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Solitary folded-winged wasps of the genus *Zethus* Fabricius (Vespidae, Zethinae) parasitised by two new species of Strepsiptera on different continents

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Abstract

Two new species of Strepsiptera from the genus *Zethus* Fabricius, 1804 (Hymenoptera: Vespidae) are described. Although the stylopisation of the genus *Zethus* has been known for almost a century, we provide the first description of its strepsipteran parasites. *Zethus brasiliensis fuscatus* R. Bohart & Stange, 1965 is parasitised by *Eupathocera zethi* **sp. nov.** in the Neotropical Region (French Guiana) and *Zethus favillaceus* Walker, 1871 by *Deltoxenos impressus* **sp. nov.** in the Afrotropical Region (Kenya). An independent switch to the same host genus is supported by molecular and morphological data. Diagnoses and detailed descriptions of the species are provided based on characters of the female cephalothorax and male cephalotheca. Diagnostic characters are discussed.

Keywords

Cephalothorax, morphology, parasite association, Strepsiptera, taxonomy, wasp parasites, *Zethus*

Introduction

Zethus Fabricius, 1804 is the most species-rich genus in Vespidae and comprises 279 out of the 363 species of Zethini (Lopes et al. 2021). Although a recent phylogenomic study supported the subfamily status of Zethinae as sister to Polistinae + Vespinae

(Piekarski et al. 2018), the original "Zethini" were revealed as a paraphyletic grouping with two major clades Raphiglossinae and Zethinae (Bank et al. 2017). The close phylogenetic relationship between Zethinae and Polistinae + Vespinae also has implications for the interpretation of the evolution of traits such as nest-building (Bank et al. 2017). Polistinae and Vespinae are well known for building nests from paper-like material (Evans and West-Eberhard 1970). Intriguingly, some members of Zethinae use old insect burrows in twigs, branches or in the ground, which is possibly an ancestral behaviour. However, other species in the subfamily construct new nests from masticated and salivated plant material (usually leaves) pasted together with a resinous substance (Bohart and Stange 1965). Advanced nesting behaviour has arguably contributed to the evolutionary success of Polistinae and Vespinae after their solitary ancestors evolved the ability to exploit masticated and salivated plant material for constructing nests (Bank et al. 2017). Some species of Zethinae even have preserved a plesiomorphic form of social behaviour, with several females sharing a nest but each one tending their own brood, but with complex interactions such as nest usurpation, nest defence, or adoption of orphaned nests (Lopes and Noll 2014; Kelstrup et al. 2023).

The genus *Zethus* is widespread throughout the New World and the Ethiopian and Oriental Regions (Nguyen and Carpenter 2017). It reaches its greatest diversity in the Neotropics, where over 230 species are known (Tan et al. 2018). The genus is characterised by the following features: propodeum with elongate orifice and acutely pointed dorsally, propodeal valvula elongate and quadrate, separate from propodeal lamella; labial palp four-segmented; mid-tibia usually with two spurs (Bohart and Stange 1965; Nguyen and Carpenter 2017; Tan et al. 2018). A recent cladistic analysis based on morphological characters supported 9 subgenera within the genus *Zethus* (Lopes et al. 2021).

Regarding interactions with other organisms, only fragmentary information is available on parasites of *Zethus* species. Pereira et al. (2018) reported 8 genera of phoretic and parasitic mites (Acari) found on *Zethus*. Salt (1927) and Salt and Bequaert (1929) recorded *Zethus pubescens* Smith, 1857, *Z. spinipes variegatus* Saussure, 1852, and *Z. romandinus* Saussure, 1852) as hosts of Strepsiptera. Almost a century later Benda et al. (2019) recorded stylopised *Zethus* sp. from French Guiana. The undescribed strepsipteran species was assigned to *Pseudoxenos* Saunders, 1872 (Xenidae), following the traditional taxonomy of Strepsiptera with species parasitising solitary wasps included in this genus. Benda et al. (2021) published a molecular phylogeny of Xenidae based on an extended dataset with a focus on species diversity. They recorded *Zethus brasiliensis fuscatus* R. Bohart & Stange, 1965 as a host for an undescribed putative species of *Pseudoxenos*. Benda et al. (2022b) published a new generic classification of Xenidae and included the undescribed species from *Z. brasiliensis fuscatus* in the genus *Eupathocera* Pierce, 1908 which was restituted from synonymy.

In the present study we describe two new species of Strepsiptera from two host species from two continents, *Z. brasiliensis fuscatus* and *Z. favillaceus* Walker, 1871. We provide diagnoses and descriptions of the species in accordance with a previous phylogenetic study (Benda et al. 2021) and an updated generic classification based on characters of the female cephalothorax and male cephalotheca Benda et al. (2022b).

Materials and methods

Taxon sampling

The material of Strepsiptera from *Zethus brasiliensis fuscatus* and *Z. favillaceus* comprised a total of 11 females, 3 empty male puparia and 1 occupied male puparium. Material from the following public and private collections was examined:

NMPC National Museum of the Czech Republic, Prague, Czech Republic;
OLML Oberösterreichisches Landesmuseum, Linz, Austria;
YNPC Yuta Nakase personal collection, Matsumoto, Japan.

Fixation and preparation

The host individuals were relaxed in water vapour and then immediately dissected. The dissected endoparasitic female and male puparium were removed from the host body and cleared using a mixture of lysis buffer ATL and proteinase K (Qiagen) at 56 °C for several hours. The cleared specimen was cleaned in distilled water several times and then stored in a vial with 96% ethanol. The female cephalothorax was air-dried or dried by using absolute ethanol and hexamethyldisilazane (HMDS method) (Heraty 1998) to prevent the cuticle from collapsing during the drying process. The female body was extracted from the cephalothorax before drying. After this step, the dried specimens were glued onto card mounting points, which were pinned afterwards.

Measurements

The width and length of the female cephalothorax, the female head capsule and the male cephalotheca were measured using a Leica S9D Stereomicroscope with a calibrated ocular micrometer. The cephalothorax length was measured from the apex of the clypeal lobe to the constriction of abdominal segment I; the cephalothorax width is the maximum distance between its lateral margins.

Photomicrography

The general habitus of stylopised host specimens and the host abdomen with protruding strepsipterans were documented with multifocus images, taken with Canon EOS 550D or 70D cameras equipped with EF 50 mm and MP-E 65 mm macro lenses. Lateral lights and a diffuser were used.

For the documentation of the original colouration of the female larval cephalothorax and the male cephalotheca, specimens glued to card mounting points were used. They were photographed with a Canon EOS 7D digital SLR equipped with a Canon MP-E 65 mm macro lens (Canon, Krefeld, Germany) fitted with a StackShot macro rail (Cognisys, Traverse City, MI, USA). Each specimen was illuminated with two

flashlights (Yongnuo Photographic Equipment, Shenzhen, China) fitted to a transparent cylinder for even and soft light. For the documentation of tiny structures on the head capsule, we used a Canon EOS 70D camera attached to an Olympus BX40 microscope. The microscope was equipped with lateral lights and a diffuser. Zerene Stacker (Zerene Systems LLC, Richland, USA) was used to process stacks of images with different focus.

