Extending the fossil record of late Oligocene non-biting midges (Chironomidae, Diptera) of New Zealand (#105715)

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Extending the fossil record of late Oligocene non-biting midges (Chironomidae, Diptera) of New Zealand

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Background: The chironomid subfamily Orthocladiinae is diverse and highly endemic in New Zealand, and thought to reflect a complex biogeographical history. This fauna has been important for developing phylogenetic and biogeographic concepts such as Brundin´s writings on Transantarctic relationships but the fossil record that would support these reconstructions has been extremely poor. Here we describe the first fossil species of Chironomidae, subfamily Orthocladiinae, from New Zealand, based on inclusions in amber from the late Oligocene Pomahaka Formation of the South Island. **Methods:** We examined newly excavated fossil tree resin (amber) from the late Oligocene Pomahaka Formation in southern New Zealand for inclusions. Amber pieces containing chironomids were prepared and morphologically investigated using light-microscopy and µCT-scanning. Specimens were taxonomically identified based on identification keys for modern adult chironomid midges. Habitus and key morphological features of each specimen were documented photographically and/or by line drawings. **Results:** Thirteen Chironomidae specimens from Pomahaka amber were identified as members of the subfamily Orthocladiinae Kieffer, which hitherto had no fossil record from New Zealand. *Bryophaenocladius zealandiae* sp. nov. Baranov is the first fossil Southern Hemisphere record of the genus *Bryophaenocladius* Thienemann, which is absent from the extant fauna of the main islands of New Zealand but may be present on the subantarctic Auckland Islands. Two specimens described as Morphotype 1 closely resemble *Bryophaenocladius zealandiae* sp. nov. but are significantly larger, either indicating intraspecific size variation or representing a separate taxon close to *B. zealandiae*. *Gymnometriocnemus extinctus* sp. nov. Baranov is the first fossil record of the widespread extant genus *Gymometriocnemus* Edwards, today present with a single endemic species in New Zealand. The new fossil taxa belong to chironomid groups requiring terrestrial or semi-aquatic habitats for larval development, which supports the notion of a humid forest swamp paleoenvironment for the Pomahaka amber source forest.

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Abstract

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Keywords: Chironomidae, fossil insects, palaeoecology, Pomahaka Formation, Zealandia

Introduction

Non-biting midges (Chironomidae) have historically served as a model group for the development of both modern phylogenetic analysis and historical biogeography (Hennig, 1960; Brundin, 1966). Studies of the Chironomidae fauna of New Zealand have played a major role in understanding transantarctic vicariance patterns (Brundin, 1966; Krosch & Cranston, 2013). In particular, phylogenetic studies of the Podonominae, southern Diamesinae and austral Orthocladiinae were seminal for understanding vicariance patterns caused by the break-up of Gondwana (Brundin, 1966; Krosch et al., 2011; Krosch & Cranston, 2013). This renders the fossil record of New Zealand's Chironomidae significant to understand biogeographic patterns in the Southern Hemisphere (Schmidt et al., 2018; Baranov, Haug & Kaulfuss, 2024).

However, our knowledge of the fossil history of Chironomidae in New Zealand has been very limited, so far. Schmidt et al. (2018) reported four specimens of *Bryophaenocladius* (Orthocladiinae) from Oligocene amber from the South Island, which are included in our descriptions herein. Baranov, Haug & Kaulfuss (2024) described three morphotypes of immature Chironomidae from Early Miocene lake sediments at Foulden Maar in South Island. Subfossil records of Chironomidae include the larvae of *Corynocera duffi* Deevey, 1955 from Holocene swamp deposits in Canterbury, South Island (Deevey, 1955) and numerous other chironomid taxa identified from various Holocene sites on South Island (Schakau, 1991; Woodward & Schulmeister, 2007; Dieffenbacher-Krall et al., 2008). Phylogenetic reconstructions have suggested that some chironomid lineages such as representatives of Podonominae survived a postulated “Oligocene bottleneck” or hypothetical complete Oligocene submersion of the New Zealand landmass (Cranston et al., 2010).

Considering this limited fossil record, it is difficult to improve our understanding of the evolutionary history of Chironomidae in New Zealand. Thus, any additional deep time records of ~~Chironomidae~~ from New Zealand are of great value. In this study, we describe two new species of non-biting midges (Chironomidae: Orthocladiinae) from Oligocene amber from the South Island of New Zealand. These new discoveries ~~extend our~~ knowledge of the past diversity and historical biogeography of Orthocladiinae in New Zealand.

Geological setting

The Chironomidae specimens studied ~~herein~~ are inclusions in amber from the estuarine late Oligocene Pomahaka Formation in southern New Zealand. Fossiliferous amber was collected from a lignite bed and associated carbonaceous mudstone in a temporary excavation pit on private farmland near Pomahaka River approx. 12 km south of Tapanui (46.04450°S, 169.22292°E) (Fig. 1). The locality is registered as G45/f0107 in the New Zealand Fossil Record File (GNS Science & Geological Society of New Zealand, 2024). Fourier-transform infrared spectroscopy analysis of amber from the site indicates an araucarian, *Agathis*-like parent plant, which is supported by finds of araucarian wood and frequent pollen of *Araucariacites australis* Cookson in Pomahaka Formation sediments (Pocknall, 1982; Lee et al., 2009; Kaulfuss et al., 2024). Within the lignite and underlying carbonaceous mudstones, amber is very common and occurs randomly distributed as mm-sized droplets to dm-sized lumps and blocks, showing no signs of sorting and abrasion by reworking and transport. Combined with reconstructions from sedimentological and palynological data (Pocknall, 1982; Lindqvist, Gard & Lee, 2016), this indicates *in situ* resin deposition and amber formation in a domed forest swamp adjacent to brackish mire or saltmarsh within an estuarine paleoenvironment. A comprehensive facies analysis of Pomahaka Formation was published by Lindqvist, Gard & Lee (2016). The late Oligocene age (Chattian, New Zealand stage Duntroonian, 27.3–25.3 ma) of Pomahaka Formation is based on palynomorph and molluscan biostratigraphy (Wood, 1956; Pocknall, 1982; Beu, Maxwell & Brazier, 1990).

Materials and Methods

Material

Thirteen Chironomidae inclusions from Pomahaka amber were studied herein, including four specimens reported as *Bryophaenocladius* Thienemann or closely related to it by Schmidt et al. (2018) and nine newly discovered specimens. Three of the *Bryophaenocladius* specimens reported by Schmidt et al. (2018) are fossilized in a single piece of amber and a further specimen in a separate piece. The collection number OU35028.2 collectively assigned to all four specimens in Schmidt et al. (2018) is here replaced by individual numbers for each specimen (OU47579–OU47582). The nine new specimens originate from a single amber piece made up of multiple thin layers formed by successive resin flows but were separated and prepared as individual pieces (Figs. 1B, C). The type material and associated specimens are deposited in the Geology Museum of the Geology Department, University of Otago (OU); collection numbers are provided below in the Systematic Paleontology section.

