Description of the male of fossil *Calomicrus eocenicus* Bukejs et Bezděk (Coleoptera: Chrysomelidae: Galerucinae) from Eocene Baltic amber using X-ray microtomography

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**Abstract.** A male representative of the extinct species *Calomicrus eocenicus* Bukejs et Bezděk, 2014 (Chrysomelidae: Galerucinae) is found and described for the first time from Eocene Baltic amber using X-ray microtomography. The aedeagus is well preserved within the body cavity of the specimen, and it is illustrated in detail. This fossil species exhibits distinct sexual dimorphism: the male has a smaller total body size, as well as a copula-adapted modification in abdominal ventrite 5 (apical margin deeply trilobed, with round medial fovea present); meanwhile the female is larger in body size and has a simple abdominal ventrite 5 (without fovea, non-incised and widely rounded apically). Similar sexually dimorphic characters are typical for extant members of the tribe Luperini, and this report is the first time that they are described in an Eocene species. The known sexually dimorphic characters present in Coleoptera within Eocene Baltic amber are briefly discussed.

**1 Introduction**

Records of Galerucinae sensu stricto (excluding Alticini) from fossil resins are scarce. Until now only four species have been described: *Leptonesiotes virkkii* Santiago-Blay, Poinar et Craig, 1996 from middle Miocene Dominican amber (20.43–13.65 Ma) (Santiago-Blay et al., 1996); *Calomicrus eocenicus* Bukejs et Bezděk, 2014 from middle Eocene Baltic amber (Bartonian, 41.2–37.8 Ma) (Bukejs and Bezděk, 2014); *Calomicroides danicus* Nadein, 2015 from upper Eocene Danish (Scandinavian) amber (37.2–33.9 Ma) (Nadein et al., 2015); and *Taimyraltica calcarata* Nadein, 2017 from Upper Cretaceous Taimyr amber (85.8–84.9 Ma) (Nadein and Perkovsky, 2018). Additionally, members of *Galerucella* Crotch, 1837; *Luperus* Geoffroy, 1762; and *Monolepta* Chevrolat, 1836 have been reported from Baltic amber without detailed descriptions (Hope, 1836; Klebs, 1910; Bachofen-Echt, 1949; Hieke and Pietrzeniuk, 1984; Spahr, 1981).

*Calomicrus eocenicus* was originally described based on a single female specimen. Unfortunately, the holotype was lost during postal shipment. In the current paper, the male of *C. eocenicus* from Baltic amber is described. The male genitalia are examined and illustrated using X-ray microtomography. The external sexually dimorphic characters are described, and the original species concept (based on the female holotype only) is revised. Finally, the available data on sexual dimorphism in fossil Coleoptera from Eocene Baltic amber are briefly discussed.
2 Geological setting

Baltic amber originally occurs within the “blaue Erde” (“Blue Earth”) horizon, which can be found throughout the Baltic Sea coastal area in Europe (Kaliningrad region, Russia; Poland; Denmark; Sweden; Germany; and Lithuania) (Engel, 2001). Although most estimates of the age of Baltic amber have suggested that it is derived from the early part of the middle Eocene (Lutetian, 47.8–41.2 Ma) based largely on K–Ar dating (Ritzkowski, 1997), palynological biostratigraphy of the specific region where the sample originated suggests a younger, late Eocene (Priabonian, 37.8–33.9 Ma) age (Aleksandrova and Zaporozhets, 2008). An intermediate, middle Eocene (mostly Bartonian, 41.2–37.8 Ma) age was recently proposed for the extinct central European resin-producing forests according to the stratigraphy of the Sambian amber deposits (Bukejs et al., 2019). Following classic views (e.g. Andrée, 1951; Poinar, 1992; Turkin, 1997), Baltic amber is thought to have been produced by *Pinus sibirica* (Göppert) Conwentz. Pinaceae along with different coniferous (Sadowski et al., 2017) and deciduous (primarily fagacean) trees, dominated the mixed forest cover of northern and central Europe in the warm-temperate, humid conditions of the Eocene. More recent research on the chemical composition of Baltic amber has also hypothesized that trees within the families Araucariaceae or Sciadopityaceae might be candidates for the production of this amber deposit (Wolfe et al., 2009; Lambert et al., 2014); however, the final chemical composition of amber can be influenced by long-term diagenetic processes, leaving the source tree for the deposit open to debate.