Scanning electron microscopy (SEM)

Dried female cephalothoraces glued to card points were mounted on a rotatable specimen holder (Pohl 2010). Each specimen was sputter coated with gold with an Emitech K 500 (Sample preparation division, Quorum Technologies Ltd., Ashford, England). The SEM micrographs were taken with an ESEM XL30 (Philips, Amsterdam, Netherlands) equipped with Scandium FIVE (Olympus, Münster, Germany).

Image processing

All images were processed and arranged into plates with Adobe Photoshop® CS5 (Adobe System Incorporated, San Jose, USA) software. CorelDraw® X8 (CorelDraw Corporation, Ottawa, ON, Canada) was used for the lettering of the plates.

Terminology and description style

The terminology used for the female cephalothorax was adopted from Benda et al. (2022), Richter et al. (2017), Löwe et al. (2016), and Kinzelbach (1971a). The cephalothorax is described in morphological orientation in figures, although its functional orientation in the host's body is inverted.

Results

Eupathocera zethi **Benda & Straka, sp. nov.**
https://zoobank.org/8AD765DD-069C-44E2-BAAA-46020E29AC56

Type material. ***Holotype*** • French Guiana: 1♀; Cayenne, Roura env., 18 Oct. 2015; Naoki Ogawa leg.; NMPC; host: *Zethus brasiliensis fuscatus* R. Bohart & Stange, 1965.

Paratypes • French Guiana: 1♀; same host specimen (collection data) as for holotype, 18 Oct. 2015; Naoki Ogawa leg.; YNPC; 1♀ + 1 empty male puparium (EMP), 2♀; 35 km S of Roura, Relais de Patawa, 16 July 2000; Ji. Kadlec leg.; OLML; same host species as holotype; 1♀; NE Mount de Kaw Fourgassie, 5. Aug. 2006; M. Snížek; OLML; same host species as holotype.

Diagnosis of female cephalothorax. This species is diagnosed by a combination of characters. It differs from all remaining species of *Eupathocera* by the presence of very

conspicuously imprinted mesal furrows indicating the pro-mesothoracic and meso-metathoracic borders on the ventral side (sbpm, sbmm; Figs 1C, 2A), and inconspicuous mandibles fused with the labial area and bearing a rounded (not distinctly raised) mandibular bulge (mdb; Fig. 3E). The clypeal surface is completely smooth with distinctly exposed sensilla, in contrast to *Eupathocera luctuosae* Pierce, 1911 and *E. insularis* (Kifune, 1983), which display a wrinkled, lamellar clypeal area, with scarcely visible sensilla. The number of clypeal sensilla is very high in the new species, more than 60. A larger number occurs only in species utilising *Sphex* L., 1758 – *Eupathocera fuliginosi* (Brethes, 1923) (more than 60) and *E. westwoodi* (Templeton, 1841) (more than 80). The border between the clypeal area and frontal region is indistinct in comparison to *Eupathocera luctuosae* Pierce, 1911 and *E. insularis* (Kifune, 1983) where it is clearly recognisable.

Description of female cephalothorax. ***Shape and colouration.*** Size of holotype cephalothorax: length 1.8 mm, width 1.74 mm; slightly variable, as long as wide or slightly wider than long, length 1.78–2.03 mm, width 1.74–1.83 mm. Abdominal segment I not protruding laterally, corner below spiracles rounded. Anterior head margin rounded, not protruding from head capsule. Thorax slightly widening posteriorly. Colouration of cephalothorax mostly dark with light brown pattern on ventral side, but mostly light brown dorsomedially with specific contrast pattern.

Head capsule. Approximately ⅓ as long as entire cephalothorax including lateral extensions. Colouration mostly dark with specific pattern with paler lateral extensions, mandibular bases and ventral labral field. Clypeal area distinctly delimited from labral area. Clypeal lobe rather indistinct but visible. Clypeal surface completely smooth with distinctly exposed sensilla. Number of clypeal sensilla slightly over 60. Border between clypeal and frontal region indistinct but still present. Frontal region smooth or very slightly wrinkled (fr, Fig. 2F). Segmental border between head and prothorax indicated by dark transverse stripe on dorsal side (sbhp, Fig. 1D), in SEM pictures visible by change in cuticular sculpture (sbhp, Fig. 3B). Head and prothorax distinctly separated by birth opening ventromedially (bo, Fig. 3A) and laterally by suture (sbhp, Fig. 3A).

Supra-antennal sensillary field. Smooth or very slightly wrinkled, with dispersed sensilla (Fig. 2C, D). Not distinctly delimited by furrow medially, but border marked by different surface structure of supra-antennal sensillary field and smooth frontal region (Fig. 3B).

Antenna. Vestigial antenna bulging, preserved as more or less clearly defined area, with distinct plates (pra, Fig. 2C, D). Antennal torulus reduced (Fig. 2C, D). Periantennal area expanded, smooth (paa, Fig. 2C, D). Distance between antennal area and supra-antennal sensillary field relatively large.

Labrum. Ventral field wider than long, elliptic, completely smooth, shiny, and pale, contrasting with dark dorsal labral field and labium. Dorsal labral field slightly arcuate, 5× wider than long in midline. Setae on dorsal field blunt, sensilla-like, spines lacking.

Mandible. Anteromedially directed at an angle of 30°, enclosed in mandibular capsule. Mandibular bulge not distinctly raised, rounded, with several inconspicuous sensilla. Cuticle of mandible smooth posteriorly, with longitudinal grooves dorsolaterally (md; Fig. 3E, F). Mandibular tooth narrow, anteriorly oriented, with or without spines (mdt; Fig. 3E, F).

