightly dorsoventrally compressed club which is markedly thicker than scape and comprising about 2/5 of total antennal length; ultimate antennomere apparently somewhat longer than scape and about 1.5 times as long as each of antenomeres 8 and 9. Pronotum about 1 and 1/3 as wide as long with subexplanate sides about as wide as antennal club, moderately convex at disk, evenly arcuate along sides, widest at the middle, lateral edges moderately undulate, with anterior and posterior angles not projecting, posterior ones nearly rounded and anterior ones with a distinct top. Scutellum strongly transverse, somewhat widening posteriorly and about twice as wide as long. Elytra complete, elongate, widest at base, almost twice as long as wide combined and nearly three times

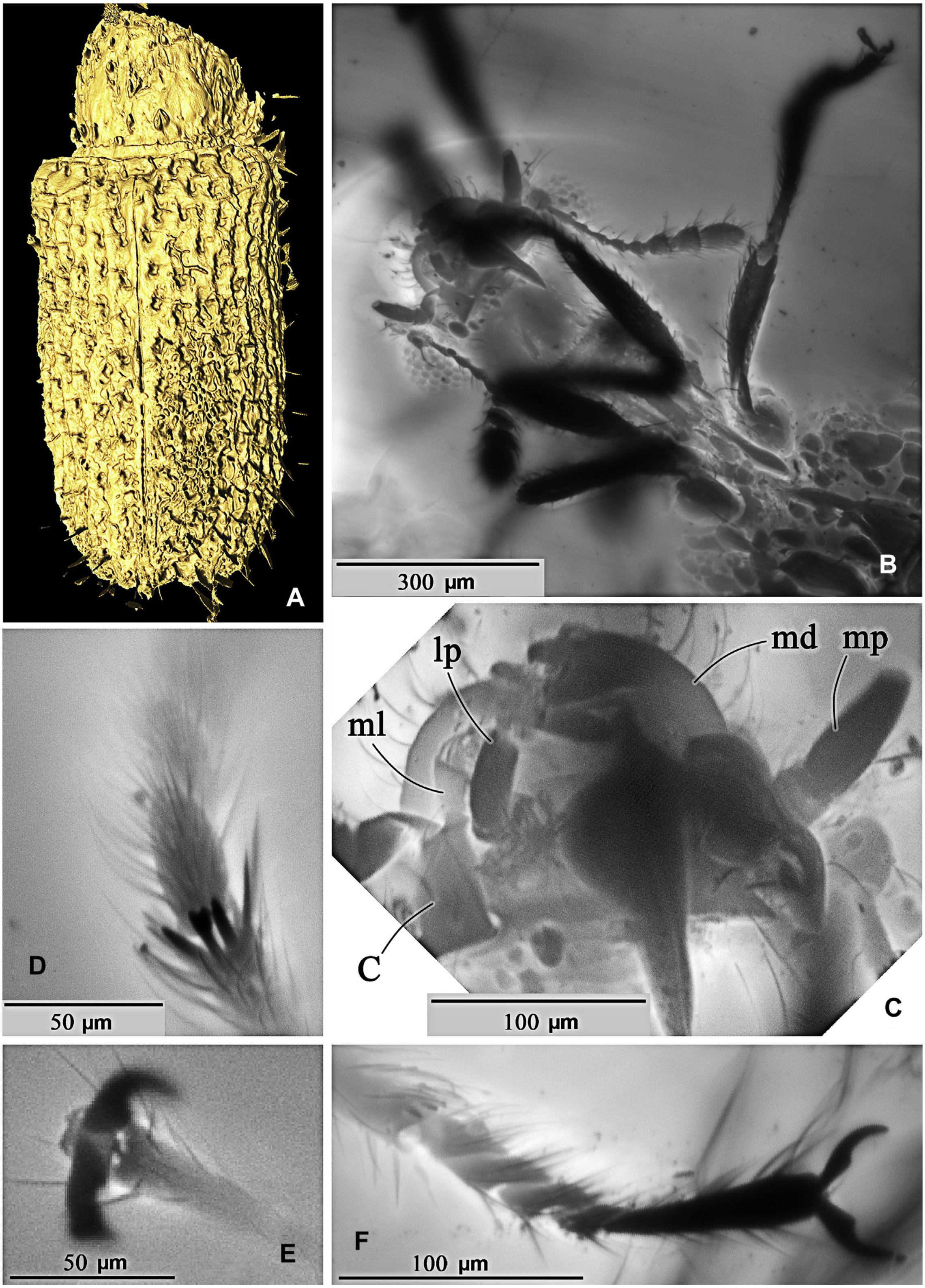

Fig. 6. CLSM images (A — Isosurface, B–F — maximum intensity projections) of *Libanopsis poinari* sp. nov., holotype "841 BC": A — body, dorsal view (body length 1.4 mm); B — anterior part of body, ventral view; C — mouthparts, ventral view; D — apex of mesotibia and mesotarsomere 1, ventral view; E — anterior tarsal claws, ventral view; F — mesotarsus, dorsal view. Abbreviations: c — cardo, li — ligula, lp — labial palpus, md — mandible, ml — maxillary lobe, mp — maxillary palpus.

as long as pronotum, subparallel-sided and their apices forming a widely rounded joint arc, rather convex and steep at sides, lateral edge slightly undulate. Pygidium with widely rounded apex and slightly exposed from under apices of elytra.

Underside of head with distinct elevation along middle behind mentum. Hypostomal sinuses moderately deep with well developed maxilla bearing 5-segmented palpus of usual structure and ultimate palpomere slightly subconical and apparently more than