Preparation and imaging

Layered pieces of amber were microscopically examined for biological inclusions and subsequently separated along surfaces of individual resin flows. In instances where this resulted in the exposure of wings at the surface of the amber piece, wings were photographed with a binocular stereomicroscope (Carl Zeiss Stemi 508 with a Canon EOS 70D digital camera) prior to further preparation. Where possible, the thin and brittle, inclusion-bearing amber shards were ground and polished to obtain dorsal, ventral and/or lateral views of inclusions. Polished amber shards, and those too small and fragile for polishing, were embedded in epoxy resin to stabilise specimens, applying the protocol provided by Sadowski et al. (2021). Epoxy-embedded amber pieces were ground and polished using a grinder/polisher machine (Buehler Eco-Met 250) and CarbiMet silicone carbide abrasive papers (CarbiMet) and/or manually using a set of wet silicone carbide abrasive papers (FEPA P #220–4000).

Specimens were studied with a Carl Zeiss AxioScope A1 compound microscope and photographed with a Canon 5D digital camera. Figures were generated with Helicon Focus (8.2.0) software and enhanced using Adobe® Photoshop CC. Line drawings were prepared with Inkscape software.

μCT-scanning

Four Chironomid specimens preserved in close proximity in nearly opaque amber could not be prepared separately and studied by light microscopy. These specimens (OU47579, OU47580, OU47581 and OU47582) were scanned on the Imaging Beamline P05 (Lytaev et al., 2014) operated by the Helmholtz-Zentrum Hereon at the PETRA III storage ring (Deutsches Elektronen Synchrotron - DESY, Hamburg, Germany), using a photon energy of 18 keV and a sample-to-detector distance of 100 mm. Projections were recorded with a custom 20 MP CMOS imaging system with an effective pixel size of 1.28 µm (Lytaev et al., 2014). For each tomographic scan, 3601 projections were recorded at equal intervals between 0 and π. Reconstruction was carried out by applying a transport of intensity phase retrieval approach and using the filtered back projection algorithm (FBP). This workflow was carried out in a custom reconstruction pipeline using Matlab (Math-Works) and the Astra Toolbox (Moosmann et al., 2014; van Aarle et al., 2015, 2016). Raw projections were binned twice for further processing, resulting in an effective pixel size of the reconstructed volume (voxel) of 2.56 µm. Scanned volumes were reconstructed using Drishti ver. 2.6.6 (Limaye, 2012). To decrease the demands for computer memory, we converted all stacks into 8-bit tiffs, downscaled all tiffs by 50% and subsequently cropped the empty space around the amber piece using Fiji 'scale' and 'crop' functions (Schindelin et al., 2012). Volumes were rendered in Drishti ver. 2.6.6 (Limaye, 2012).

Terminology and taxonomy

Our morphological terminology is based on Sæther (1980) and Marshall et al. (2017). Specimens were identified using the keys provided by Cranston, Oliver & Sæther (1989), Langton & Pinder (2007), Albu (1974), Andersen & Schnell (2000), Armitage (1987), Du, Wang & Saether (2011), Epler (2012), Freeman (1959), Hazra & Das (2011), Kaczorowska & Gilka (2002), Lin, Qi & Wang (2012), Makarchenko & Makarchenko (2006), Moubayed & Lods-Crozet (2022), Moyubayed & Langton (2023), Pinder & Armitage (1986), Saether (1973, 1977, 1983), Sublette & Wirth (1980), Wang, Sæther & Andersen (2001) ,Wang, Liu & Epler (2004), Wang, Andersen & Sæther (2006) and Willassen (1996).

Leg measurements of specimens are mainly approximated values only, due to the difficulty of measuring the variously oriented legs in the amber.

Results

Systematic paleontology

Order **Diptera** Linnaeus, 1758
Family **Chironomidae** Newman, 1838
Subfamily **Orthocladiinae** Kieffer, 1911
Genus ***Bryophaenocladius*** Thienemann, 1934
Bryophaenocladius zealandiae **sp. nov. Baranov**
(Figs. 1C, 2–7; Table 1)

Holotype. OU47576, adult male, complete specimen in a piece of translucent, yellowish-orange amber with dimensions of 8 × 4 × 0.5 mm. Head and thorax covered by cloudy coating (“Verlumung”) ventrally and dorsally, and parts of the thorax and abdomen obscured by numerous bubbles (Figs. 1C, 2, 4, 5).

Paratypes. OU47540, OU47572 and OU47575, adult males, generally well preserved but some morphology obscured by air bubbles (Fig. 3)

Associated specimens. OU47579 in a semi-translucent piece, OU47580, OU47581 and OU47582 together in one nearly opaque piece, mostly obscured by detritus and air bubbles; all adult males (Figs. 6, 7).

Derivation of name. The specific epithet refers to the largely submerged continent Zealandia.

Type locality and horizon. Temporary lignite pit, site G45/f0107, near Tapanui, southern New Zealand; Pomahaka Formation, late Oligocene (Chattian, New Zealand stage Duntroonian).

Diagnosis. The new species can be easily distinguished from any living and fossil *Bryophaenocladius* species based on the combination of the tapering anal point, hyaline at its distal part, and anvil-shaped inferior volsella, together with gonostylus gently curving distally (Andersen & Schnell, 2000; Baranov, Andersen & Hagenlund, 2015; Wang, Sæther & Andersen, 2001; Wang, Liu & Epler; 2004; Wang, Andersen & Sæther, 2006; ; Seredszus and Wichard, 2007).

Description

Habitus: Total length 1.2–1.7 mm, wing length 0.71–0.96 mm (n = 5). Overall light yellowish-brown coloration, with thorax and pedicelli darker than the rest of the body.

Head: Eyes bare, kidney-shaped, without dorsomedial extension. Palpomeres’ length in µm (n = 2, OU47572, OU47575): 2nd: 23, 3rd: 48–60, 4th: 80, 5th: 92–93 (Fig. 2A). Clypeus square, with at least 8 setae (paratype OU47575). Palpomere three with a possible small distal protrusion, but condition of specimens not permitting corroboration of that. Antennae with 13 flagellomeres, (flagellomeres measurable on holotype only, length in μm): Fl1: 13.766, Fl2: 16, Fl3: 25, Fl4: 25, Fl5: 19, Fl6: 21, Fl7: 21, Fl8: 17, Fl9: 15, Fl10: 24, Fl11: 15, Fl12: 23, Fl13: 166, AR = 0.7.

Thorax: Acrostichals ~~setae~~ strong and decumbent, ~~holotype OU47576: 5 visible, paratype OU47540: 8 visible~~. Dorsocentrals (holotype OU47576) uniserial, at least 7 on each side; scutellars uniserial, at least 8. Postnotum bare. Anepisternum and epimeron without leaf-shaped setae.

Legs: Leg elements lengths as listed in Table 1. Terminal tarsomeres without pulvilli, shape of all the flagellomeres cylindrical. Foreleg tibial spurs 10–31 μm (n = 2), midlegs without tibial spur, hindtibia with two spurs, short 6–16 μm (n = 3), long 17–31 μm (n = 3) hindtibia comb made of 7–9 (n = 3) setae. Tarsomeres without pseudospurs. Lateral spines compressed to the shaft of the tibial spurs.

Table 1. Length (in μm) of leg segments of *Bryophaenocladius zealandiae* sp. nov. Baranov, males (measured on different numbers of specimens, depending on the preservation of the leg elements of the fossil). Values are given as min–max range and mean.