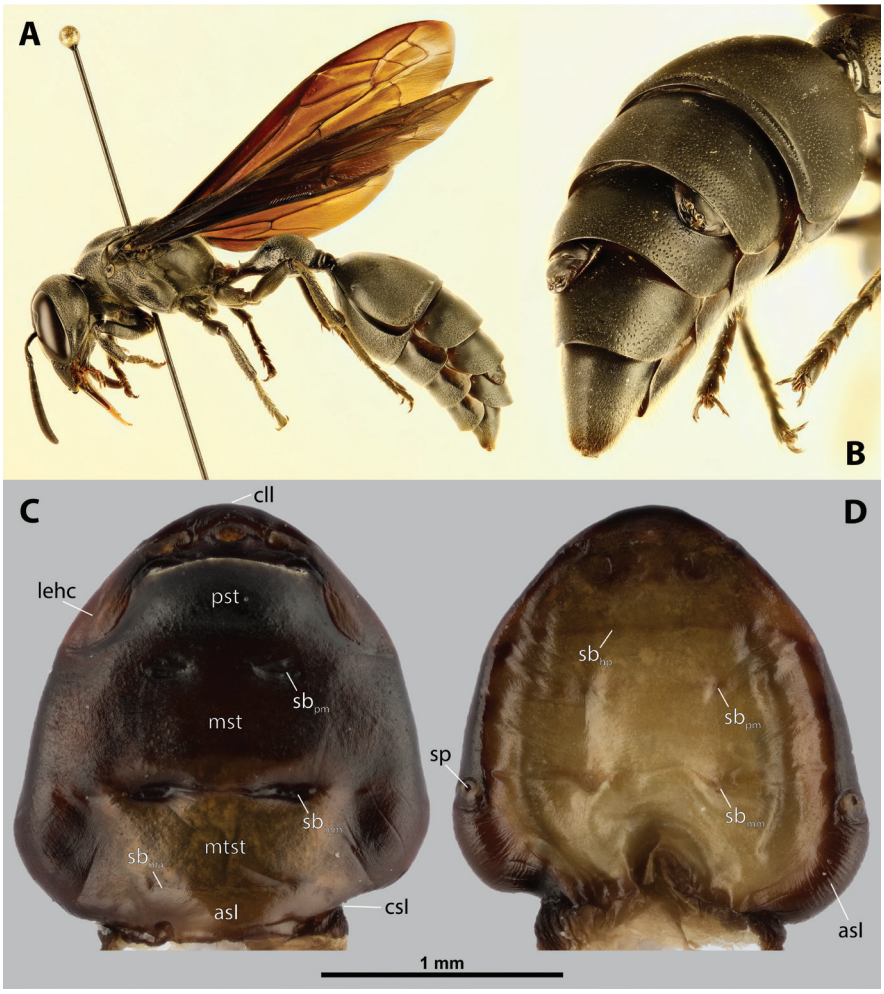

Figure 1. *Eupathocera zethi* Benda & Straka, sp. nov., host, female cephalothorax **A** *Zethus brasiliensis fuscatus* R. Bohart & Stange, 1965 stylopised by *E. zethi* sp. nov., lateral view **B** detail of host abdomen of *Z. brasiliensis fuscatus*, with two female cephalothoraces **C–D** holotype of *E. zethi* sp. nov., ♀ (NMPC) from *Z. brasiliensis fuscatus* **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: asI – abdominal segment I, cll – clypeal lobe, csI – constriction of abdominal segment I, lehc – lateral extension of head capsule, mst – mesosternum, mtst – metasternum, pst – prosternum (prosternal extension), sbhp – segmental border between head and prothorax, sbma – segmental border between metathorax and abdomen, sbmm – segmental border between mesothorax and metathorax, sbpm – segmental border between prothorax and mesothorax, sp – spiracle.

Maxilla. Distinctly reduced and only very slightly protruding, not projecting beyond mandible anteriorly. Partially fused to labial area, both regions not clearly separated. Cuticle reticulated, with smooth areas, not distinctly wrinkled (mx; Fig. 3E, F). Vestige of palp inconspicuous, forming small bulge with impression, located medially on ventral side of maxilla. Submaxillary groove indistinctly produced posterolaterally towards maxillary base.

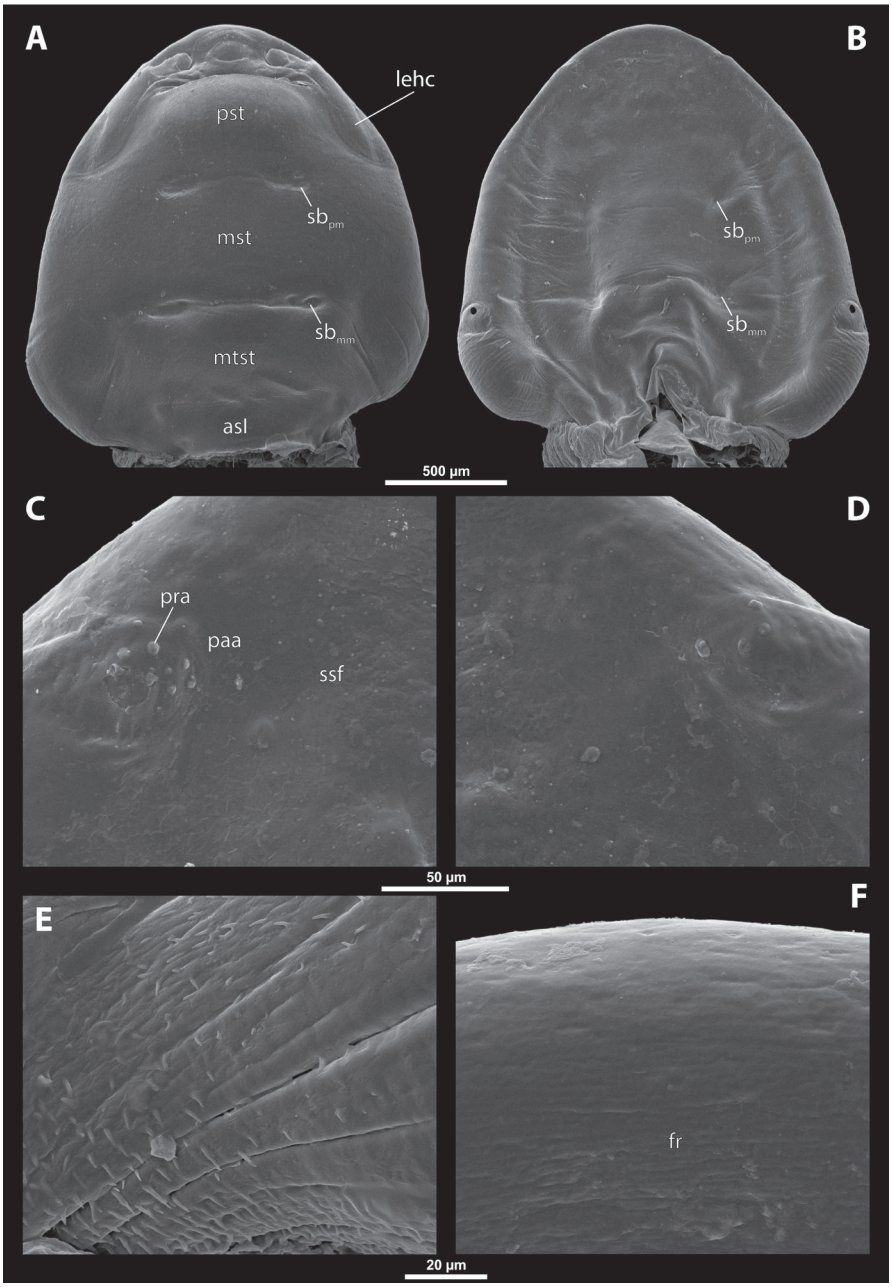

Figure 2. Holotype of *Eupathocera zethi* Benda & Straka, sp. nov., ♀ (NMPC), SEM micrographs of cephalothorax **A** ventral side **B** dorsal side **C** right vestigial antenna, dorsal side **D** left vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of anterior border of cephalothorax, dorsal side. Abbreviations: asI – abdominal segment I, fr – frontal region, lehc – lateral extension of head capsule, mst – mesosternum, mtst – metasternum, paa – periantennal area, pra – plate of vestigial antenna, pst – prosternum (prosternal extension), sbmm – segmental border between mesothorax and metathorax, sbpm – segmental border between prothorax and mesothorax, ssf – supra-antennal sensillary field.