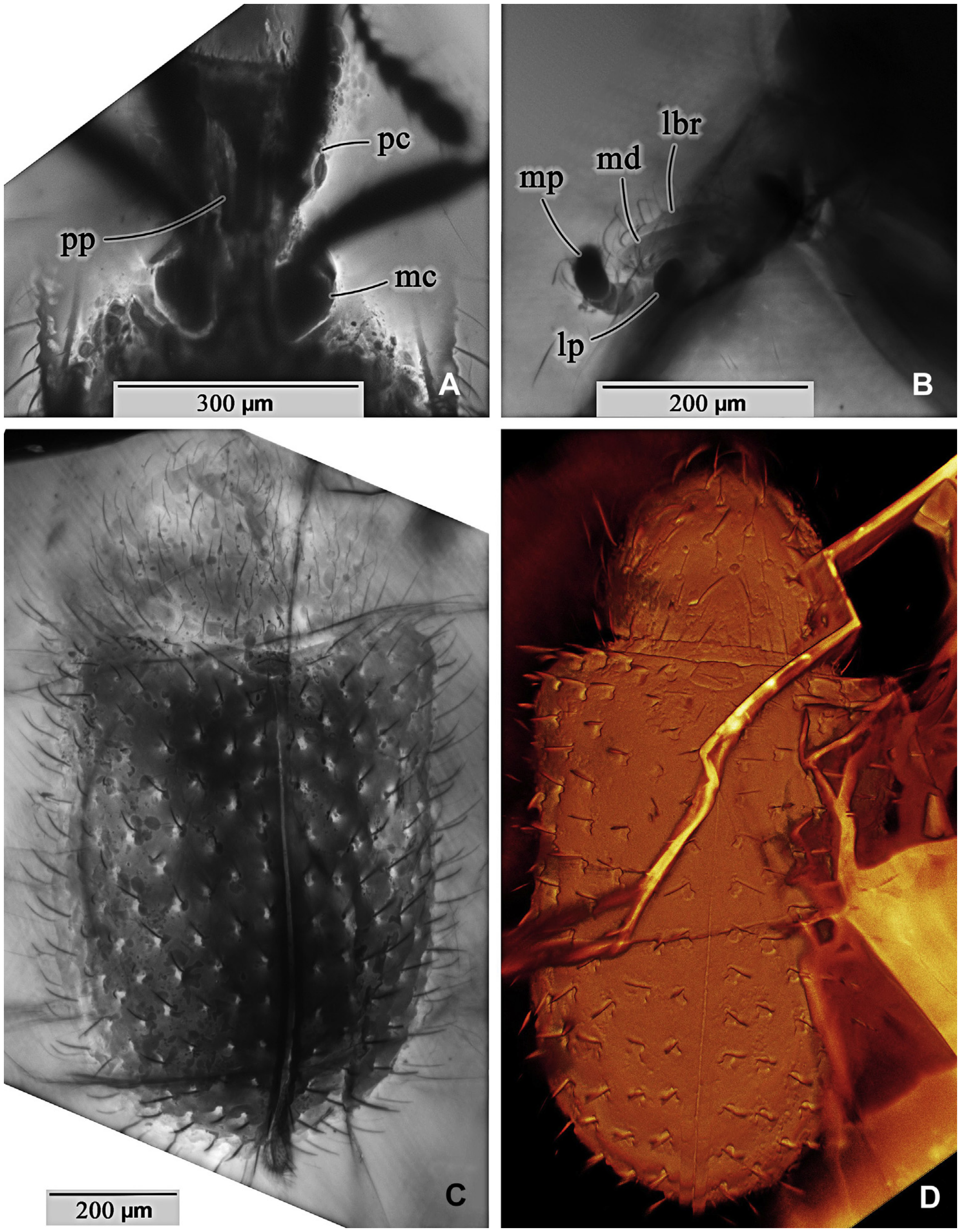

Fig. 7. CLSM images (A,B,D – maximum intensity projections; C – volume rendering) of species of *Libanopsis* gen. nov. : A – prosternum and mesoventrite of holotype of *L. poinari* sp. nov., "841 BC", ventral view; B – maxillary and labial palpi of the same specimen, ventral view; C – body of holotype of *L. slipinskii* sp. nov., "NBS 4C", dorsal view; D – body of holotype of *L. impexa* sp. nov., "474 B", dorsal view (body length 1.3 mm). Abbreviations: lbr – ligula, lp – labial palpus, mc – mesocoxa; md – mandible, mp – maxillary palpus, pc – procoxa, pp – prosternal process.

three times as long as thick. Mentum subpentangular, four times as wide as long. Labial palpi 3-segmented and with ultimate palpomere subcylindrical and about 2.5 times as long as wide. Prosternum gently and narrowly convex along the middle with rather long and narrow intercoxal process. Procoxae not widely transverse. Procoxal cavities widely open posteriorly. Pronotosternal sutures slightly evident at outer end of procoxal cavities. Distances between pro- and mesocoxae obvious. Mesoventrite moderately short, about half as long as prosternum, apparently of usual structure. Mesocoxae subtriangularly oval. Metaventrite slightly convex and with median excavation at its posterior edge between metacoxae. Metepisterna subtriangular and widened anteriorly. Metacoxae transverse. Abdomen with five ventrites; ventrite 1 apparently longest, ventrite 2 somewhat shorter, ventrites 3–5 apparently comparable in length and hypopygidium widely rounded at apex. Epipleura at anterior third of elytra rather wide (somewhat wider than antennal club) and gradually narrowing and becoming obsolete at apex.

Legs moderately long and narrow. All trochanters of heteromeroid rather than elongate type, particularly metatrochanter. Femora of usual shape, narrow and comparable in shape, pro- and mesofemora about 3.0 times as long as wide, metafemur about 2.5 times as long as wide. Tibiae narrow, about as long as femora and slightly widening apically. Tarsi with four first tarsomeres with one lobe; tarsomere 1 somewhat longer than each of tarsomeres 2–4; tarsomere 5 slightly longer than tarsomeres 2–4 combined and very

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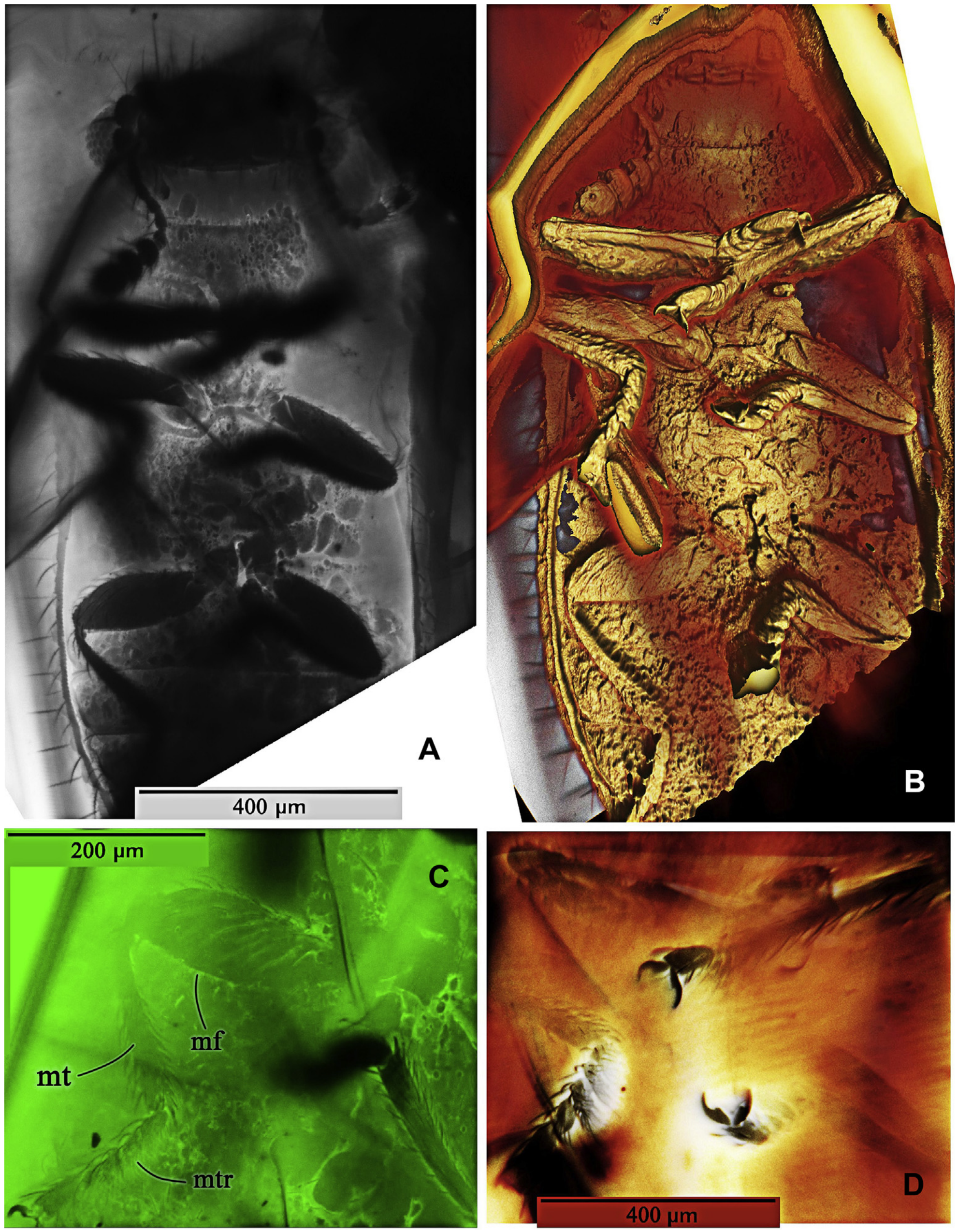

Fig. 8. CLSM images (A,C – maximum intensity projections; B – Isosurface, Orthoslice and volume rendering combined image; D – volume rendering) of *Libanopsis impexa* sp. nov., holotype, "474 B": A, B – body, ventral view; C – posterior leg, ventral view; D – pro- and mesotarsi, ventral view. Abbreviations: mf – metafemur, mt – metatibia, mtr – metatarsus.

thin at base and at apex markedly more than twice as wide as at base; claws rather long and slightly swollen (with serration) at base.