Leg	Femora	Tibia	Ta1	Ta2	Ta3	Ta4	Ta5
Foreleg	255–547 350 (n=5)	255–570 370 (n=5)	136–260 206 (n=3)	58–100 78 (n=3)	50–84 67 (n=3)	36–45 41 (n=3)	50–65 55 (n=3)
Midleg	255–543 400 (n=6)	248–656 374 (n=4)	136–287 202 (n=3)	66–145 96 (n=3)	67–82 76 (n=3)	57–88 70 (n=3)	44–84 63 (n=3)
Hindleg	305–541 419 (n=6)	290–607 454 (n=6)	202–459 286 (n=6)	81–177 131 (n=5)	85–111 100 (n=5)	45–73 57 (n=5)	43–60 52 (n=4)

Wings: 0.71–0.96 mm long (n = 5). Anal lobe strongly reduced. Costal extension ca. 85 µm long (n=1). Cu1 slightly sinuate. Squama fully fringed, with at least 11 setae (n = 1, holotype) (Figs. 3A, B). Wing membranes without macrotrichia, with coarse punctuation.

Hypopygium: Anal point hyaline, bare, 23–40 µm long (n = 3), parallel-sided for the most of the length, widening distally. Gonocoxite 60–140 µm long (n = 3), with large, axe-shaped inferior volsella (Figs. 5A–D, 7C–D). Gonostylus gently curved distally, 43 µm long (n = 1, holotype). Megasetae present, crista dorsalis absent (Figs. 5A–D).

Taxonomic notes

The new species is attributed to the genus *Bryophaenocladius* based on the combination of bare eyes, bare wings, fringed squama, ~~anepisternum and epimeron without leaf-shaped setae~~, lateral spines compressed to the shaft of the tibial spurs, pulvilli absent, acrostichal setae strong and decumbent, comb present on the hindtibia, and anal point hyaline and well developed (Cranston, Oliver & Sæther, 1989). In the absence of a modern, comprehensive revision of the genus *Bryophaenocladius* it is difficult to ascertain relations between the new fossil species and other species of *Bryophaenocladius.* The general shape of the hypopigium, particularly the long, distally expanding anal point, is highly reminiscent of *B. beuki*, Baranov, Andersen & Hagenlund, 2015 from Baltic amber (Baranov, Andersen & Hagenlund, 2015). Among extant taxa, the hypopigium of the new species is quite similar to *B. psilacrus* Makarchenko & Makarchenko, 2006 in the axe-shaped inferior volsella, the long, hyaline anal point, and the gently curving gonostylus, without crista dorsalis, as well as to *B. vernalis* (Goetghebuer, 1921) (Brundin, 1956; Makarchenko & Makarchenko, 2006).

Morphotype 1 cf. *Bryophaenocladius zealandiae*

(Figs. 1C, 8, Table 2)

Material. OU47573 and OU47574, both complete and fairly well visible within yellowish-orange translucent amber.

Description

Habitus: Total length 2 mm, wing length 1 mm. Colour: dark brown head and body, and legs of the lighter-brown colour. **Head:** Eyes bare, kidney-shaped, without dorsomedial extension. Palpomeres' length in µm (n = 2): 2nd: 26, 3rd: 45–77, 4th: 46–52, 5th: 83–100 (Figs. 7A–D). Clypeus square. Palpomere three is definitely with a conical protrusion on the distal end. **Thorax:** Acrostichals setae strong and decumbent, 8 present (n = 2). Dorsocentrals present but difficult to count, uniserial, at least 4. Postnotum bare. Anepisternum and epimeron without leaf-shaped setae. **Legs:** Leg elements lengths as listed in Table 2. Terminal tarsomeres without pulvilli, shape of all the flagellomeres cylindrical. Foreleg tibial spurs 16 µm (n = 2), midlegs without tibial spur, hindtibia with two spurs, short 27 µm (n = 1), long 30–46 µm (n = 2) hindtibia comb made of 6–8 (n = 2) setae. Tarsomeres without pseudospurs. Lateral spines compressed to the shaft of the tibial spurs. **Wings:** 1 mm long (n = 2). Details of venation not observable. **Hypopygium:** Only visible in lateral aspect Anal point hyaline, bare, ca 50 µm long (n = 1). Gonocoxite ca. 150 µm long (n = 1).

Table 2. Length (in µm) of leg segments of morphotype 1 cf. *Bryophaenocladius zealandiae* (measured on two specimens). Values are given as min–max range and mean.

Leg	Femora	Tibia	Ta1	Ta2	Ta3	Ta4	Ta5
Foreleg	471–518	461–606	325–415	111–115	101–109	72–136	46–79
Midleg	409–511	471–561	178–250	93–144	65–117	33–49	50–51
Hindleg	489–565	530–570	293–340	153–189	95–142	52–79	62–72

Taxonomic notes

This morphotype is highly reminiscent of *Bryophaenocladius zealandiae* sp. nov., but is 30–50% larger than this new species. It is unclear if this is an indication of naturally large intraspecific size variation or if these two specimens (OU47573, OU47574) represent a different taxon. Should they indeed be members of *Bryophaenocladius zealandiae* sp. nov., this will corroborate that this species has a distal projection on the end of palpomer 3, supporting affinity with the subgenus *Odontocladius* Albu, 1974.

Genus ***Gymnometriocnemus*** Edwards, 1932

***Gymnometriocnemus* (*Gymnometriocnemus* s.str.*) extinctus* sp. nov. Baranov**

(Figs. 1C, 9–12, Tables 3, 4)

Holotype. OU47546; male, well preserved, except for missing thorax, in a piece of semi-translucent, yellow amber (7 × 5 × 0.5 mm) with abundant small air-bubbles (Figs. 1C, 9, 10).

Paratypes. OU47577, OU47578; females, complete and well visible in semi-translucent pieces of yellow-brown amber (Figs. 1C, 11, 12).

Derivation of name. After latin exstinguō or extinguō**,** meaning extinct.

Type locality and horizon. Temporary lignite pit, site G45/f0107, near Tapanui, southern New Zealand; Pomahaka Formation, late Oligocene (Chattian, New Zealand stage Duntroonian).

Diagnosis. This fossil species can be distinguished from the rest of the known species of *Gymnometriocnemus* on the basis of the combination of the following characters: subapical setae of the antenna weak, gonostylus expanding distally, without obvious crista, but with an sub-oval expansion ventrally, megasetae short and sturdy, inferior volsella subrectangular, narrowing distally, strong sclerotized virga absent (Freeman, 1959; Hergstrom, 1974; Sæther, 1969, 1983; Sasa & Kikuchi, 1995; Stur & Ekrem, 2015; Makarchenko & Makarchenko, 2019). Of all species of *Gymnometriocnemus*, *G.* (*G.* st. str.) *extinctus* sp. nov. is most reminiscent of *G.* (*G.* st. str.) *marionensis* Sæther, 1969, but differs in having much shorter and sturdier megasetae (Sæther, 1969; Stur & Ekrem, 2015).

Description

Adult male (OU47546)

Habitus: Total length 2.3 mm, wing length 1.5 mm. Colour: dark brown across the parts of the body.

Head: Eyes bare, presence of the dorsomedial extension impossible to ascertain. Palpomeres' length in µm (n = 1). 3rd: 115, 4th: 126–152, 5th: 143 (Fig. 8B). Clypeus square with at least 10 setae. Antennae with 13 flagellomeres, (flagellomeres measurable on holotype only, length in µm): Fl1: 27, Fl2: 30, Fl3: 19, Fl4: 31, Fl5: 30, Fl6: 31, Fl7: 25, Fl8: 30, Fl9: 27, Fl10: 28, Fl11: 28, Fl12: 31, Fl13: 360, AR=1.1 Flagellomere 13 with a crown of gentle sensillae and stronger subapical setae (Fig. 9C).