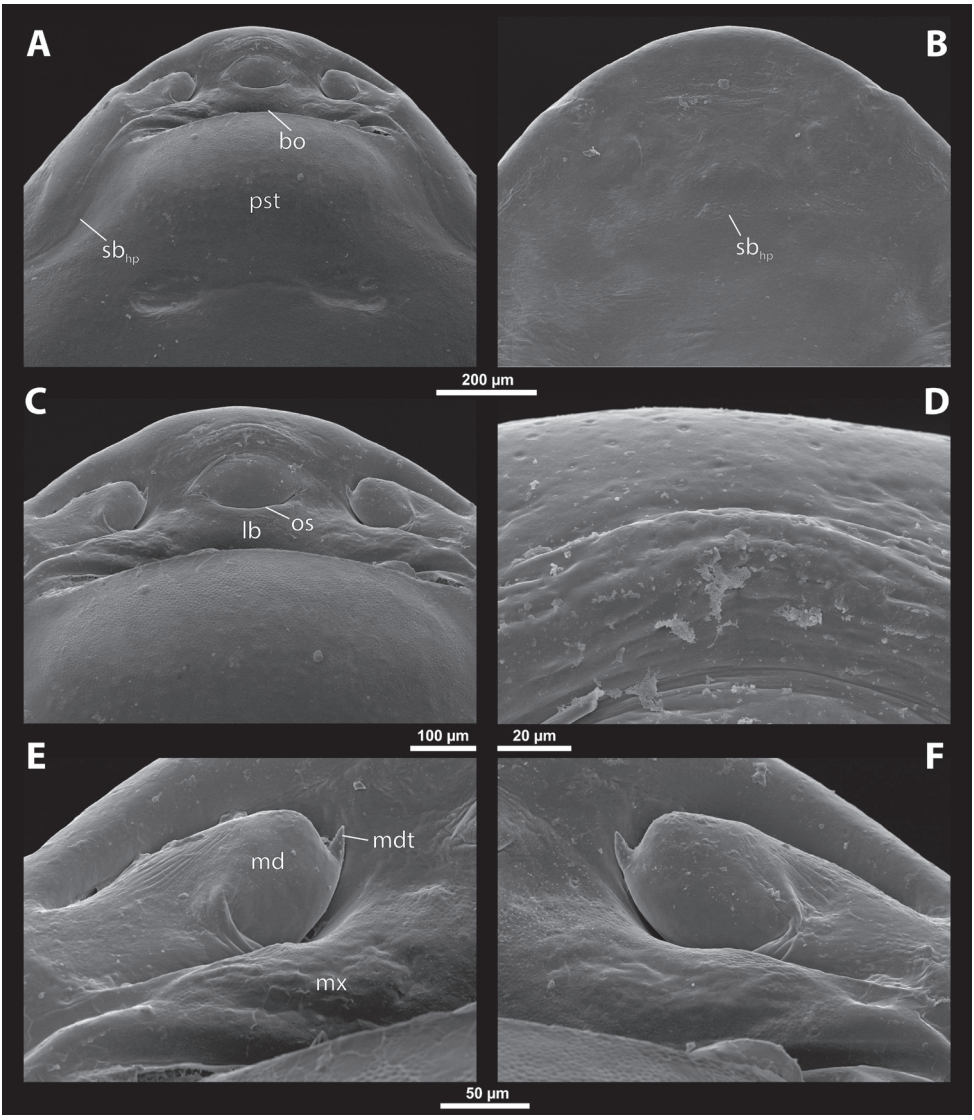

Figure 3. Holotype of *Eupathocera zethi* Benda & Straka, sp. nov., ♀ (NMPC), SEM micrographs of cephalothorax **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: bo – birth opening, lb – labial area, md – mandible, mdt – mandibular tooth, mx – vestige of maxilla (maxilla), pst – prosternum (prosternal extension), os – mouth opening, sbhp – segmental border between head and prothorax.

Labium. Labial area not distinctly recognisable between maxillae, flat, slightly longer than wide in midline (lb; Fig. 3C). Anteriorly delimited by mouth opening, posteriorly by birth opening. Cuticular surface very slightly reticulated.

Mouth opening. Slightly arcuate, sclerotised along margin (os; Fig. 3C).

Thorax. Pro-mesothoracic and meso-metathoracic borders distinct on ventral side, separated by mesal furrows (sbpm, sbmm; Figs 1C, 2A). On dorsal side separated by less conspicuous dark mesal furrows (sbpm, sbmm; Figs 1D, 2B). Border between metathorax and abdomen marked by change in cuticular surface structure or pigmentation. Cuticle of thoracic segments reticulate on ventral side, often with scattered small papillae. Dorsal side of thorax predominantly smooth, only slightly wrinkled. Prosternal extension undifferentiated. Prosternum bulging, distinctly elevated above head medially and laterally (pst; Fig. 3A). Shape of meso- and metathorax unmodified, transverse. Prosternum and mesosternum on ventral side with dark colouration, but metasternum medially pale. All thoracic segments pale on dorsal side, but dark laterally.

Abdominal segment I and spiracles. Setae and cuticular spines present on lateral region of abdominal segment I (Fig. 2E). Spiracles on posterior ~ ⅓ of cephalothorax slightly elevated, with anterolateral or anterodorsal orientation. Cephalothoracic part of abdominal segment I below spiracles dark on dorsal side, medially paler on ventral side (asI; Figs 1C, 2A).

Etymology. The name refers to the host genus *Zethus*. From Greek *Zethus* – the son of Zeus in ancient Greek mythology. Adjective.

***Deltoxenos impressus* Benda & Straka, sp. nov.**
https://zoobank.org/9FE257A5-8010-4607-9285-7364594430E2

Type material. ***Holotype.*** Kenya • 1♀; Mwingi, Kangonde vadi, 18 Apr. 2007; M. Halada leg.; OLML; host: *Zethus favillaceus* Walker, 1871.

Paratypes. Kenya • 2♀; 1♀ + 2 empty male puparia; 1♀ + 1 male puparium; 1♀ + 1 male puparium + 2 empty male puparia; same locality and host as for holotype, 18 Apr. 2007; M. Halada leg.; OLML.

Diagnosis of female cephalothorax. This species is easily distinguished from other representatives of the genus *Deltoxenos* by mandibles distinctly anteromedially directed (angle of 65°) and by conspicuous impressions on the surface of the lateral extensions at the site of the reduced compound eyes. These impressions are best visible on SEM images (im; Fig. 6A) but also recognizable with a light microscope. The surface of the lateral extensions is slightly wrinkled or smooth in other species of *Deltoxenos*. The anterior head margin is rounded, and the clypeal lobe is merged with the head capsule, in contrast to *Deltoxenos rueppelli* (Kinzelbach, 1971), where the clypeal lobe protrudes distinctly from the head capsule but is blunted on top. On the ventral side the clypeal area is very smooth and lacks sensilla (cl; Fig. 6D), whereas sensilla are present ventrally in *Deltoxenos bidentatus* (Pasteels, 1950) and *D. rueppelli*. In contrast to *Deltoxenos bequaerti* (Luna de Carvalho, 1956), the cuticle of the thoracic segments on the ventral side is reticulate, with scattered inconspicuous or more distinct and pigmented papillae, mainly visible on the metasternum. In contrast, the thoracic segments on the ventral side are evenly scattered with conspicuous papillae in *D. bequaerti*.