Libanopsis impexa **Kirejtshuk, sp. nov.**

Figs. 5 C; 7 D; 8; 9; 10 A

Derivation of name. The epithet of this species, the Latin "*impexus*" means bald, simple, coarse, unvarnished, unembellished, uncombed; tousled.

Holotype. "474 B", sex unclear; complete specimen; inclusion in a thin subquadrangular piece of more or less homogeneous light amber (3.4 mm long and 1.6 mm wide). The piece of amber was prepared and embedded between two round microscope cover slips in a Canada Balsam medium. The dorsal integument of the specimen is more or less clear along the centre but with some "milky" covering and apparently organic debris along its sides; its underside is rather obscured with some "milky" deposit, although the head and part of thoracic sclerites are visible.

Diagnosis. This new species is similar to *L. poinari* sp. nov., but differs from it and all congeners in the characters mentioned below in the key to species. In addition, see the diagnosis of the previous species and following species.

Notes. Taking into consideration the great similarity of this new species with the type species of the genus, common characters shared by both are omitted in the description below. The specimen seems to have a genital capsule exposed from under abdominal segment VII (probably male anal sclerite).

Description of holotype. Body length 1.3 mm, width 0.6 mm, elongate, rather convex dorsally and slightly ventrally; dorsum dark brown; underside dark brown; appendages reddish with lighter tarsi and darker tarsal claws; dorsum with uniform, rather long and sparse, thick erect brownish hairs (setae) somewhat longer than distance between their insertions; underside apparently with fine, short and moderately dense hairs. Interspaces between punctures on elytra more than twice as great as one puncture diameter, rather elevated, forming irregular smooth ridges.

Head subflattened dorsally and with clear elevations over antennal insertions; anterior edge of frons transverse and beneath is a short transverse labrum. Eyes rather large, coarsely facetted and globular, distance between them about 5 times as great as eye transverse diameter. Antennae moderately long and not reaching the posterior edge of pronotum; antennomere 1 (scape) rather large and subglobular; antennomere 2 large and sublobular, somewhat smaller than scape and somewhat longer than wide; antennomere 3 narrow and longest one in flagellum, longer than

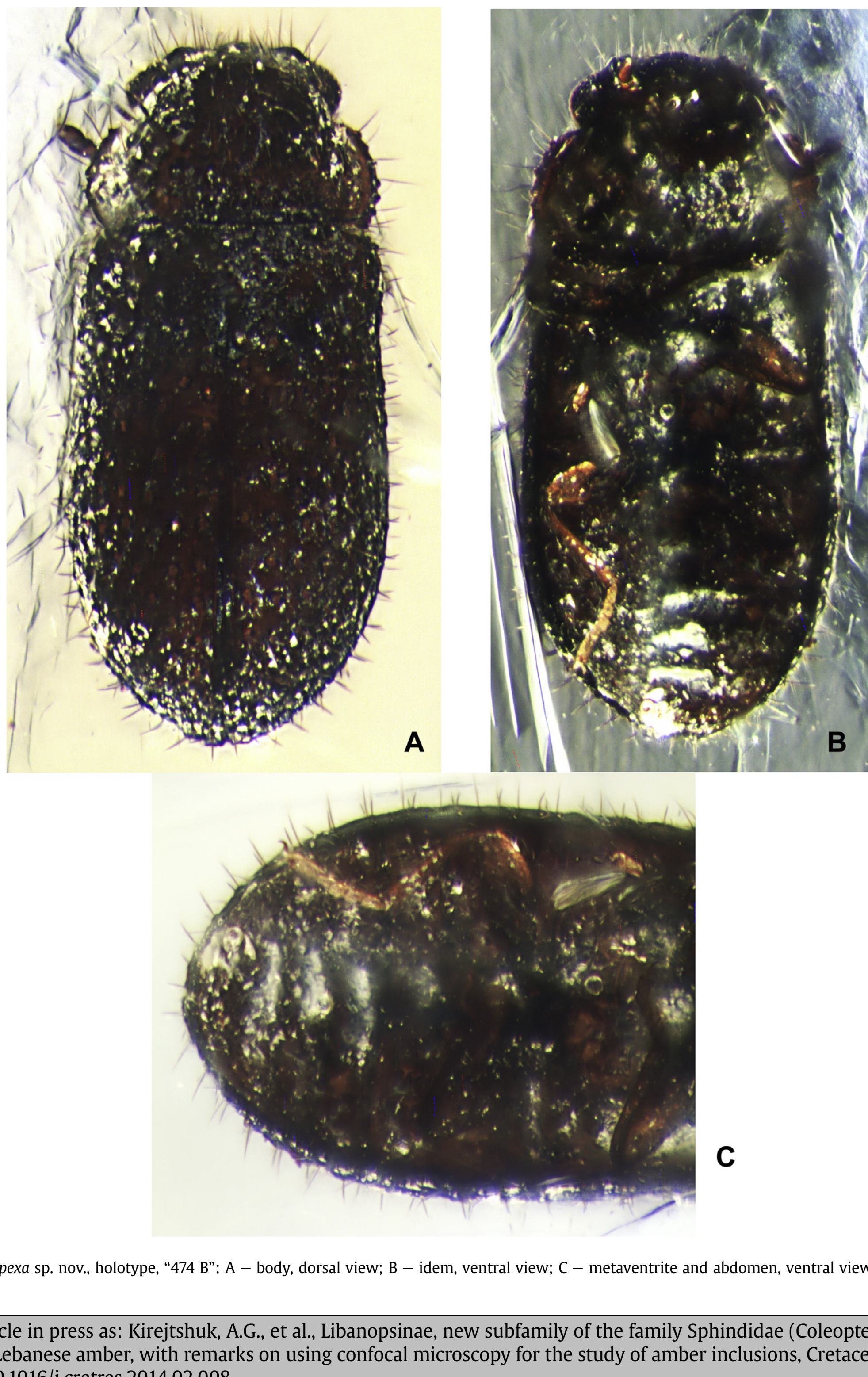

Fig. 9. *Libanopsis impexa* sp. nov., holotype, "474 B": A – body, dorsal view; B – idem, ventral view; C – metaventrite and abdomen, ventral view. Body length 1.3 mm.

scape and slightly longer than ultimate antennomere; antennomere 4 similar to antennomere 3 but somewhat shorter; antennomeres 5 and 6 similar and each of them slightly shorter than antennomere 4; antennomere 7 as long as each of two previous ones and much thicker at apex, antennomeres 8–10 forming a loose and slightly dorso-ventrally compressed club which is somewhat wider than scape and about 1/3 of entire antennal length; ultimate antennomere subequal in length with scape and about 1.5 times as long as each of antennomere 8 and 9. Pronotum about 1 and 2/3 as wide as long with subexplanate sides that are wider than antennal club, moderately convex at disk, evenly arcuate along sides, widest at middle, posterior and anterior ones with a distinct top. Elytra complete, elongate, widest at base, together about 1 and 3/5 as long as wide and somewhat more than three times as long as pronotum, subparallel-sided and their apices forming a joint arc. Pygidium with widely rounded apex and apparently slightly exposed from under apices of elytra.

Underside of head subflattened behind mentum. Prosternum gently convex and wide along the middle with rather long and narrow intercoxal process. Pronotosternal suture clearly visible between outer end of procoxal cavities. Distances between pro- and mesocoxae comparable. Mesoventrite slightly shorter than prosternum. Metaventrite rather convex. Abdomen with five ventrites; ventrites 1 apparently longest, ventrite 2 somewhat shorter, ventrites 3 and 4 apparently comparable in length and hypopygidium somewhat longer than two previous ones and widely rounded at apex. Epipleura at anterior third of elytra rather wide (about twice as wide as antennal club).