Thorax: Most of the thorax, except for anteropronotum and part of the scutum, missing. Strong anteropronotal setae present (at least two), three dorsocentrals visible.

Legs: Leg elements lengths as listed in Table 3. Foreleg tibial spurs 30 µm (n = 1), presence and number of other spurs impossible to ascertain. Pulvilli absent.

Table 3. Length (in µm) of leg segments of *Gymnometriocnemus extinctus* sp. nov. Baranov, male holotype OU47546.

Leg	Femora	Tibia	Ta1	Ta2	Ta3	Ta4	Ta5
Foreleg	704	741	423	175	222	161	217
Midleg	596	520	468	124	76	-	-
Hindleg	615	783	-	-	-	-	-

Wings: 1.5 mm long (n = 1). Wing membrane densely covered with macrotrichia. Cu1 slightly sinuate, costal extension pronounced. Squama appearently bare (Figs. 10A–B).

Hypopygium: With numerous long setae, gonocoxite 114 µm long (n = 1). Gonostylus ca. 70 µm long (n = 1), expanding distally, without obvious crista, but with sub-oval expansion ventrally, megasetae short and sturdy. Anal point short, cresting top of tergite IX, with several long setae (Figs. 10C–D). Inferior inferior volsella inferior volsella subrectangular, narrowing distally (Figs. 10C–D). Presence of virga impossible to ascertain, but since hypopigium is partially transparent, we can rule out presence of extremely strong and sclerotized virga.

Adult female (OU47577, OU47578), tentatively associated with male on the basis of the similarity of the wing structure and setation of the anteropronotum

Habitus: Total length 0.9–1.0 mm, wing length 0.63–0.72 mm. Colour: similar to the male holotype.

Head: Eyes bare, reniform. Palpomeres length in µm (n = 2): 2nd: 29 (n = 1, OU47578), 3rd: 40–41, 4th: 41–42, 5th: 75–82 (Figs. 12A–B). Clypeus square with at least 11 setae. Antennae with 5 flagellomeres, (n = 2, length in µm): Fl1: 76 (n = 1, OU47577), Fl2: 19–29, Fl3: 26–28, Fl4: 22–27, Fl5: 39–40. Flagellomere 5 with a weak but distinct subapical seta (Figs. 12A–B). Pedicellus cup-shaped.

Thorax: Acrostichals setae strong and decumbent, 5–8 (n = 2). Dorsocentrals bisereal, upper row 5, lower row 8. Postnotum bare. Anteropronotum 4. Anepisternum and epimeron without leaf-shaped setae. Prealars 3, humerals 4. Scuterals uniserial, 6.

Legs: Leg elements lengths as listed in Table 4. Foreleg tibial spurs 14–15 µm (n = 2), midtibial spur 11–15, hindtibial spur 26–37 (length in µm). Hindtibial comb made of 8–9 strong seate. Pulvilli absent, empodium feathery.

Table 4. Length (in µm) of leg segments of *Gymnometriocnemus extinctus* sp. nov. Baranov, female paratypes (measured on different numbers of specimens, depending on the preservation of leg elements of the fossil). Values are given as min–max range.

Leg	Femora	Tibia	Ta1	Ta2	Ta3	Ta4	Ta5
Foreleg	312	308	109–139	56–70	48–50	28–34	26–46
Midleg	238–282	259–266	92–100	39–49	33–34	23–29	38–47
Hindleg	267–269	211–296	145–171	55–69	70–89	37–40	42–50

Wing: 0.63–0.72 mm long (n = 2). Wing membrane densely covered with macrotrichia. Cu_1 slightly sinuate. Squama bare, costal extension pronounced (Fig. 11C). Wing very similar to male holotype (OU47546). Halters dark-brown in their entirety.

Female genitalia: Cerci very small, gonapohysis VIII divided into small mesal lobe and narrow dorsomesal lobe (Figs. 12C–D). Gonocoxite relatively small, with at least 5 strong setae. Tergite IX rounded, undivided (Figs. 12C–D).

Taxonomic notes

This species is attributed to the genus *Gymnometriocnemus* (subgenus *Gymnometriocnemus*) based on the combination of bare eyes, apical flagellomere with subapical sensillae, wing covered with macrotrichia with pronounced costal extension and bare squama, anteropronotals present, pulvilli absent, presence of crest-like anal point on tergite IX and overall extremely high density of setae on the body (Freeman, 1959; Sæther, 1983; Cranston, Oliver & Sæther, 1989; Stur & Ekrem, 2015). Since the hypopigium and Tergite VIII are partially translucent in the male specimen, and the extremely large and sclerotized virga is not visible, we find its presence unlikely. Since a large and sclerotized virga does not appear to be present, we propose that the new species is a representative of the subgenus *Gymnometriocnemus* s. str. Edwards, 1932 rather than of *Raphidocladius* Sæther, 1983, as the synapomorphy of the latter is the presence of a strong virga (Sæther, 1983; Stur & Ekrem, 2015). This new species can be differentiated from the New Zealand endemic genus *Pterosis* Sublette & Wirth, 1980, which closely resembles

Gymnometriocnemus, by the subapical seta, characteristic for *Gymnometriocnemus* but absent in *Pterosis* (Sublette & Wirth, 1980; Sæther, 1983; Stur & Ekrem, 2015). We tentatively associate two females (OU47577, OU47578) with the male holotype OU47546 on the basis of matching wing venation and setation patterns, patterns of anteropronotal setation and general congruence of the female genital morphology with the genus *Gymnometriocnemus* (Sæther, 1977).

Discussion

Faunal affinities and biogeography

Chironomids as a group are fairly old, with the oldest representatives occurring in the uppermost Triassic of Europe (203 mya) (Ansorge, 1999), although based on dated phylogenies the group is likely significantly older, at least 250 mya (Cranston et al., 2011). The oldest Orthocladiinae fossils are known from Lower Cretaceous Lebanese amber (Veltz, Azar & Nel, 2007). A long geological history and rich fossil record has made Chironomids a suitable model group for historical biogeographic analyses. Following Willi Hennig´s work on phylogenetic systematic ~~(Hennig, 1959)~~, Lars Brundin became interested in applying principles of cladistic analysis and an emerging understanding of plate tectonics to the analysis of Chironomidae distribution in the Southern Hemisphere (Brundin, 1966). Brundin came to the conclusion that the majority of Chironomidae distribution patterns in Australia, Southern Neotropics and New Zealand can be explained by the break-up of Gondwana ~~(Brundin, 1966)~~. Since then, however, our understanding of the assembly of New Zealand´s biota has become more nuanced. In particular the role of dispersal has become more widely appreciated (e.g., Trewick, 2000; Sanmartin et al., 2001).

The composition of the New Zealand Orthocladiinae fauna, and Chironomidae ~~in general,~~ reflects a complex history influenced by both trans-Tasman and trans-Antarctic dispersal and vicariance following the break-up of Gondwana (Krosch & Cranston, 2013; Krosch et al., 2011, 2015). The two new species from Pomahaka amber are among the first fossil records of Orthocladiinae from New Zealand and allow a valuable but very limited first glimpse of the late Oligocene fauna.