3 Material and methods

The material examined is deposited in the Palaeontology Collection of the Royal Saskatchewan Museum (Regina, Saskatchewan, Canada) (RSKM specimen number prefix).

Observations of the studied beetle specimen were made using a Nikon SMZ745T stereomicroscope. Photographs were taken using a Visionary Digital imaging system, consisting of a Canon EOS 5D camera with a Canon MP-E 65 mm macrophotography lens, attached to an automated camera lift with studio flash lighting. Extended depth of field at high magnifications was achieved by combining multiple images from a range of focal planes using Helicon Focus 6.8.0 software, and the resulting images were edited to create figures using Adobe Photoshop CS5.

The X-ray micro-CT observations of specimen RSKM_P3300.139 were conducted at Daugavpils University, Daugavpils, Latvia (DU), using a Zeiss Xradia 510 Versa system. Scans were performed with a polychromatic X-ray beam at an energy of 40 kV and power of 3 W. Sample-to-detector distance was set to 17.5 mm, and source-to-sample distance was 47.7 mm. Tomographic slices were generated from 3001 rotational steps through a 360° rotation, using a 4× objective, and the exposure time during each projection was set to 5 s. Acquired images were binned (2 × 2 × 2), giving a voxel size of 4.87 µm. Images were imported into the Dragonfly PRO (version 4.1) software platform for interactive segmentation and 3D visualization.

4 Systematic palaeontology

Family *Chrysomelidae* Latreille, 1802
Subfamily *Galerucinae* Latreille, 1802
Tribe *Luperini* Gistel, 1848
Subtribe *Luperina* Gistel, 1848
Genus *Calomicrus* Dillwyn, 1829
*Calomicrus eocenicus* Bukejs et Bezděk, 2014

Material examined

Collection number RSKM_P3300.139, male. A complete beetle is included in an elongate, transparent, yellow amber piece with dimensions of 41 mm × 19 mm × 8 mm, and preserved without supplementary fixation. Syninclusions: one specimen of Hymenoptera, a few stellate Fagaceae trichomes, numerous small detritus particles and gas vesicles.

Strata

Baltic amber from Eocene amber-bearing Blue Earth layers (a primarily Bartonian age is interpreted for the extinct central European resin-producing forests, according to Bukejs et al., 2019).

Locality

Yantarony settlement (formerly Palmnicken), Sambian (Samland) Peninsula, Kaliningrad region, Russia.

Note

Specimen RSKM_P3300.139 is morphologically similar to the female holotype of *Calomicrus eocenicus* and is identified as male, showing sexually dimorphic characters of abdominal ventrite 5 and a distinctly smaller body size (see Discussion).

Description of male

Measurements: total body length 3.5 mm; pronotum length 0.75 mm, pronotum maximum width 1.0 mm; elytra length 2.7 mm, elytra maximum width 1.9 mm.
Figure 1. *Calomicrus eocenicus* Bukejs et Bezděk, RSKM_P3300.139, photomicrographs, habitus: (a) dorsal view; (b) ventral view. Scale bars = 1.0 mm.

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Figure 2. *Calomicrus eocenicus* Bukejs et Bezděk, RSKM_P3300.139, X-ray micro-CT renderings, habitus: (a) dorsal view; (b) ventral view; (c) frontal view; (d) left lateral view. Scale bars = 1.0 mm.
Body elongate, slender, slightly convex dorsally and ventrally; reddish-brown as preserved, shiny, with weak metallic lustre; glabrous dorsally, with very fine, short, recumbent setation ventrally (distinctly visible on pygidium and abdomen).