Tarsi with four first tarsomeres with one lobe; tarsomere 1 somewhat longer than each of tarsomeres 2–4; tarsomere 5 slightly longer than tarsomeres 2–4 combined, at base only slightly narrower than at apex.

Libanopsis limosa Kirejtshuk, sp. nov.

Figs. 10 B, C; 11

Derivation of name. The epithet of this species refers to the dark and subdued colouration of this new species masking by thick layer of “milky cover”, the Latin “*limosus*” means muddy, dirty, miry, silty, oozy, shady.

Holotype. “J-6 F”, probable male; specimen missing the distal part of the left antenna and somewhat broken distal part of the elytra; inclusion in a thin subquadrangular piece of more or less homogeneous light amber (2.2 × 1.2 × 1.0 mm). The piece of amber was prepared and embedded in parallelogram bar (17.2 × 4.0 × 2.2 mm) with Canada Balsam medium. Part of the dorsum and most of the underside of the specimen are covered with a thick layer of “milky deposit” and most of the remaining integument is scarcely visible because of various organic matter above it.

Diagnosis. This new species differs from its congeners in the characters mentioned in the key to species below. Also, see the diagnoses of the previous and following species. *Libanopsis limosa* sp. nov. differs from two previous species in the somewhat more convex pronotum with very narrow subexplanate sides and widest portion in the basal third. The two following new species, in contrast to the two previous ones and *L. limosa* sp. nov., are markedly more robust.

Notes. Taking into consideration the great similarity of this new species with the type species of the genus, common characters shared by both are omitted in the description below. The specimen has a genital capsule exposed from under abdominal segment VII (male anal sclerite) with long setae, which precludes the genital capsule from being retracted inside the abdomen.

Description of holotype. Body length 1.2 mm, width 0.6 mm, height 0.3 mm, elongate, rather convex dorsally and subflattened ventrally; dorsum blackish brown with dark (dark brown) appendages; with uniform, rather long and sparse, comparably thin and erect whitish hairs (setae) somewhat longer than distance between their insertions; underside apparently with fine, short and moderately dense hairs (slightly visible on prosternum and distal part of abdomen). Interspaces between punctures on elytra more than two puncture diameters, probably more or less smooth; interspaces between punctures on elytra slightly vaulted.

Head apparently subflattened dorsally with slight elevations over antennal insertions; anterior edge of frons transverse. Eyes rather large, coarsely facetted and globular, distance between them about 4 times as great as eye transverse diameter. Antennae moderately long and not reaching the posterior edge of pronotum; antennomere 1 (scape) moderately large and slightly compressed dorsoventrally; antennomere 2 large and slightly compressed dorsoventrally, somewhat smaller than scape and longer than wide; antennomere 3 narrow and longest in flagellum, longer than scape and longer than ultimate antennomere; each of antennomeres 4–6 rather short; antennomeres 8–10 forming a loose and slightly dorsoventrally compressed club which is somewhat thicker than scape and composing about one third of the total antennal length; ultimate antennomere somewhat shorter than scape and about 1.5 times as long as each of antennomeres 8 and 9. Pronotum almost twice as wide as long with very narrow subexplanate sides (much less than width of antennal club, rather convex at disk, evenly arcuate along sides, widest at basal third, with anterior and posterior angles not projecting and with a rounded top. Scutellum not visible. Elytra complete, elongate, widest at base, each elytron less than 3 times (nearly 2.5 times) as long as wide and apparently three times as long as pronotum, broadly arcuate with their apices forming a widely rounded joint arc, rather convex and steep at sides, lateral edge slightly undulate.

Underside of head apparently flattened behind mentum. Ultimate maxillary palpomere slightly subconical and apparently more than three times as long as wide. Mentum and labial palpi invisible. Prosternum gently and narrowly convex along the middle with rather long and narrow intercoxal process. Distances between procoxae somewhat greater than that between mesocoxae. Metaventrite and metepisterna apparently as in two previous species. Abdomen with ventrites 1 longest, ventrite 2 somewhat shorter than ventrite 1, ventrites 3–5 apparently comparable in length with hypopygidium rather widely rounded to subtruncate at apex. Epipleura gradually narrowing as in two previous species. Heavily sclerotised anal sclerite broken on one side and rather exposed from under segment VII. Exposed parameres with apices curved medially.

Femora and tibiae of usual shape. Tarsi with four first tarsomeres with one lobe; tarsomere 1 somewhat longer than each of tarsomeres 2–4; tarsomere 5 slightly longer than tarsomeres 2–4 combined and at base only slightly thinner than at apex; claws rather long and dentate at base.

Libanopsis straminea sp. Kirejtshuk, nov.

Fig. 12

Derivation of name. The epithet of this species refers to the colouration of this species; the Latin “stramineus” means straw coloured.

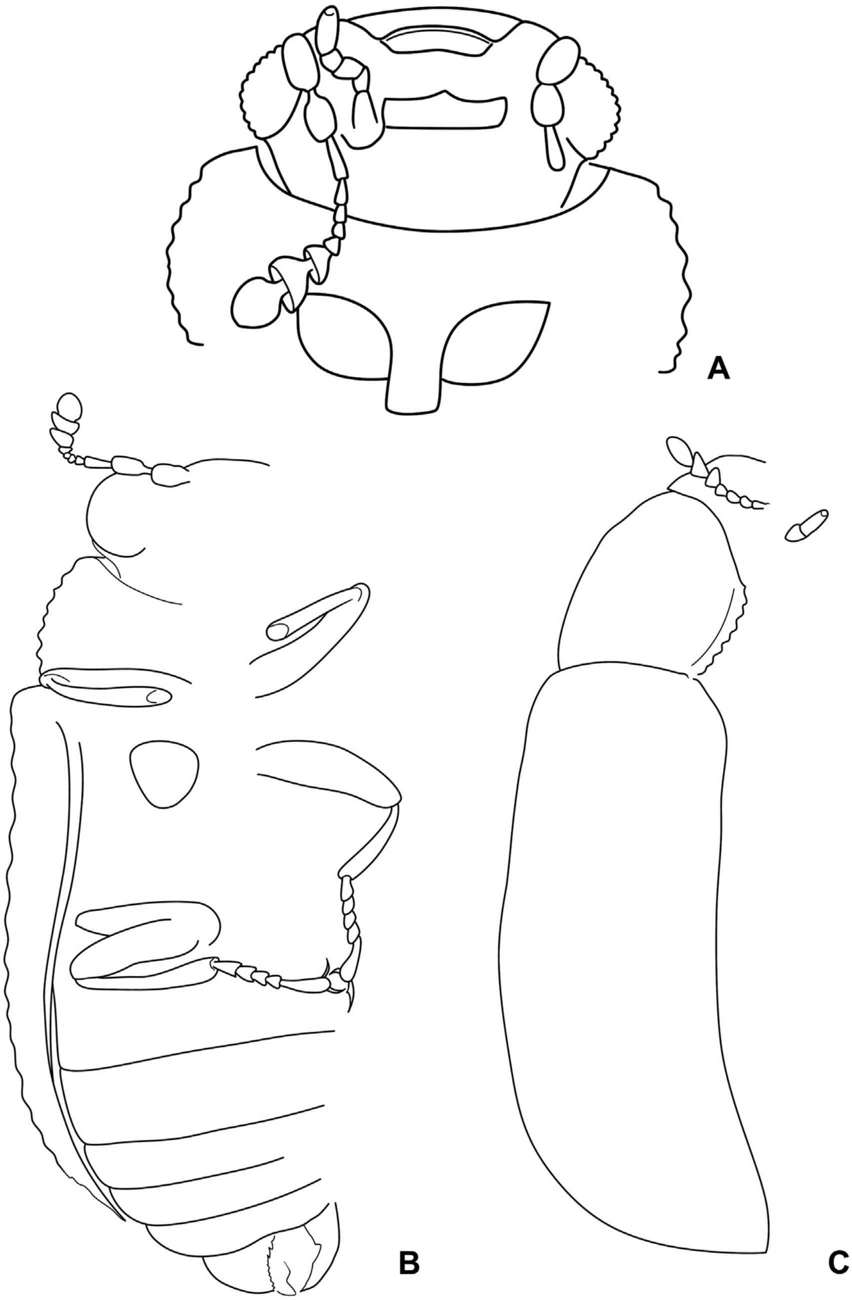

Fig. 10. Species of *Libanopsis* gen. nov.: A – head and prothorax of holotype of *L. impexa* sp. nov., "474 B", ventral view; B –body of *L. limosa* sp. nov., "J-6 F", ventral view; C – idem, lateral view. Body length of holotype of *L. impexa* sp. nov. 1.3 mm. Body length of holotype of *L. limosa* sp. nov. 1.2 mm.