Bryophaenocladius has a near worldwide distribution but it appears to be absent from Australia (but larvae have been reported from orchards in western Australia; Cranston, 1996) and New Zealand. When we first recorded *Bryophaenocladius* from New Zealand Oligocene amber

~~(Schmidt et al., 2018),~~ we noted that there are no formal records of this genus from the extant fauna of New Zealand (Boothroyd & Forsyth, 2011; Ashe & O'Connor, 2012). We also noted that the BOLD V4 system has barcoding records of *Bryophaenocladius* in New Zealand (Schmidt et al., 2018) but, on closer examination, these belong to the two BOLD BINs BOLD:AAM6273 and BOLD:AAG1021. Representatives of these BINs all cluster around the Holarctic species *Bryophaenocladius ictericus* (Meigen, 1830). It is likely that this species has been historically introduced to New Zealand with agricultural produce, as *Bryophaenocladius* larvae are associated with agricultural plants (Cranston, 1987).

While there appear to be no native species of *Bryophaenocladius* on the main islands of New Zealand, it is highly likely that the monotypic *Kuschelius dentifer* Sublette & Wirth, 1980, endemic to the sub-Antarctic Auckland Islands, is in fact a species of *Bryophaenocladius.* Sublette & Wirth (1980) erected the genus *Kuschelius* as intermediate between *Chaetocladius* Kieffer and *Bryophaenocladius*, and distinguished *K. dentifer* from species of *Bryophaenocladius* by the presence of apical setae on the terminal flagellomere of the antenna and slightly diverted spines on the tibial spur of the hind leg (Fig 13 A-E). However, these characters in combination with the structure of the hypopigium and the presence of the distal projection on the distal end of palpomere three fit well within the current definition of *Bryophaenocladius*, subgenus *Odontocladius* Albu, 1974 (Albu, 1974; Armitage, 1987; Moubayed & Langton, 2023). As pointed out by Sæther (1982) and Armitage (1987), *K. dentifer* is almost certainly a *Bryophaenocladius*, very similar to *B. brincki* (Freeman, 1955) originally described from South Africa. Molecular data on *K. dentifer* are not yet available.*Bryophaenocladius zealandiae* sp. nov. ~~Baranov~~ from Pomahaka amber now records the genus *Bryophaenocladius* in Zealandia in the late Oligocene (~26 mya) and documents its post Oligocene extinction in New Zealand, at least on the main islands. The only two other fossils of *Bryophaenocladius* are from Eocene Baltic amber. A modern review of *Bryophaenocladius* and additional fossils are needed to decipher the biogeographic history of this genus.

The genus *Gymnometriocnemus* identified here from Pomahaka amber includes 17 extant species in all major biogeographical regions except Antarctica, with the actual number of global species expected to be much higher (Stur & Ekrem, 2015). Subgenus *Gymnometriocnemus*, to which the new fossil species from Pomahaka amber is assigned, includes 11 species, with *G.*

wilsoni Freeman, 1961 occurring in Australia and a single species, the endemic *G. lobifer* (Freeman, 1959) (sometimes misspelled as *Gymnometriocnemis lobifer*) occurring in New Zealand on both the North and South Islands (Ashe & O´Connor, 2012). *Gymnometriocnemus* (*G.*s.str.*) extinctus* sp. nov. Baranov described here from the late Oligocene is the first fossil record of this genus globally. Its discovery in amber from the Pomahaka Formation indicates a long presence of *Gymnometriocnemus* in New Zealand.

Paleoecology of non-biting midges from Pomahaka amber

It is notable that both newly discovered species of midges from Pomahaka amber belong to Chironomidae groups whose extant representatives have larvae that develop mostly in terrestrial and semi-aquatic habitats (Moller Pillot, 2013), unlike the aquatic habitat seen in larvae of most other chironomids. Larvae of both *Bryophaenocladius* and *Gymnometriocnemus* develop in wet mosses or decaying leaves or similar wet habitats (Strenzke, 1957; Moller Pillot, 2013). Terrestrial and semi-terrestrial Chironomidae are relatively common in various amber deposits worldwide, probably due to their association with mosses and other microhabitats at the bark of the resin-producing trees or on the nearby forest floor (Solórzano-Kraemer et al., 2018). There are two fossil species of *Bryophaenocladius*, *B. beuki* Baranov, Andersen & Hagenlund, 2015 and *B. circumclusus* Seredszus & Wichard, 2007 and a probable larva of this genus (Baranov et al., 2019), all from Eocene Baltic amber. Until now no fossil *Gymnometriocnemus* were known but there are numerous other fossil representatives of Chironomidae whose extant representatives develop in terrestrial habitats, such as *Parametriocnemus, Paraphaeocladius, Pseudorthocladius, Smittia* and *Pseudosmittia* (Zelentsov et al., 2012; Baranov, Andersen & Hagenlund, 2015). High prevalence of the Chironomids with terrestrial larvae in certain amber deposits probably reflects high humidity in amber forest habitats, as relatively high and constant humidity is required by these groups of Chironomids to finish larval development (Strenzke, 1957; Armitage, Cranston & Pinder, 1995, Zelentsov et al., 2012). The finding of terrestrial or semi-aquatic midges in Pomahaka amber is consistent with the paleo-environmental reconstruction. The amber-bearing lignites of Pomahaka Formation formed by in-situ growth and decomposition of wetland forest trees and litter in domed forest swamps (Lindqvist, Gard & Lee, 2016) and the palynomorph assemblage from the lignites includes ferns, shrubs, herbs and reeds associated with moist and damp habitats and overall indicates high humidity and high rainfall throughout the year (Pocknall, 1982).

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

Viktor Baranov conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Joerg Hammel conducted μCT scanning, analysed related data, authored or reviewed drafts of the article, and approved the final draft.

Daphne E. Lee authored or reviewed drafts of the article, and approved the final draft.

Alexander R. Schmidt discovered and prepared some of the amber fossils and commented on the manuscript, conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Uwe Kaulfuss conducted fieldwork, prepared some of the amber fossils, conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding the data availability: All specimens included in this work are housed at the Geology Museum of the Geology Department, University of Otago (OU), under accession numbers: *Bryophaenocladius zealandiae* sp. nov. Baranov, OU47576 (holotype); OU47540, OU47572, OU47573, OU47574, OU47575 (paratypes); OU47579, OU47580, OU47581, OU47582 (associated material). Morphotype 1, cf. *Bryophaenocladius*, OU47573, OU47574. *Gymnometriocnemus* (*G.) extinctus* sp. nov. Baranov, OU47546 (holotype); OU47577, OU47578 (paratypes)

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Figure 1 Late Oligocene Pomahaka amber. (A) Map of amber locality G45/f0107 near Tapanui, southern New Zealand. (B) Typical appearance of layered, fossiliferous Pomahaka amber. (C) Epoxy-embedded pieces of Pomahaka amber with newly discovered Chironomidae inclusions.

Figure 2 *Bryophaenocladius zealandiae* sp. nov. Baranov, holotype OU47576. (A) Habitus, dorsal view. (B) Habitus, ventral view. (C) Antenna, ventral view. (D) Tibial spurs. Abbreviations: *Ti1*, foreleg tibia; *Ti2*, midleg tibia; *Ti3*, hindleg tibia.

Figure 3 *Bryophaenocladius zealandiae* sp. nov. Baranov, paratypes. (A, B) Habitus and hypopygium of paratype OU47540. (C, D) Hypopygium and habitus of paratype OU47575. (E) Habitus of paratype OU47572.