Other characters that distinguish *D. impressus* sp. nov. from *D. bidentatus* and *D. rueppelli*: ventral field wider than long, elliptic, nearly circular versus more flattened and not nearly circular; dorsal field arcuate, slightly raised versus dorsal field distinctly arcuate and distinctly raised; mandible anteromedially directed at angle of 65° versus a maximum of 45° in *D. rueppelli*; mandibular bulge distinctly raised, elongated, slightly curved laterally versus mandibular bulge slightly raised and anteriorly directed; cuticle of mandible completely smooth versus almost completely wrinkled.

Description of female cephalothorax. ***Shape and colouration.*** Size of holotype cephalothorax: length 1.06 mm, width 0.9 mm. Cephalothorax variable in size but always distinctly longer than wide, length 0.9–1.06 mm, width 0.74–0.9 mm. Promesothoracic and meso-metathoracic segmental border only slightly constricted laterally (Fig. 4C, D). Abdominal segment I not protruding laterally, corner below spiracles rounded. Anterior head margin rounded, not distinctly protruding from remaining head capsule. Thorax elongated, very slightly widening posteriorly. Cephalothorax with conspicuously contrasting light and dark colour pattern.

Head capsule. Ca ⅔ as long as entire cephalothorax including lateral cephalic extension. Colouration forming specific pattern with dark brown anterior part and pale lateral extensions. Surface of lateral extensions at site of reduced compound eyes smooth, with conspicuous impressions visible on SEM images (im; Fig. 6A). Clypeal area well delimited from labral area, arcuate, clypeal lobe merged with head capsule. Surface completely smooth with slightly more than 40 distinctly exposed sensilla on dorsal side (cls; Fig. 5F). Ventral side of clypeal area smooth and lacking sensilla (cl; Fig. 6D). Border between clypeal and frontal region indistinct but still present. Frontal region smooth (fr; Fig. 6B). Segmental border between head and prothorax indicated by distinct mesal furrow on dorsal side (sbhp; Fig. 6B) and by dorsal transverse stripe of frontal and occipital papillae (p; Fig. 6B). Head and prothorax distinctly separated by birth opening ventromedially (bo; Fig. 6A) and laterally by suture (sbhp; Fig. 6A).

Supra-antennal sensillary field. Completely smooth, with dispersed sensilla (Fig. 2C, D). Distinctly delimited by furrow on medial side (fssf; Fig. 6B), surface of supra-antennal sensillary field and frontal region with same sculpture.

Antenna. Preserved as poorly defined area, with several distinct rounded plates and an inconspicuous cavity (Fig. 5C, D). Antennal torulus reduced. Periantennal area expanded, smooth (paa; Fig. 5C, D). Border between antennal area and supra-antennal sensillary field indistinct.

Labrum. Ventral field wider than long, elliptic, nearly circular. Dorsal field arcuate, slightly raised, laterally narrower than medially, 6× wider than long in midline (vlf, dlf; Fig. 6C, D). Dorsal field with 16 sensilla inserted in cavities.

Mandible. Mandibles anteromedially directed at angle of 65°, enclosed in mandibular capsule. Mandibular bulge distinctly raised, elongated, slightly curved laterally, with several sensilla (mdb; Fig. 6E, F). Cuticle of mandible completely smooth. Mandibular tooth slightly curved, pointed apically, almost completely lacking spines (mdt; Fig. 6E, F).

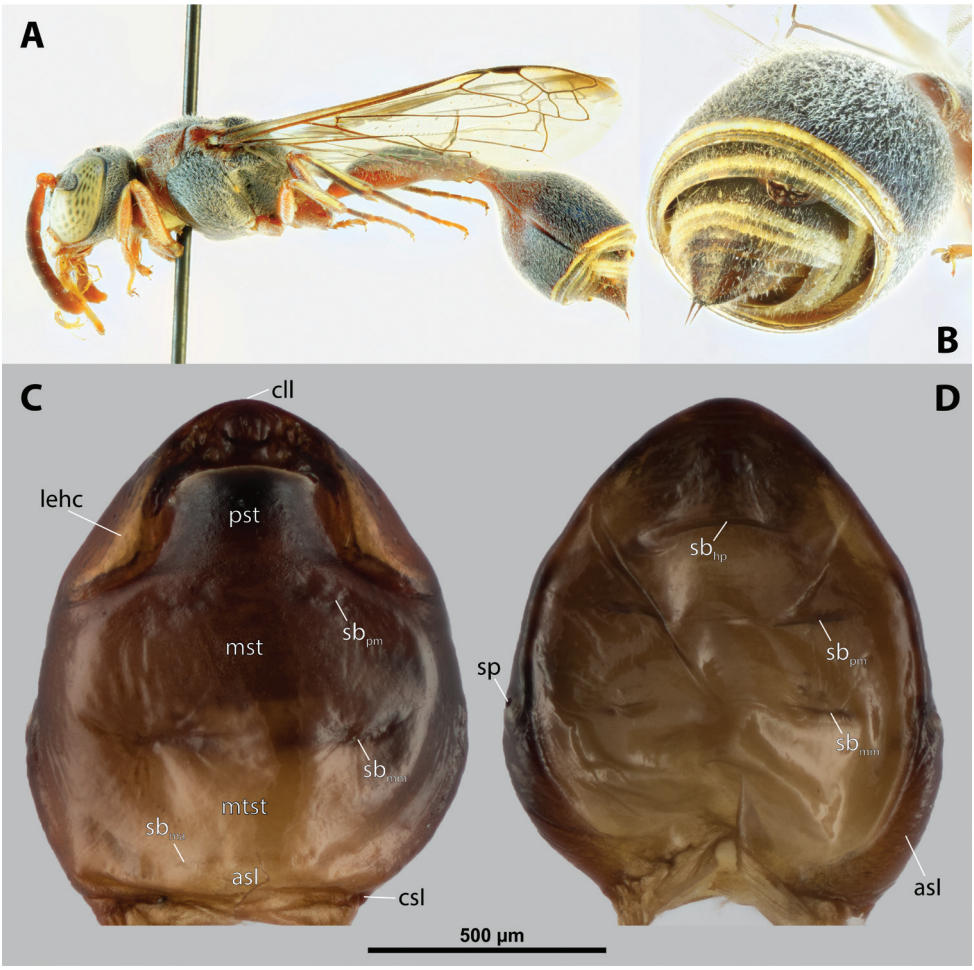

Figure 4. *Deltoxenos impressus* Benda & Straka, sp. nov., host, female cephalothorax **A** *Zethus favillaceus* Walker, 1871 stylopised by *D. impressus* sp. nov., lateral view **B** detail of host abdomen of *Z. favillaceus*, with female cephalothorax **C–D** holotype of *D. impressus* sp. nov., ♀ (OLML) from *Z. favillaceus* **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: asI – abdominal segment I, cll – clypeal lobe, csI – constriction of abdominal segment I, lehc – lateral extension of head capsule, mst – mesosternum, mtst – metasternum, pst – prosternum (prosternal extension), sbhp – segmental border between head and prothorax, sbma – segmental border between metathorax and abdomen, sbmm – segmental border between mesothorax and metathorax, sbpm – segmental border between prothorax and mesothorax, sp – spiracle.