Holotype. "TAR-101 A", probable female; specimen with exposed pieces of both hindwings and with missing portion of pronotum and dorsal side of head; inclusion in a thin subquadrangular piece of homogeneous light amber (3.1 mm long and 4.0 mm wide) with small dark wood filament at base of left elytron and fine threads of organic matter at its apex (one thread long and oriented obliquely adjacent to the specimen. There are also some layers of organic matter along the right side of the distal part of the specimen. The piece of amber was prepared and embedded between two square microscope cover slips in Canada Balsam. The dorsum of the specimen is covered with a thin layer of air making it scarcely possible to observe outlines of sclerites and sculpture patterns of the integument; the underside has a thick "milky" covering on the head, distal part of prothorax, mesothorax and abdomen.

Diagnosis. This new species differs from the congeners in the characters mentioned in the below key to species. Besides, this new species differs from congeners in the somewhat larger and lighter body and appendages, two types of setae on dorsum, probably very shallow punctures on dorsum. This new species has the dorsum clearly more convex than that in *L slipinskii* sp. nov. Finally, the integument of base of head, prosternum and metaventrite of *L. straminea* sp. nov. rather smooth and without visible pubescence.

Notes. Taking into consideration the great similarity of this new species with the type species of the genus, most characters shared by both are omitted in the description below.

Description of holotype. Body length 1.8 mm, width 0.7 mm, elongate oval, moderately convex dorsally and subflattened ventrally;

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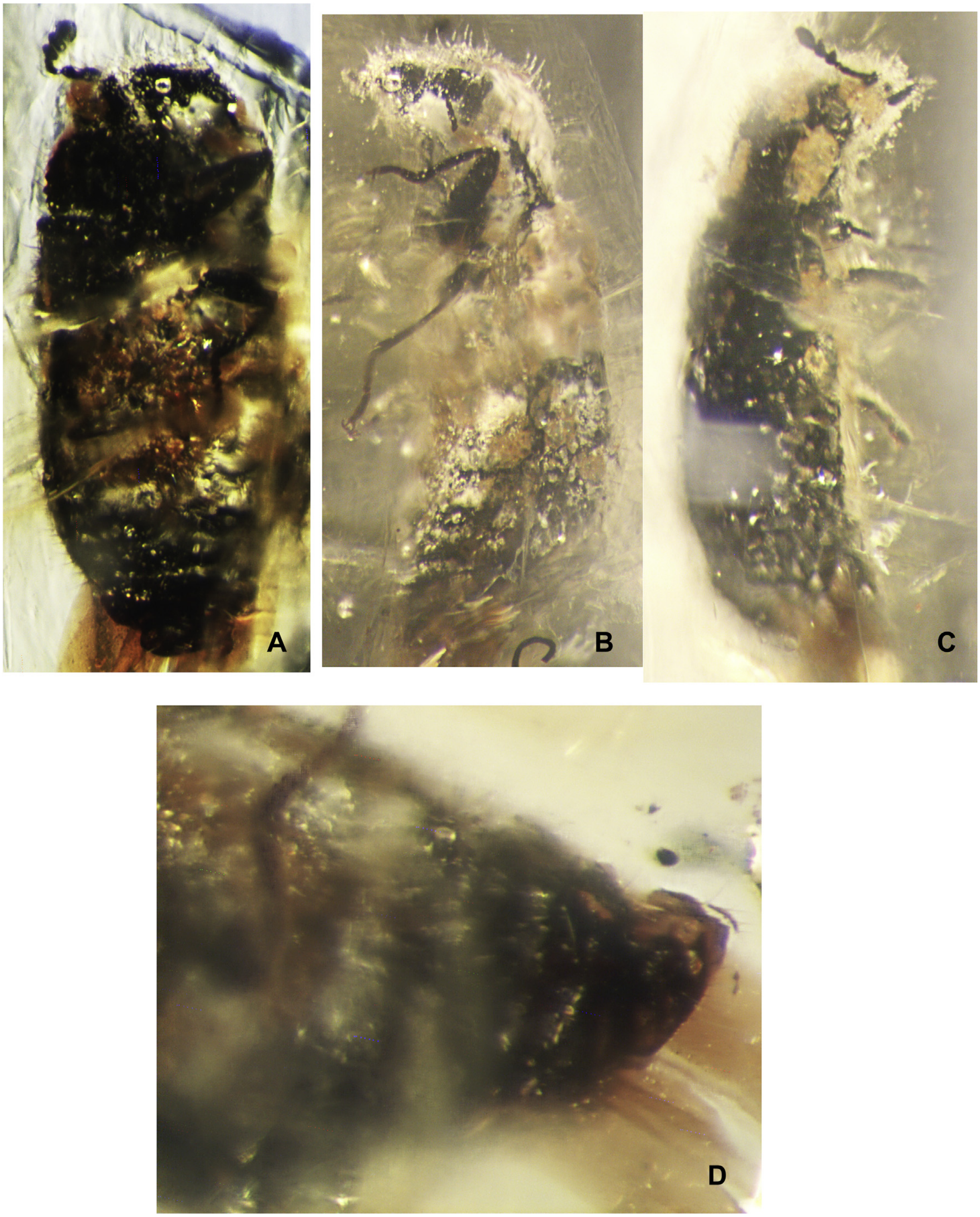

Fig. 11. *Libanopsis limosa* sp. nov., holotype "J-6 F": A – body, ventral view; B, C – idem, lateral view; D – abdomen, ventral view. Body length 1.2 mm.

dorsum dark brown; underside and appendages reddish; dorsum with rather long and sparse and thick brownish hairs (setae), among which are some erect setae; some arcuate setae are curved in the middle and orientated toward the surface of the integument, these setae are somewhat longer than the distance between their insertions; underside without visible hairs.

Dorsum apparently with subuniform, large, diffuse and rather sparse suboval large punctures which could be shallow and indistinct; sculpture on interspaces between punctures invisible. Visible integument of head base, prosternum and metaventrite without clear punctation, smooth.