Figure 4 *Bryophaenocladius zealandiae* sp. nov. Baranov, wing of holotype OU47576. (A) Photomicrograph. (B) Line drawing. Abbreviations: *An*, anal vein; *B*, brachiolum; *C*, costal vein; *ce*, costal extension; *Cu1*, cubital vein 1; *Cu2*, cubital vein 2; *M1+2*, medial vein 1+2; *M3+4*, medial vein 3+4; *R1*, radial vein 1; *R2+3*, radial vein 2+3; *R4+5*, radial vein 4+5; *RM*, radial medial crossvein; *Sc*, subcostal vein; *Sq*, squama.

Figure 5 *Bryophaenocladius zealandiae* sp. nov. Baranov, hypopigium of holotype OU47576. (A) Photomicrograph, dorsal. (B) Line drawing, dorsal. (C) Photomicrograph, ventral. (D) Line drawing, ventral. Abbreviations: *AP*, anal point; *Gc*, gonocoxite; *Gs*, gonostylus; *IVo*, inferior volsella; *TVIII*, abdominal tergite 8; *TIX* abdominal tergite 9.

Figure 6 ***Bryophaenocladius zealandiae*** **sp. nov. Baranov, associated specimen OU47579**. (A) Habitus. (A') Overview of the amber piece containing the specimen. (B) Habitus, opposite side of the body.

Figure 7 ***Bryophaenocladius zealandiae*** **sp. nov. Baranov, uCT scans of associated specimens.** (A) Habitus of specimens OU47580 and OU47581 in the same amber piece. (B) Habitus of specimen OU47580, dorso-lateral view. (C) Hypopigium of specimen OU47581. (D) Hypopigium (OU47581), marked. Abbreviations: *AP*, anal point; *Gc*, gonocoxite; *Gs*, gonostylus; *TIX*, abdominal tergite 9. (E) Overview of the amber piece containing specimens OU47580, OU47581 and OU47582.

Figure 8 Morphotype 1 cf. ***Bryophaenocladius zealandiae***. (A) Habitus of specimen OU47574. (B) Habitus of specimen OU47573. (C) Palpomere 3 (OU47574), arrow marks distal protrusion. (D) Hypopigium, lateral view (OU47574).

Figure 9 ***Gymnometriocnemus extinctus*** **sp. nov. Baranov, holotype OU47546, male**. (A) Habitus. (B) Head. (C) Last flagellomere apical setae marked by arrow. (D) Hypopigium, ventral view.

Figure 10 ***Gymnometriocnemus extinctus*** **sp. nov. Baranov, holotype OU47546.** (A) Photomicrograph of wing. (B) Line drawing of wing. (C) Photomicrograph of hypopigium, dorsal view. (D) Line drawing of hypopigium, dorsal view. Abbreviations: *AP*, anal point; *Gc*, gonocoxite; *Gs*, gonostylus; *IVo*, inferior volsella; *TIX*, abdominal tergite 9; *C*, costal vein; *Cu1*, cubital vein 1; *M1+2*, medial vein 1+2; *M3+4*, medial vein 3+4; *R1*, radial vein 1; *R2+3*, radial vein 2+3; *R4+5*, radial vein 4+5.

Figure 11 ***Gymnometriocnemus extinctus*** **sp. nov. Baranov, female paratypes.** (A, B) Habitus and wing of specimen OU47577. (C) Habitus of specimen OU47578.

Figure 12 *Gymnometriocnemus extinctus* sp. nov. Baranov, female paratypes. (A) Head of specimen OU47577. (B) Head of specimen OU47578; b’ close-up of last flagellomere with apical setae. (C) Female genitalia (OU47578). (D) Female genitalia, marked (OU47578). Abbreviations: *A8*, abdominal segment 8; *Ce*, cerci; *Gca*, gonocoxite apodem; *Gc*, gonocoxite (8); *Gp8*, gonapophysis 8; *TIX*, tergite 9.

Figure 13. Holotype (adult male, number NZAC02044947) of *Kuschelius dentifer* Sublette and Wirth, 1980. (A). Wing. (B) Head, arrow marks a apical protrusion of the 3d palpomere. (C) Hypopigium. (D) Midtibia with spurs and the comb. (E) Hindtibia with the spurs and comb. All photos in this plate are made by Dr. Leanne Elder, licensed under CC BY 4.0 and used with the photographer’s explicit permission.

Figure 1

Figure 1 Late Oligocene Pomahaka amber.

(A) Map of amber locality G45/f0107 near Tapanui, southern New Zealand. (B) Typical appearance of layered, fossiliferous Pomahaka amber. (C) Epoxy-embedded pieces of Pomahaka amber with newly discovered Chironomidae inclusions.