Head hypognathous, relatively small, without distinct punctures; head together with eyes slightly wider than anterior margin of pronotum; vertex slightly convex. Compound eyes directed laterad, oval in outline, moderately large, prominent, entire, with distinct facets; vertical diameter 1.3× transverse diameter. Distance between eyes equal to about 1.4× transverse diameter of one eye. Antennal calli wide and elongate, slightly oblique, apparently contiguous; supracallinal sulcus distinct. Frontal ridge narrow, and sharp. Clypeus strongly transverse, with anterior margin concave. Labrum transverse, about 1.4× length of clypeus; anterior margin almost straight. Antennae filiform, slender, with 11 antennomeres; covered with fine, pale, semierect setae; antennae rather long, extending to reach posterior one-third of elytral length; antennomeres cylindrical, elongate; scape

Figure 3. Calomicrus eocenicus Bukejs et Bezděk, RSKM_P3300.139, X-ray micro-CT renderings: (a) elytra, dorsal view, with clipping planes exposing details beneath pronotum and right elytron; (b) pterothorax without legs, ventral view; (c) habitus without legs, caudal view. Scale bars = 0.5 mm. Abbreviations: f – fovea; p – pygidium; v1–v5 – abdominal ventrites 1–5 respectively.
Figure 4. *Calomicrus eocenicus* Bukejs et Bezděk, RSKM_P3300.139, X-ray micro-CT renderings, aedeagus: (a) dorsal view; (b) ventral view; (c) right lateral view; (d) left lateral view. Scale bars = 0.1 mm.

1.4× times as wide as antennomere 3; pedicle shortest, about 0.9× as long as antennomere 3; antennomeres 3–10 slightly dilated apically; antennomere 11 nearly spindle-shaped, with pointed apex, 3.3× as long as wide. Relative length ratios of antennomeres 1–11 equal to 9 : 6 : 7 : 8 : 8 : 8 : 8 : 9 : 8 : 11. Antennal insertion positioned posterior to anterior border of eyes. Distance between antennal sockets nearly equal to diameter of one socket.

**Prothorax.** Pronotum transverse, about 1.33× as wide as long, widest in anterior one-third of pronotal length, and slightly narrowed both anteriad and posteriad. Lateral margins widely rounded, with pronounced bordering; posterior margin slightly convex, with narrow bordering; anterior margin straight in dorsal view, arcuate in frontal view. Pronotal disc impunctate; lateral regions with sparse, very fine and effaced punctuation (distinctly smaller than elytral punctures). Posterior angles distinct, nearly orthogonal; anterior angles indistinct, not protruding laterally. Anterior and posterior setiferous pores bearing long setae. Prohypomera and prosternum without distinct punctuation; procoxal cavities open posteriorly; prosternal process sharp, very narrow, about 0.1× as wide as transverse diameter of procoxa.

**Pterothorax.** Scutellum shield small, triangular, impunctate, and glabrous. Elytra weakly convex, subparallel-sided, slightly narrowed posteriad, and widest in posterior one-third of elytral length; elytral anterior margin distinctly wider than pronotal posterior margin; covered with small, irregular punctures, in moderately dense arrangement, with distance between punctures equal to 1–3× diameter of one puncture. Humeral calli well developed, prominent. Elytral apices rounded. Metathoracic wings present, but details not observable in micro-CT scan data. Epipleura wide, gradually narrowed posteriorly, not reaching elytral apex, and covered with fine punctures. Metaventrite moderately convex, with distinct median line; covered with rugosity and very fine punctures. Metepisternum with rugosity and fine punctures, about 3.7× as long as wide; anterior and posterior margins oblique, lateral margins concave.

Legs slender, moderately long; covered with short, semierect setae. Procoxae widely oval and slightly transverse, 1.3× as wide as long, very narrowly separated; mesocoxae widely oval, narrowly separated by about 0.2–0.3× transverse diameter of mesocoxa; metacoxae narrowly elongate, transverse, moderately separated by about 0.5× transverse diameter of metacoxa. Femora slightly flattened and slightly dilated medially; metafemora and metatibiae equal in length; metathorax about 3× as long as wide. Tibiae almost straight, parallel-sided, without spines apically; metatibiae about 11× as long as wide. Tarsi long; metatarsus about 0.7× as long as metatibia; all tarsomeres 3 deeply bilobed; pro-
tarsomeres 1–3 slightly dilated apically, with protarsomere 1 about 0.3× as wide as long, protarsomere 2 about 0.6× as wide as long, and protarsomere 3 about 1.1× as wide as long. Relative length ratios of metatarsomeres 1–4 equal to 12 : 5 : 5 : 7. Claws thin, with large denticle basally.