Antennae moderately long and not reaching the posterior edge of pronotum; antennomere 1 (scape) rather large and subglobular; antennomere 2 large and elongate oval, somewhat smaller than scape and somewhat longer than wide; antennomere 3 rather narrow and the longest in the flagellum, apparently comparable in length with the scape and slightly shorter than ultimate antennomere; each of antennomeres 4–6 half as long as antennomere 3; antennomere 7 somewhat longer and at apex wider than antennomeres 3–6 (subconical) but shorter than antennomere 3, antennomeres 8–10 forming a loose and slightly dorso-ventrally compressed club which is about twice as wide as scape and

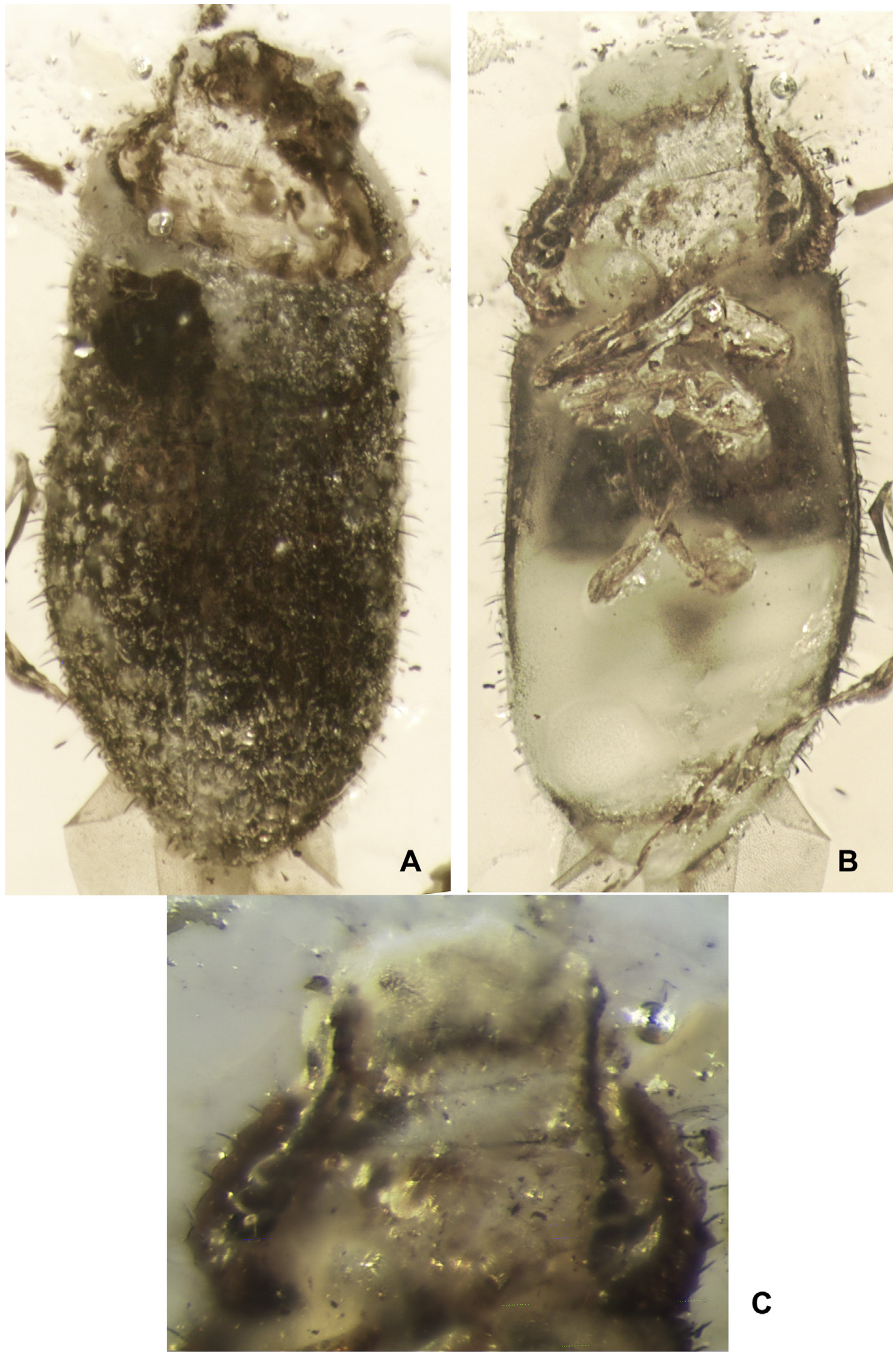

Fig. 12. *Libanopsis straminea* sp. nov., holotype "TAR 101 A": A – body, dorsal view; B – idem, ventral view; C – anterior part of body, ventral view. Body length 1.8 mm.

comprising about 2/5 of total antennal length; ultimate antennomere apparently somewhat longer than scape and about 1.5 times as long as each of antenomeres 8 and 9. Pronotum widest in posterior third, with subexplanate and undulate sides about as wide as antennal club. Scutellum not visible. Elytra complete, elongate, about 1.5 times as long as wide combined, subparallel-sided in basal 2/3 with their apices forming an angle, moderately convex and gently sloping, lateral edge slightly undulate. Pygidium with widely rounded apex and not exposed from under apices of elytra.

Prosternum gently and slightly convex along the middle and with intercoxal process slightly widened at truncate apex. Pronotosternal sutures not visible. Distances between procoxae about as wide as antennal club. Metaventrite slightly convex. Abdominal ventrites 1 apparently longest, ventrites 2–5 apparently comparable in length and hypopygidium widely rounded at apex. Epipleura at anterior third of elytra rather wide (about as wide as antennal club).

Pro- and mesofemora apparently more than 3.0 times as long as wide, metafemur nearly 3.0 times as long as wide. Tarsi with four

Fig. 13. *Libanopsis slipinskii* sp. nov., holotype "NBS 4C": A – body, dorsal view; B – idem, ventral view; C – anterior part of body, ventral view. Body length 1.1 mm.

first tarsomeres with one lobe; tarsomere 1 somewhat longer than each of tarsomeres 2–4; tarsomere 5 slightly longer than tarsomeres 2–4 combined and very thin at base; at apex about twice as thick as at base; base of claws not visible.

Libanopsis slipinskii **Kirejtshuk, sp. nov.**

Figs. 7 C; 13; 14

Derivation of name. The epithet of this new species is devoted to Adam S. Ślipiński who provided the senior author with numerous consultations on the comparison of fossil species examined with members of the Recent fauna.

Holotype. "NBS-4 C", probably male; complete specimen; inclusion in a thin subquadrangular piece of more or less homogeneous light amber (2.5 mm long and 2.0 mm wide) with some brown pieces of organic matter along the right side of the distal part of the specimen. The piece of amber was prepared and embedded between two round microscope cover slips in Canada Balsam. The dorsal integument of the specimen is somewhat soiled and its underside bears a layer of "milky deposit" (rather thick at sides) making it scarcely possible to observe outlines of sclerites and the sculpture of the integument.

Diagnosis. This new species differs from its congeners in the characters mentioned in the key to species below and in the diagnoses of the previous new species. This new species is the smallest and most robust among its congeners, and also has longest and most dense setae on its dorsum, as well as having unique proportions of the antennomeres.

Notes. Taking into consideration the great similarity of this new species with the type species of the genus, most characters shared by both are omitted in the description below.

Description of holotype. Body length 1.1 mm, width 0.6 mm, elongate oval, rather convex dorsally and subflattened ventrally; dorsum dark brown to blackish; underside and appendages dark brown; dorsum with uniform, rather long and sparse, thick erect brownish hairs (setae) of different length, most of which as well as having unique proportions of the distance between their insertions; underside apparently with very sparse, thin long hairs. Dorsum with subuniform, large, diffuse, rather shallow indistinct in outline and rather sparse punctures; interspaces between them slightly greater than two puncture diameters, smooth and not elevated. Underside with slightly visible, fine and sparse punctation and probably with smooth interspaces.

Head subflattened dorsally and with clear elevations over antennal insertions. Eyes moderately large, coarsely facetted and globular, distance between them slightly more than five times as great as eye transverse diameter. Antennae moderately long and reaching the posterior edge of pronotum; antennomere 1 (scape) and 2 not clearly visible but apparently large and sublobular, antennomere 3 narrow and longest one in flagellum, somewhat longer than ultimate antennomere; antennomeres 4–7 comparable in length and each of them about half as long as antennomere 3; antennomere 7 somewhat thicker than antennomeres 3–6 at apex (subconical), antennomeres 8–10 forming a loose and slightly dorsoventrally compressed club comprising about 2/5 of total antennal length; ultimate antennomere only slightly longer than each of antenomeres 8 and 9.