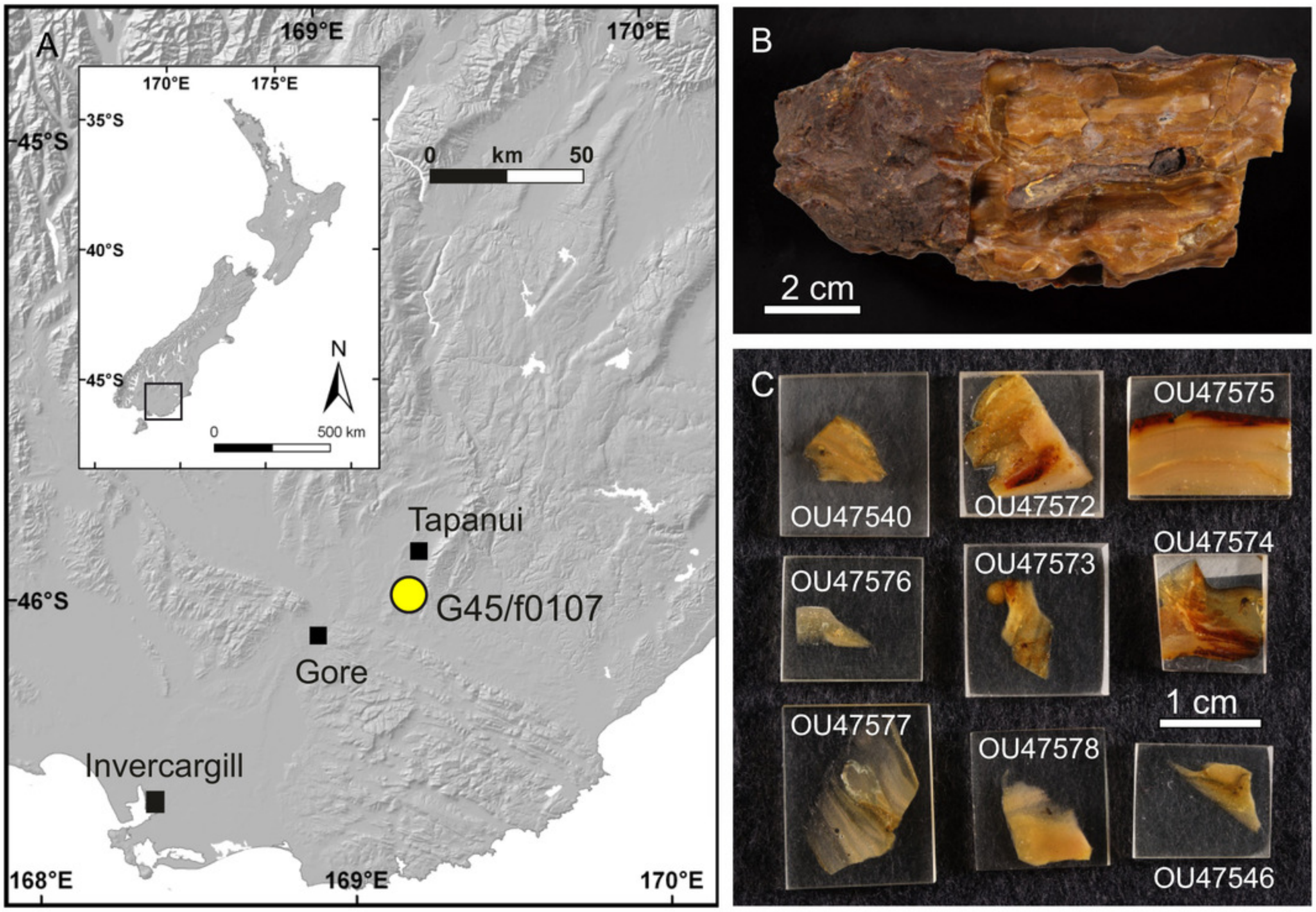

Figure 2

Figure 2 *Bryophaenocladius zealandiae* sp. nov. Baranov, holotype OU47576.

(A) Habitus, dorsal view. (B) Habitus, ventral view. (C) Antenna, ventral view. (D) Tibial spurs. Abbreviations: *Ti1*, foreleg tibia; *Ti2*, midleg tibia; *Ti3*, hindleg tibia.

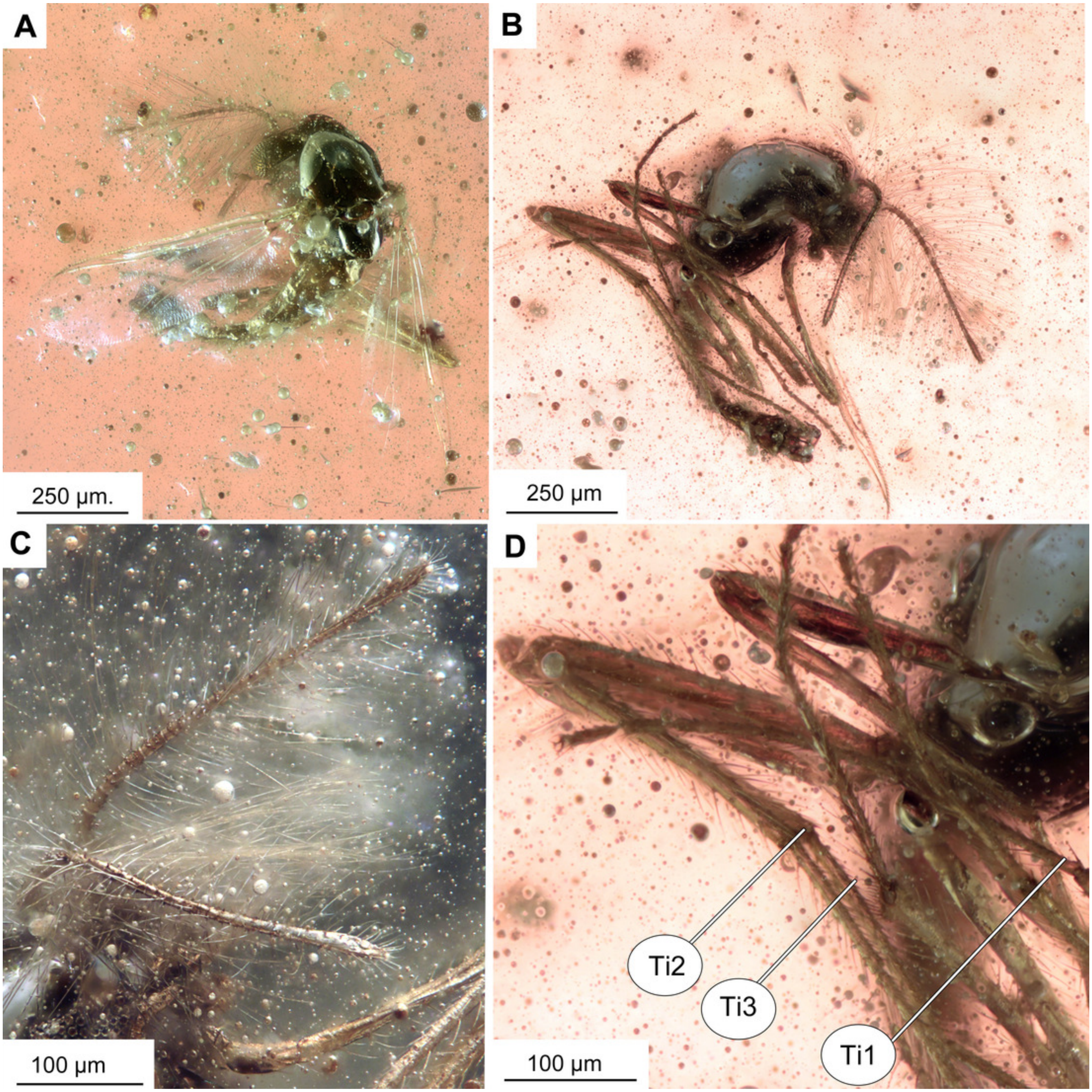
A
250 µm.
B
250 µm
C
100 µm
D
Ti2
Ti3
Ti1
100 µm

Figure 3

Figure 3 *Bryophaenocladius zealandiae* sp. nov. Baranov, paratypes.

(A, B) Habitus and hypopygium of paratype OU47540. (C, D) Hypopygium and habitus of paratype OU47575. (E) Habitus of paratype OU47572.