Maxilla. Only slightly raised, almost fused with labial area (mx; Fig. 6C). Cuticle smooth, with longitudinal furrow. Apical maxillary region not projecting beyond mandibular apex. Basal part firmly connected with labium and not overlapping with mandible (mxb; Fig. 6C). Vestige of palp indistinct. Maxillary base distinctly produced anterolaterally as submaxillary groove. Space between prothoracic extension and head extended (sbhp, mxb; Fig. 6A).

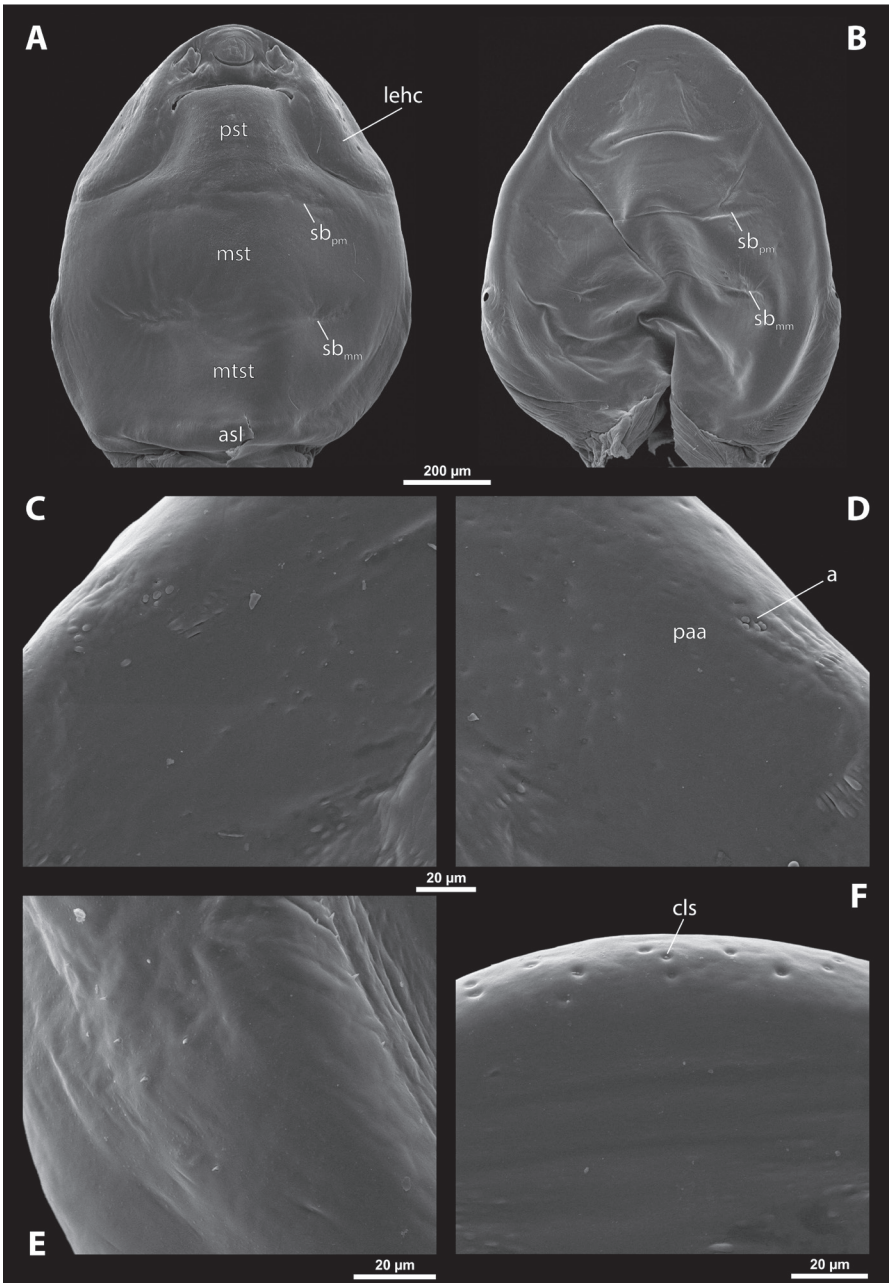

Figure 5. Holotype of *Deltoxenos impressus* Benda & Straka, sp. nov., ♀ (OLML), SEM micrographs of cephalothorax **A** ventral side **B** dorsal side **C** right vestigial antenna, dorsal side **D** left vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of anterior border of cephalothorax, dorsal side. Abbreviations: a – vestigial antenna, asI – abdominal segment I, cls – clypeal sensillum, lehc – lateral extension of head capsule, mst – mesosternum, mtst – metasternum, paa – periantennal area, pst – prosternum (prosternal extension), sbmm – segmental border between mesothorax and metathorax, sbpm –segmental border between prothorax and mesothorax.