Abdomen with five visible ventrites, densely covered with fine punctures. Ventrite 5 with deep, oval fovea medially; posterior margin trilobed (median lobe moderately narrow with rounded apical margin; incisions short). Relative length ratios of ventrites 1–5 equal to 18 : 10 : 5 : 6 : 12 (measured medially). Pygidium evenly covered with dense, fine punctures.

Aedeagus (Fig. 4) lanceolate, with sharp triangular apex; in lateral view, aedeagus moderately curved, with apical one-third of length thin, and middle part wide; in ventral view, aedeagus with wide, V-shaped, longitudinal furrow in middle part of length, and with longitudinal, medial carina in apical one-fifth of length. Tegmen Y-shaped.

5 Discussion

Sexual dimorphism can be an important feature useful in morphological, taxonomic, and phylogenetic studies. Sexual dimorphism consists of both primary and secondary sexual characteristics. The former include the features of the reproductive system (e.g. chromosomes, gonads, and genitalia), while the latter are very diverse and include different external (morphological) and internal (e.g. hormonal) adaptations. Secondary characteristics develop within each sex to facilitate ecological (e.g. feeding), behavioural (e.g. copulation, migration, offspring care) and other differences between specimens belonging to each sex. Features such as wing development, antennal length ratios, the form and size of sense organs or mandibles, tarsal setation and dilatation, horns, teeth, and different structural modifications of the exoskeleton (like furrows, foveae, projections on pronotum and elytra etc.), total body size, and colour variability are among the many well-known sexually dimorphic characters in Recent Coleoptera (Arrow, 1951; Crowson, 1981; Klausnitzer, 2002; Gullan and Cranston, 2014).

Sexual dimorphism in extant members of the tribe Luperini (Coleoptera: Galerucinae) is characterized as follows: males always have a trilobed abdominal ventrite 5, often with the middle lobe impressed; meanwhile, abdominal ventrite 5 is always simple and entire in females; often the males have slightly wider first tarsomeres and longer antennae (e.g. Wagner, 2003; Bezděk, 2015). Rarely the males of some luperin genera have also modified head, elytra or legs (Mohamed-said and Furth, 2013). However, the sexually dimorphic characters present on the abdomens of fossil representatives of Luperini have never been described. The description of Diatrotica exesa Wickham, 1911 is brief, and sexually dimorphic characters are not mentioned (Wickham, 1911). The holotype of Leptonesiotes virkkii Santiago-Blay, Poinar et Craig, 1996 is probably male, based on the structure of middle tibiae, but the terminal part of the abdomen is missing within this specimen (Santiago-Blay et al., 1996). The abdomen is also not visible in the holotype of Calomicroides danicus Nadein, 2015 (Nadein et al., 2015).

Calomicroides eocenicus was originally described from a single female (Bukejs and Bezděk, 2014), without any known male counterparts. The male described herein is morphologically similar to the known female. However, sexual dimorphism in this fossil species is distinct: the male has an abdominal ventrite 5 with a trilobed apical margin and with deep, round fovea medially, as well as having a smaller total body size; in the female, abdominal ventrite 5 is without fovea, and widely rounded apically, without incisions, while the total body size is significantly larger.

In general, amber specimens differ from compression fossils, where the sex of specimens is rarely determinable, and reports of sexually dimorphic characters are the exception, rather than the rule. Consequently, three-dimensionally preserved inclusions in fossil resins have the capacity to provide unique data on sexual dimorphism in extinct lineages. Sexual dimorphism in fossil beetles has usually been documented as an incidental observation in taxonomic papers (e.g. Lyubarsky and Perkovsky, 2015; Jiang et al., 2019). It has only received more extensive coverage in a few papers focused on groups such as fossil silvanid beetles like Protolitiota antennatus Liu, Ślipiński, Wang et Pang, 2019, from Cretaceous Burmese amber (Cai and Huang, 2019). Both the larger synthetic work and the taxonomic papers on amber inclusions have demonstrated that the antiquity of sexually dimorphic characters in Coleoptera can be traced back to the Cretaceous.