Pronotum about 1 and 5/6 as wide as long with subexplanate sides more than twice as wide as antennal club, moderately convex at disk, evenly arcuate along sides, widest at the middle, lateral edges moderately undulate, with anterior and posterior angles not projecting, posterior ones nearly rounded and anterior ones with a distinct top. Scutellum strongly transverse, somewhat widening posteriorly and about twice as wide as long. Elytra complete, elongate, about 1 and 2/3 as long as wide combined and nearly three times as long as pronotum, subparallel-sided in basal 2/3 with their apices forming an almost widely rounded joint arc with slight angle at top, rather convex and steep at sides, lateral edge slightly undulate. Pygidium widely truncate at apex and seemingly not exposed from under apices of elytra.

Underside of head not visible because of "milky deposit". Ultimate maxillary palpomere at least three times as long as wide and slightly narrowing apically. Prosternum gently and slightly convex along the middle and with intercoxal process parallel-sided at apex. Pronotosternal sutures not visible. Distances between pro- and mesocoxae apparently comparable. Mesoventrite about half as long as prosternum. Abdominal ventrite 1 apparently longest, apparently more than twice as long as each of ventrites 2–5; hypopygidium widely truncate at apex. Anal segment. Epipleura at anterior third of elytra rather wide (somewhat wider than antennal club).

Pro- and mesofemora apparently about 3.0 times as long as wide, metafemur about 2.5 times as long as wide. Tarsomere 1 somewhat longer than each of tarsomeres 2–4; tarsomere 5 slightly longer than tarsomeres 2–4 combined and very thin at base and at apex markedly more than twice as thick as at base; claws rather long and slightly swollen (with serration) at base.

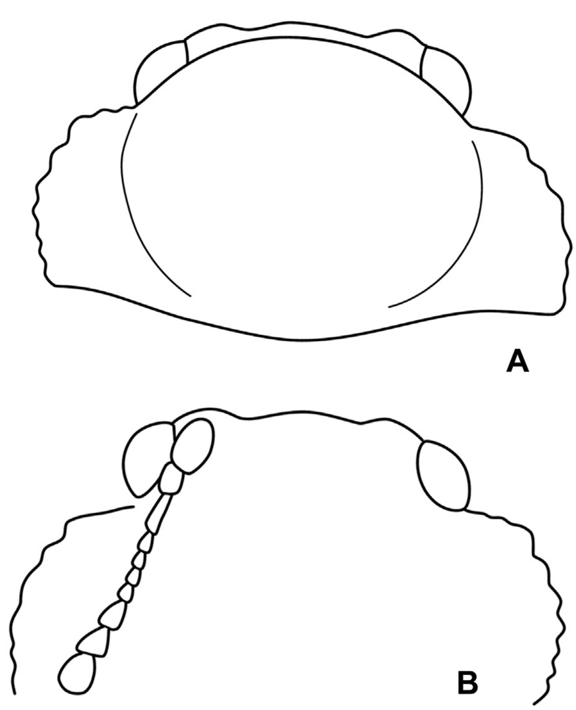

Fig. 14. *Libanopsis slipinskii* sp. nov., holotype "NBS 4C": A – head and pronotum, ventral view; B – head and ptothorax, ventral view. Body length 1.1 mm.

Key to the species

1. Head and pronotum slightly transverse; pronotum at most twice as wide as long … ……….…… 2

- Head and pronotum strongly transverse; pronotum more than three times as wide as long ……………………………… …………… 4

2 (1). Appendages nearly black; body less slender: each elytron less than three times (nearly 2.5 times) as long as wide; pronotum with rather narrowly subexplanate sides; ultimate tarsomere at base slightly narrower than at apex; tarsal claw distinctly dentate at base. Figs. 10 B, C; 11 ………………… ……………………… *L. limosa* sp. nov.

- Appendages brownish to reddish; body more slender; each elytron about three times as long as wide or longer; tarsal claws with a small serration …………………………………… ………………… ………… 3

3 (2). Pronotum less than 1.5 times as wide as long with subexplanate sides about as wide as antennal club; elytra regularly subparallel-sided; interspaces between punctures on elytra strongly elevated forming irregular smoothed ridges; underside of head somewhat elevated along the middle; distance between mesocoxae less than width of antennal club; metaventrite longer than prosternum with intercoxal process; ultimate tarsomere much thinner than at apex (with difference in thickness almost three times).

Figs. 4; 5 A, B; 6; 7 A, B .... *L. poinari* sp. nov.

- Pronotum more than 1.5 times as wide as long with subexplanate sides wider than antennal club; elytra widest at distal third; underside of head flattened; interspaces between punctures on elytra slightly vaulted; distance between mesocoxae greater than width of antennal club; metaventrite and prosternum with intercoxal process subequal in length; ultimate tarsomere only slightly thinner than at apex. Figs. 5 C; 7 D; 8; 9; 10 A .... *L. impexa* sp. nov.

4 (2). Elytral apices forming nearly a regular arc at apices; body about twice as long as wide; pronotum widest at the middle and with subexplanate sides much wider than antennal club; antennae longer (reaching posterior edge of prosternum), with thinner antennomeres, and rather loose and scarcely dorsoventrally compressed antennal club which is more than three times as long as wide; most setae of dorsum markedly more than twice as long as interstices between them. Figs. 7 C; 13; 14 .... *L. slipinskii* sp. nov.

- Elytral apices forming an angle at apices; body about 2.5 times as long as wide; pronotum widest at basal third and with subexplanate sides narrower than antennal club; antennae shorter (not reaching posterior edge of prosternum), with thicker antennomeres, and nearly compact and dorsoventrally compressed antennal club which is about twice as long as wide; setae of dorsum markedly not more than twice as long as interstices between them. Fig. 12 .... *L. straminea* sp. nov.

Discussion

Systematics

As demonstrated in the Comparison (see above), the new subfamily bears a combination of characters which can scarcely demonstrate a close relationship with any recent group in the family Sphindidae. In some sense the rank of this group is somewhat premature and only suggests the closest relatives for further elaboration of an equilibrium system of the superfamily. Sen Gupta and Crowson (1979) regarded this family as a member of a basal cucujoid branch and many authors prefer to consider it together with the Protocucujidae in a sister group relationship (McHugh, 1993; Ślipiński, 1999; Kirejtshuk, 2000; Leschen et al., 2005 etc.). The new subfamily could be a third member of this group. A more detailed diagnosis and comparison took this factor into consideration. Thus, the current placement of Libanopsinae subfam. nov. is indicative of its closest relation to the recent subfamilies of Sphindidae. Another possibility is to consider this new subfamily as equal to all recent groups combined as tribes in the subfamily (Sphindinae). However such changes of the family system or system within the larger cucujoid complex need a clearer definition of the characters including whether they are plesio- or apomorphic. This analysis is planned after further descriptions of some other Lower Cretaceous groups of Cucujoidea. At this level of our knowledge it is suspected that the unilobed maxillae and epipleura of *Libanopsis* species that steeply slope externally, their strongly transverse scutellum widening posteriorly with a long subtruncate apex and sparse erect setae on the dorsal integument seem to be (aut) apomorphies. On the other hand, the tarsal formula 5-5-5 of Libanopsinae subfam. nov. could be plesiotypic in comparison with 4-segmented male metatarsi in all recent sphindid groups and Protocucujidae. Other characters need further study, although such diagnostic characters as number of the antennomeres in antennal club and number of flagellomeres, level of separation of all pairs of coxae and so on seem to be a sequence of homoplasy in structural transformations.