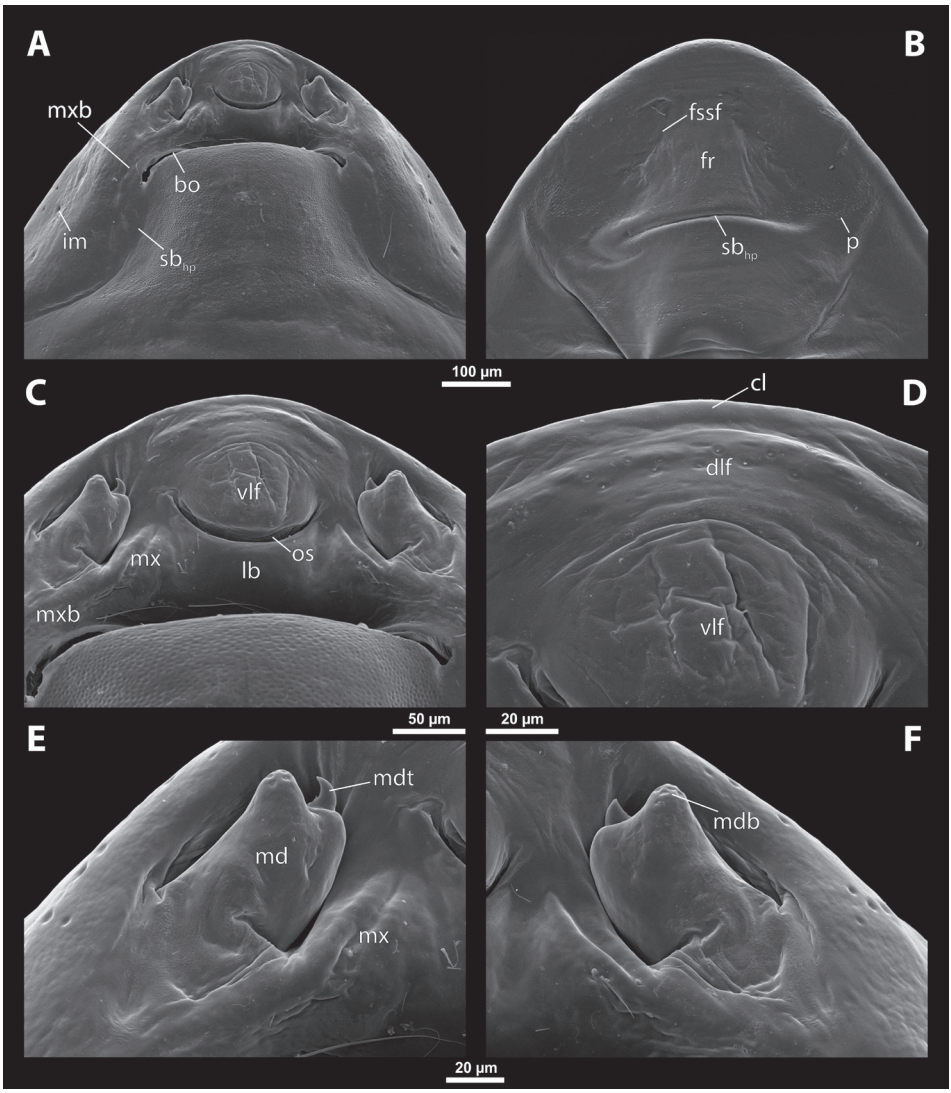

Figure 6. Holotype of *Deltoxenos impressus* Benda & Straka, sp. nov., ♀ (OLML), SEM micrographs of cephalothorax **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: bo – birth opening, cl – clypeal area, dlf – dorsal labral field of labral area, im – impression, lb – labial area, md – mandible, mdb – mandibular bulge, mdt – mandibular tooth, mx – vestige of maxilla (maxilla), mxb – maxillary base (at mandible base), os – mouth opening, p – papilla, sbhp – segmental border between head and prothorax, vlf – ventral labral field of labral area.

Labium. Labial area quite indistinct between maxillae, delimited anteriorly by mouth opening and posteriorly by birth opening (lb; Fig. 6C). Flat, approximately as long as wide. Cuticular surface smooth.

Mouth opening. Widely arcuate, almost semicircular, sclerotised along margin (os; Fig. 6C).

Thorax. Pro-mesothoracic and meso-metathoracic borders visible ventrally as slightly imprinted mesal furrows (sbpm, sbmm; Figs 4C, 5A). On dorsal side separated by conspicuous dark mesal furrows, distinctly contrasted with pale thoracic segments (sbpm, sbmm; Figs 4D, 5B). Border between metathorax and abdomen indicated by ventral ridge on ventral side or indicated by change in colour and cuticular sculpture. Cuticle of thoracic segments on ventral side reticulate with scattered inconspicuous or more distinct pigmented papillae, mainly visible on metasternum. Prosternum conspicuously dark, mesosternum brown, and metasternum light brown. Prosternal extension undifferentiated. Dorsal side of thorax usually completely smooth, rarely with papillae on prothorax. All thoracic segments dorsally pale, but dark laterally. Meso- and metathorax transverse, rarely slightly elongated.

Abdominal segment I and spiracles. Setae and cuticular spines present on lateral region of abdominal segment I posterior to spiracle (Fig. 5E). Spiracles on posterior ~ ⅔ of cephalothorax slightly elevated, with anterolateral orientation. Cephalothoracic part of abdominal segment I below spiracles dark brown and medially light brown on both sides (asI, Fig. 4C).

Diagnosis of male cephalotheca. This species is distinguished from other representatives of the genus *Deltoxenos* by a combination of the following characters: clypeal lobe distinctly arcuate in frontal view versus conspicuously bulging as in *D. rueppelli*; shape of cephalotheca rounded in frontal view, very slightly flattened (Fig. 7C) versus more flattened in *D. rueppelli*; frontal region with distinct impression versus impression not visible in *D. rueppelli* and *D. bequaerti*; occipital bulge not protruding from elliptic shape of cephalotheca versus occipital bulge protruding in *D. rueppelli* and *D. bequaerti*.

Other characters that distinguish *D. impressus* sp. nov. from *D. rueppelli*: clypeal lobe as wide as mandible versus clypeal lobe distinctly wider than mandible; gena completely dark (gn; Fig. 7C) versus light brown.

Description of male cephalotheca. ***Shape and colouration.*** In frontal view rounded, very slightly flattened, elliptic, length 0.6 mm, width 0.86 mm, in lateral view protruding anteriorly, pointed apically (Fig. 7C, D). Colouration predominantly dark with paler areas.

Cephalothecal capsule. Compound eyes visible, pale to dark, with dark individual cornea lenses. Clypeal lobe distinctly arcuate in frontal view, prominent in lateral view (cl; Fig. 7C, D). Clypeal area completely dark, clypeal sensilla indistinct. Clypeal lobe as wide as mandible.

Frontal region with paired furrow of supra-antennal sensillary field, distinct impression and slightly raised occipital bulge (ssf, fi, ob; Fig. 7C). Occipital bulge not protruding from elliptic outline of cephalotheca. Gena completely dark (gn; Fig. 7C). Diameter of genae between maxillary base and compound eye approximately 3× larger than diameter of vestigial antenna.

Supra-antennal sensillary field. Dark, kidney-shaped and bulging, not delimited medially by distinct furrow (ssf; Fig. 7). Dark sensilla well visible.