There are several characteristics that can be used for sex determination in fossil species within different beetle groups known from Baltic amber, and these appear to have been stable since at least the Eocene. Dimorphic characteristics include:

- modification of abdominal ventrites: shape of the last abdominal segments in Cantharidae (e.g. Kuśka and Kupryjanowicz, 2005; Fanti and Sontag, 2019 among others), and in Staphylinidae (Bogri et al., 2018); ventrite 1 with median, oval plaque in Monotomidae (Bukejs and Alekseev, 2015a);
- modification of antennomeres in Ripiphorinae (Batelka et al., 2020);
- pronotal shape in Cerambycidae (Vitali, 2006);
- elytral shape in Staphylinidae (Shavrin and Yamamoto, 2019);
- relative lengths of antennae in Cerambycidae (Vitali, 2018) and in Cantharidae (Parisi and Fanti, 2019);
- lateral pronotal lobes in Cantharidae (e.g. Parisi and Fanti, 2019);
– shape of protarsi and ventral surface of protarsomeres in Carabidae (Gamboa and Ortuño, 2015, 2018; Schmidt, 2015), Chrysomelidae (Bukejs and Konstantinov, 2013), Dytiscidae (Balle et al., 2010), and Melandryidae (Bukejs and Alekseev, 2015b);

– exposed distal parts of male and female genitalia in Aderidae (Alekseev and Grzymala, 2015), Anthicidae (Telnov and Bukejs, 2019), Cantharidae (Kazantsev, 2013), Chrysomelidae (Bukejs and Chamorro, 2015; Bukejs et al., 2015, 2016), Cupedidae (Kirejtshuk, 2005), Ischaliidae (Alekseev and Bukejs, 2017), Lycidae (Kazantsev, 2019), Melandryidae (Alekseev, 2014), Pyrochroidae (Bukejs et al., 2019), Scirtidae (Heuss, 2008; Klausnitzer, 2012), Staphylinidae (Jaloszyński et al., 2018; Brunke et al., 2019; Shavrin and Yamamoto, 2019), Tenebrionidae (Nabozhenko et al., 2019a, b; Telnov et al., 2019; Alekseev et al., 2020a), Zopheridae (Alekseev and Bukejs, 2016) and other groups.

Recent technological advances have allowed researchers to identify the sex of specimens with greater confidence and expand their study of features with significance to sex determination in amber inclusions. X-ray microtomography data obtained from lab-based machines or synchrotron beamlines have allowed sex determination in fossils without visibly exposed genitalia. The optically hidden, internal genitalia have been successfully studied in fossil species of Carabidae (Schmidt et al., 2016, 2017, 2019; Schmidt and Michalik, 2017), Latridiidae (Reike et al., 2017), Leiodidae (Perreau and Tafforeau, 2011; Perreau, 2012; Perreau and Perkovsky, 2014), Merophsiidae (Reike et al., 2020), Mycetophagidae (Alekseev et al., 2020b), Tenebrionidae (Nabozhenko et al., 2020), and other coleopteran families. To date, the sexual dimorphism that has been discovered in Eocene Baltic amber beetles can be characterized as “evolutionarily stable” and limited to characters known in present-day relatives; no new sexually dimorphic characters have been discovered in Eocene fossils. The continued search and accurate study of the Baltic amber beetle assemblage may possibly yield future discoveries in this respect, enabling us to provide further details on the morphology of fossil beetles and the range of variation present within individual taxa.

Author contributions. AB designed the study. AB and JB identified the specimen and performed systematic placement. AB prepared specimen description and plates. KK performed µCT measurements, reconstructed µCT data and created volume renderings and 3D models. VIA prepared the geological background and suggested ideas for discussion. VIA, AB and JB drafted the discussion. RCM located the specimen and prepared photomicrographs and plates. All authors drafted the manuscript and contributed to the writing and discussion.

Competing interests. The authors declare that they have no conflict of interest.

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