The recent cladistic comparison of imaginal and larval characters of extant coleopterous groups (Lawrence et al., 2011) shows that cucujoids can be interpreted as four clades with different links and both Sphindidae and Protocucujidae fall in the same clade at the base of the infraorder Cucujiformia. The family Protocucujidae in the cladogram of these authors was placed together with Helotidae Chapuis; 1876, most part of Erotylidae Latreille; 1802, Byturidae Gistel, 1848 and some others. At the same time the family Sphindidae were placed in another clade together with Hobartiidae Sen Gupta and Crowson, 1966; Boganiidae Sen Gupta et Crowson, 1966; Cryptophagidae Kirby, 1826; Priasilphidae Crowson, 1973; another part of Erotylidae (genus *Toramus* Grouvelle, 1916); Cucujidae Latreille, 1802; Agapythidae Sen Gupta and Crowson, 1969 and Silvanidae Kirby, 1837. Thus, the latter clade includes about a fourth of the family groups of Cucujoidea and joins forms with very different adult structural characters. A strict analysis of these characters will become possible after the descriptions at least major Mesozoic groups of cucujoids. Hunt et al. (2008), after comparison of some nucleotide sequences of many extant groups of Coleoptera, concluded that the Sphindidae is a family sharing a sister group relationship with a clade comprising the superfamilies Tenebrionoidea and Lymexyloidea, while all cucujiformian superfamilies are presented in this interpretation as a intermixed aggregate. The list of contradictory interpretations ("phylogenies") of the cucujiformian beetles in current literature can be essentially continued. Nevertheless, Buder et al. (2008) after comparison of different molecular data concluded that "Two cucujoid families, i.e., the Silvanidae (or at least *Oryzaephilus*) and Sphindidae, almost consistently form the basalmost clades of the entire cucujoid-tenebrionoid(-cleroid) assemblage", placing the cleroids within mixture of cucujoids and tenebrionoids. It is interesting to note that the cleroids (Peltidae and Trogossitidae) appeared in the fossil record much earlier (Lower Jurassic) than both tenebrionoids (Mid Jurassic) and cucujoids (Upper Jurassic–Lower Cretaceous) (Kirejtshuk and Ponomarenko, 2013), i.e. the sequence of appearance of these superfamilies in fossils is opposite to that in the conclusions of many recent neontologists. It is thought, therefore, that somewhat more probable approach to the real phylogeny will become possible only after increasing of our knowledge on late Mesozoic cucujiformians.

The bionomics, larval development and ecology of the extinct species of the new subfamily could be similar to those in recent groups, which seem to be exclusively associated with Myxomycota. Discovering of five species in the same resource (Lebanese amber) suggests that resin-producing trees in the Lower Cretaceous "Lebanese" forest could be the target of regular infections by Myxomycota.

On CLSM technique for the study of arthropod inclusions in amber

About ten years ago the confocal laser scanning microscopy (CLSM) was accidentally found to be extremely appropriate for the study of amber inclusions (Böker and Brocksch, 2002). Various ancient plant, insect and fungal samples preserved in amber have been studied using this technique (Clark and Daly, 2010; Speranza et al., 2010). The application of CLSM methods for the study of inclusions in amber is still not widespread, however in accordance with previous studies (cited above) our results show that this method is rather promising, somewhat comparable with electron microscopy and x-ray microtomography or sometimes in certain aspects even surpassing them. The main advantages of the CLSM

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method are: 1) comparable low price of study, 2) noninvasiveness, 3) effortlessness and speed of 3D-reconstructions, 4) capacity of study of the same sample under both CLS and optic microscopes.

Amber is known to have a rather strong autofluorescence signal at the exposure of blue laser (length of wave 405 nm). The cuticle of many arthropods possesses a capacity to autofluorescence as well (Valdecasas, 2008; Chetverikov, 2012 and papers cited therein). According to our observations a rather distinct autofluorescent signal can be recorded from the integument of small well preserved insects in high quality amber inclusions examined under CLSM. Usually the bright background (autofluorescence of amber) and clear outlines of the studied inclusion are observed on the obtained CLSM images even if the amber is opaque and the inclusion is not perfectly seen under conventional light microscope. Later the digital CLSM data can be processed in graphic editors and impressive 3D models obtained comparable with those obtained using microtomographic methods. When studying very old material or amber with numerous crevices, the autofluorescence may be completely absent because of a deep degradation of initial structure of insect cuticle and replacement of the latter by totally non-fluorescent matter. Even in such cases, rarely it is possible to obtain good reconstructions of the insect surface using various modern filters embedded in modern graphic editors *e.g.* Amira®, Imaris® or Fiji Open Source Image Processing Package (Schindelin et al., 2012).

Results of CLSM study of amber inclusions drastically depends on the medium used for mounting specimens. The samples prepared in Canada Balsam can be studied under CLSM, but those mounted in epoxy resin cannot, because the autofluorescence of the epoxy resin totally masks the signal from the inclusion under examination. A similar observation has been recently achieved in the course of CLSM study of the exoskeletons of eriophyoid mites (Acariformes, Eriophyoidea): the Hoyer's medium (includes Hum Arabic, chloralhydrate, glycerin and water) is the best choice for this case, but any media including the Iodine and polyvinyl chloride based Heinze medium are not appropriate as it totally destroys (if Iodine media) or essentially diminishes (if Heinze medium) the quality of autofluorescent signal (Chetverikov, 2012, 2013; Chetverikov et al., 2012, 2013). Therefore using Canada Balsam for mounting the specimens in amber for CLSM study is recommended as preferential. However inclusions in some amber pieces from Mexico (Oligocene-Miocene) produced a rather slight autofluorescent signal which was not enough to study the specimens under examination.

It is also important to note that in comparison with the above mentioned eriophyoid mites, which are very tiny (about 200–300 μm long) and have thin cuticle, the autofluorescent signals from the studied samples in amber (beetles), recent insects, acari and many other terrestrial and aquatic arthropods in dry or preserved media that were studied by previous authors (Valdecasas and Abad, 2011; Haug et al., 2011 and papers cited) demonstrate much stronger autofluorescence and resistance to photobleaching probably because of the larger body-size and thickness of integuments. The photobleaching of samples of the eriophyoid mites occurs over 40–60 s even at low laser intensity (8–12%), but the samples of the beetles in amber and dry or fixed arthropods can be scanned by lasers with high intensity (35–50%) over 5–10 min without visible degradation of the quality of obtained images. This circumstance provides an opportunity to get images of high quality with lower rates of scanning and maximum resolution available in certain CLS microscope.

Acknowledgements

The authors sincerely thank Adam S. Ślipiński (CSIRO Ecosystem Sciences, Division of Entomology, Canberra) and Alexey V. Kovalev (Zoological Institute of the Russian Academy of Sciences, St. Petersburg) for valuable discussion on attribution of the fossil group here described. Besides, they thank Polina A. Kirejtshuk (the first author's daughter) for the assistance in preparing the illustrations for this paper. They are also grateful to the technicians at the Centre of Microscopy and Microanalysis of Saint-Petersburg State University, namely N.S. Ovchinnikova and K.A. Benken, for their advices concerning the CLSM technique. Important assistance was received from George Poinar Jr. (Oregon State University), who made the manuscript more fluent and transparent and also provided valuable comments. The study was partly carried out under the framework of the programme of the Presidium of the Russian Academy of Sciences "Problems of the origin of life and formation of the biosphere". The first author is supported by the Russian Foundation of Basic Research (grant 12-04-00663a). This paper is a contribution to the team project "Biodiversity: Origin, Structure, Evolution and Geology" granted to DA by the Lebanese University. The authors greatly appreciate valuable comments on an earlier version of the manuscript received from anonymous reviewers.

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Please cite this article in press as: Kirejtshuk, A.G., et al., Libanopsinae, new subfamily of the family Sphindidae (Coleoptera, Cucujoidea) from Lower Cretaceous Lebanese amber, with remarks on using confocal microscopy for the study of amber inclusions, Cretaceous Research (2014), http://dx.doi.org/10.1016/j.cretres.2014.02.008

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