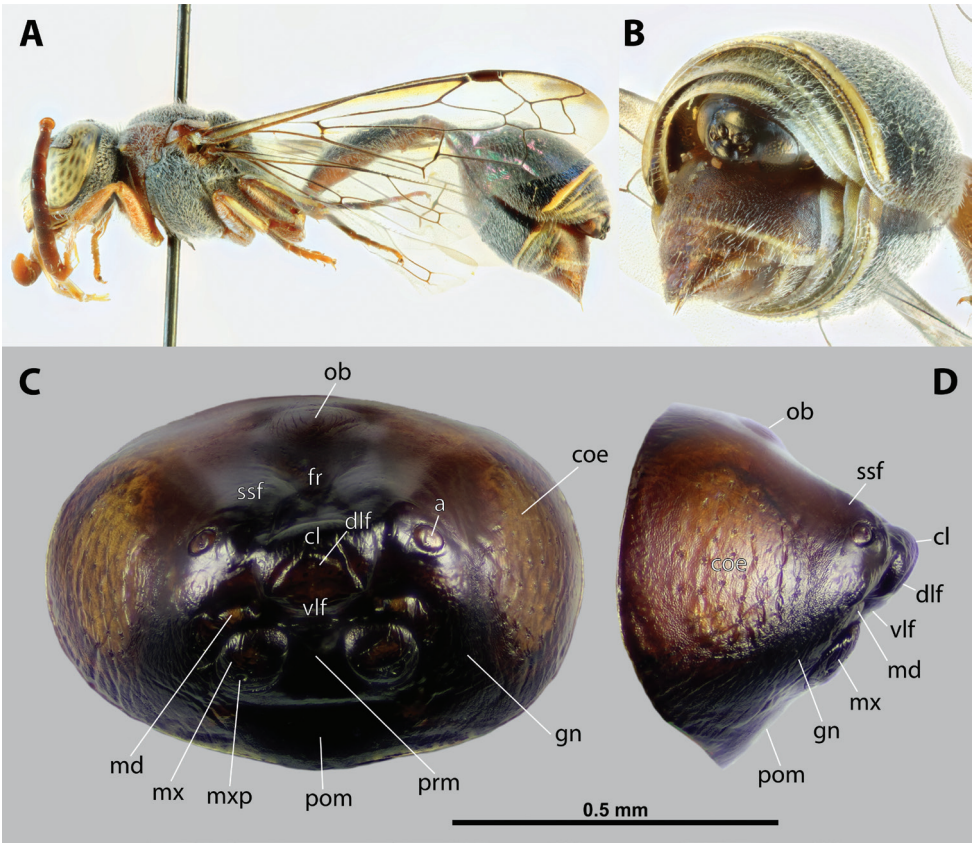

Figure 7. *Deltoxenos impressus* Benda & Straka, sp. nov., host, male cephalotheca **A** *Zethus favillaceus* Walker, 1871 stylopised by *D. impressus* sp. nov., lateral view **B** detail of host abdomen of *Z. favillaceus*, with male cephalotheca **C–D** paratype of *D. impressus* sp. nov., ♀ (OLML) from *Z. favillaceus* **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: a – vestigial antenna, cl – clypeal area, coe – compound eye, dlf – dorsal labral field of labral area, fr – frontal region, gn – gena, md – mandible, mx – vestige of maxilla, mxp – vestige of maxillary palp, ob – occipital bulge, pom – postmentum, prm – praementum, ssf – supra-antennal sensillary field, vlf – ventral labral field of labral area.

Antenna. Of standard shape, dark, small, with small plates or sensilla and complete torulus (a; Fig. 7C). Periantennal area not clearly delimited from supra-antennal sensillary field. Small plates and sensilla present.

Labrum. Labral area distinct, slightly less dark medially. Dorsal field arcuate, with dispersed setae visible (dlf; Fig. 7C). Ventral field elliptic.

Mandible. Nearly medially directed (md; Fig. 4E). Colouration predominantly dark but slightly lighter medially. Distance between mandibles very distinctly exceeding mandibular length. ***Maxilla.*** Distinct, prominent, completely dark with inconspicuous paler spot anteriorly. Wide at base, approximately 2× as wide as mandible (mx; Fig. 7C). Vestige of palp present, conspicuous (mxp; Fig. 7C).

Labium and hypopharynx. Labium distinct between and below maxillae, completely dark. Praementum and postmentum separated by furrow. Hypopharyngeal protuberance present but very indistinct (hyp; Fig. 7C). Mouth opening well visible, not covered by ventral labral field, distinctly arcuate.

Etymology. From the Latin substantive *impressio*, meaning an impression or hole. The specific epithet refers to conspicuous impressions on the lateral extensions of the female cephalothorax. Adjective.

Discussion

Although *Zethus* belongs to the most diverse and widespread genera of the family Vespidae (with 279 described species), very little is known about the spectrum of its parasites. The evolution of the highly diverse genus is also insufficiently known, even though an extensive phylogeny at the subgeneric level was presented recently (Lopes et al. 2021). From all established nine subgenera of *Zethus*, three are known to be parasitised by Strepsiptera (*Madecazethus*, *Zethus*, *Zethusculus*) (Table 1). Not surprisingly, both xenid species described in this work from different continents parasitise species of phylogenetically very distinctly separated *Zethus* subgenera. The host of *Deltoxenos impressus* sp. nov. belongs to the most ancestral subgenus *Madecazethus*, which is distributed in the Afrotropical realm and part of the Palearctic region. In clear contrast, *Eupathocera zethi* sp. nov. parasitises species of the subgenus *Zethusculus*, which is deeply nested within *Zethus* and is distributed in the New World.

Although parasites can be good models to track the biogeography of their hosts on the species and population level on a small geographical scale (Štefka et al. 2011), the situation is more complicated on higher taxonomic levels. In the case of the evolution of Xenidae, switches to a new host lineage, often connected with long-distance dispersal, play a prominent role (Benda et al. 2022b). Many xenid subgroups are rather opportunistic with some level of host group conservatism rather than host-parasite coevolution. Previously, we indicated many other parallel switches to the same group of Hymenoptera (e.g. Ammophilini, Sphecini, *Ancistrocerus* Wesmael, 1836) (Benda et al. 2021).

Table 1. Overview of *Zethus* species stylopised by Xenidae with general information about distribution, parasitic species, and original study.

Species	Subgenus	Distribution	Strepsiptera species	Publication
Zethus brasiliensis fuscatus R. Bohart & Stange, 1965	*Zethusculus*	French Guiana	*Eupathocera zethi* sp. nov.	Benda et al. (2021), this study
Zethus favillaceus Walker, 1871	*Madecazethus*	Kenya	*Deltoxenos impressus* sp. nov.	this study
Zethus pubescens Smith, 1857	*Madecazethus*	Republic of South Africa	Unknown	Salt and Bequaert (1929)
Zethus spinipes variegatus Saussure, 1852	*Zethus*	Texas, Florida (USA)	Unknown	Salt and Bequaert (1929)
Zethus romandinus Saussure, 1852	*Zethusculus*	Peru	Unknown	Salt and Bequaert (1929)

Within Vespidae, *Polistes* Latreille, 1802 − a widely distributed and large genus of social wasps, with 237 described species (Silveira et al. 2021) − is parasitised by the maximum number of species of Strepsiptera. The genus typically associated with it, *Xenos* Rossi, 1793, presently comprises 33 described species of the Old and New World (Benda et al. 2022b). In contrast, other very diverse vespid genera such as *Mischocyttarus* Saussure, 1853 or *Zethus* have a scarce record of being parasitised by strepsipterans. *Mischocyttarus* is a large genus of the New World that comprises approximately 250 described species (Silveira 2008), but only three strepsipteran parasites are described (Benda et al. 2022a). Like in the case of *Zethus*, there are probably many more undescribed species of Strepsiptera that have escaped notice due to their inconspicuousness or rarity.

The xenid genus *Eupathocera* has a very wide range of hosts. It parasitises wasps from three families: Sphecinae, Ammophilinae (Sphecidae); *Tachytes* (Crabronidae: Crabroninae); *Zethus* (Vespidae: Zethinae); and *Pachodynerus* (Vespidae: Eumeninae) (Benda et al. 2022b). According to the phylogeny of Benda et al. (2021), *Eupathocera* from *Zethus* is the sister lineage of the monophyletic group parasitising digger wasps with a divergence of about 20 million years ago. Folded-winged wasps of the genus *Zethus* could have played a key role in the evolution of Xenidae during the switch from solitary wasps (Eumeninae) to sphecid wasps (Sphecidae) that occurred in parallel in the New and Old World (Benda et al. 2019). Due to a wide host range connected with morphological variability within the genus, the diagnosis of *Eupathocera zethi* sp. nov. is complicated and a combination of characters is required. In contrast, *Deltoxenos impressus* sp. nov. can be very easily distinguished from other *Deltoxenos* species by the orientation of the mandibles and lacking impressions at the site of the compound eyes. Although we were not able to obtain DNA sequences due to the age of the material, the species can be easily assigned to a genus using a key to the genera of Xenidae (Benda et al. 2022b).

Our work and data in the literature (Salt and Bequaert 1929) suggest that the genus *Zethus* is more frequently parasitised than expected and more vigilance is needed when handling material of its species. It is also very likely that both described species have a distinctly larger distribution than we recorded in this work, which is tentatively suggested by the distribution area of *Zethus favillaceus* that ranges from South Africa to Saudi Arabia (Tan et al. 2018).

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