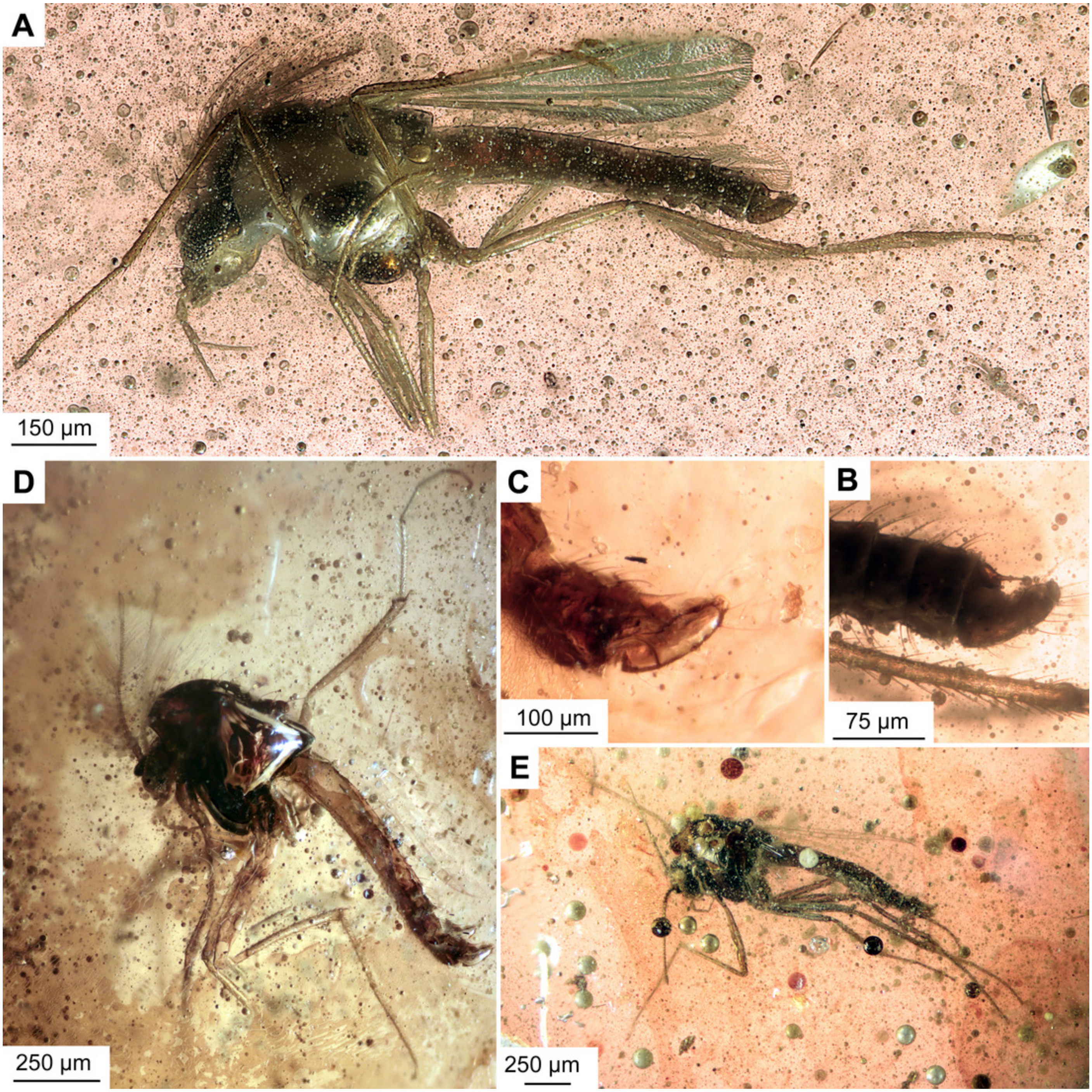
A
150 µm
D
250 µm
C
100 µm
B
75 µm
E
250 µm

Figure 4

Figure 4 *Bryophaenocladius zealandiae* sp. nov. Baranov, wing of holotype OU47576.

(A) Photomicrograph. (B) Line drawing. Abbreviations: *An*, anal vein; *B*, brachiolum; *C*, costal vein; *ce*, costal extension; *Cu1*, cubital vein 1; *Cu2*, cubital vein 2; *M1+2*, medial vein 1+2; *M3+4*, medial vein 3+4; *R1*, radial vein 1; *R2+3*, radial vein 2+3; *R4+5*, radial vein 4+5; *RM*, radial medial crossvein; *Sc*, subcostal vein; *Sq*, squama.

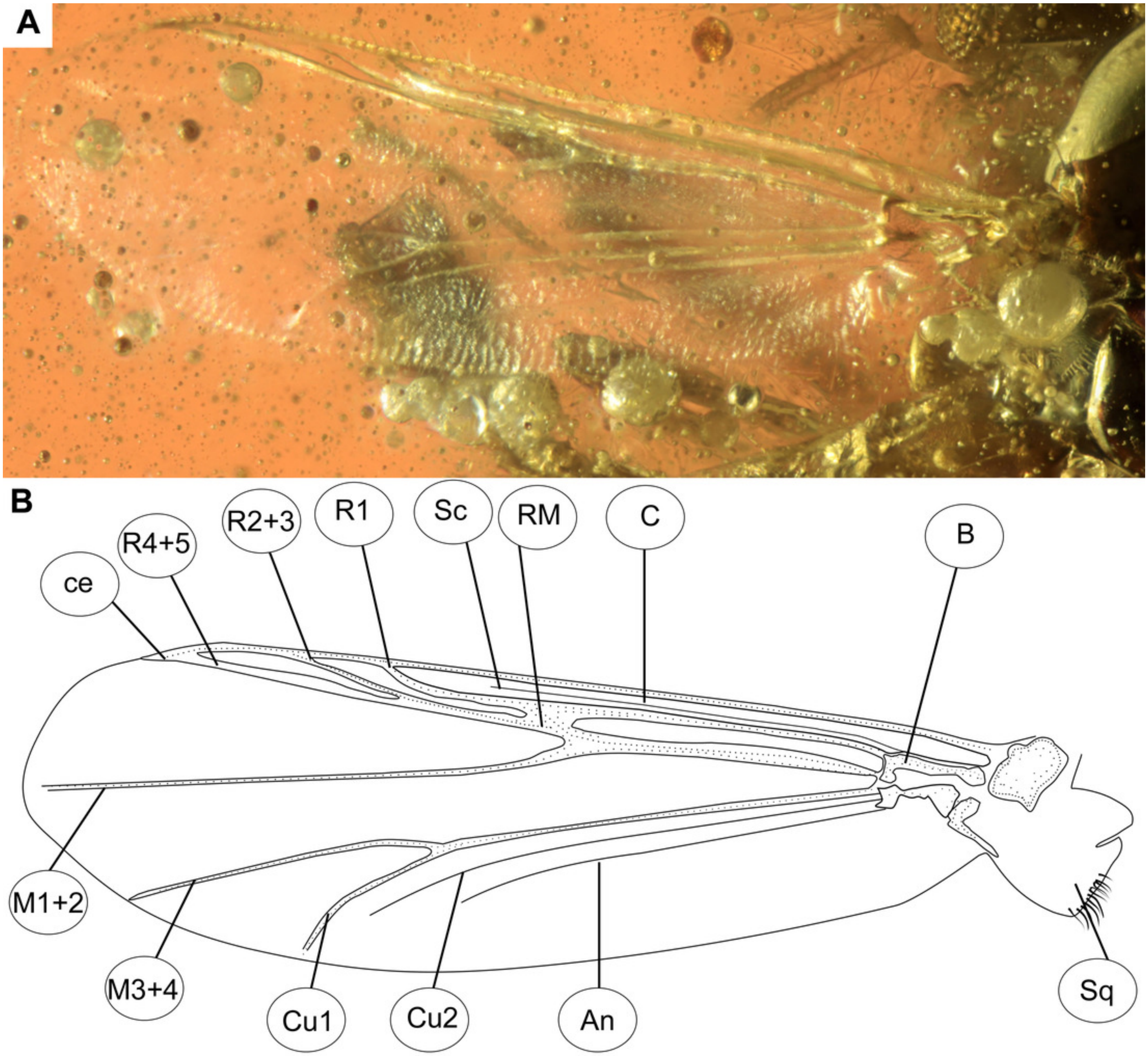
A
B
ce
R4+5
R2+3
R1
Sc
RM
C
B
M1+2
M3+4
Cu1
Cu2
An
Sq

Figure 5

Figure 5 *Bryophaenocladius zealandiae* sp. nov. Baranov, hypopigium of holotype OU47576.

(A) Photomicrograph, dorsal. (B) Line drawing, dorsal. (C) Photomicrograph, ventral. (D) Line drawing, ventral. Abbreviations: *AP*, anal point; *Gc*, gonocoxite; *Gs*, gonostylus; *IVo*, inferior volsella; *TVIII*, abdominal tergite 8; *TIX* abdominal tergite 9.

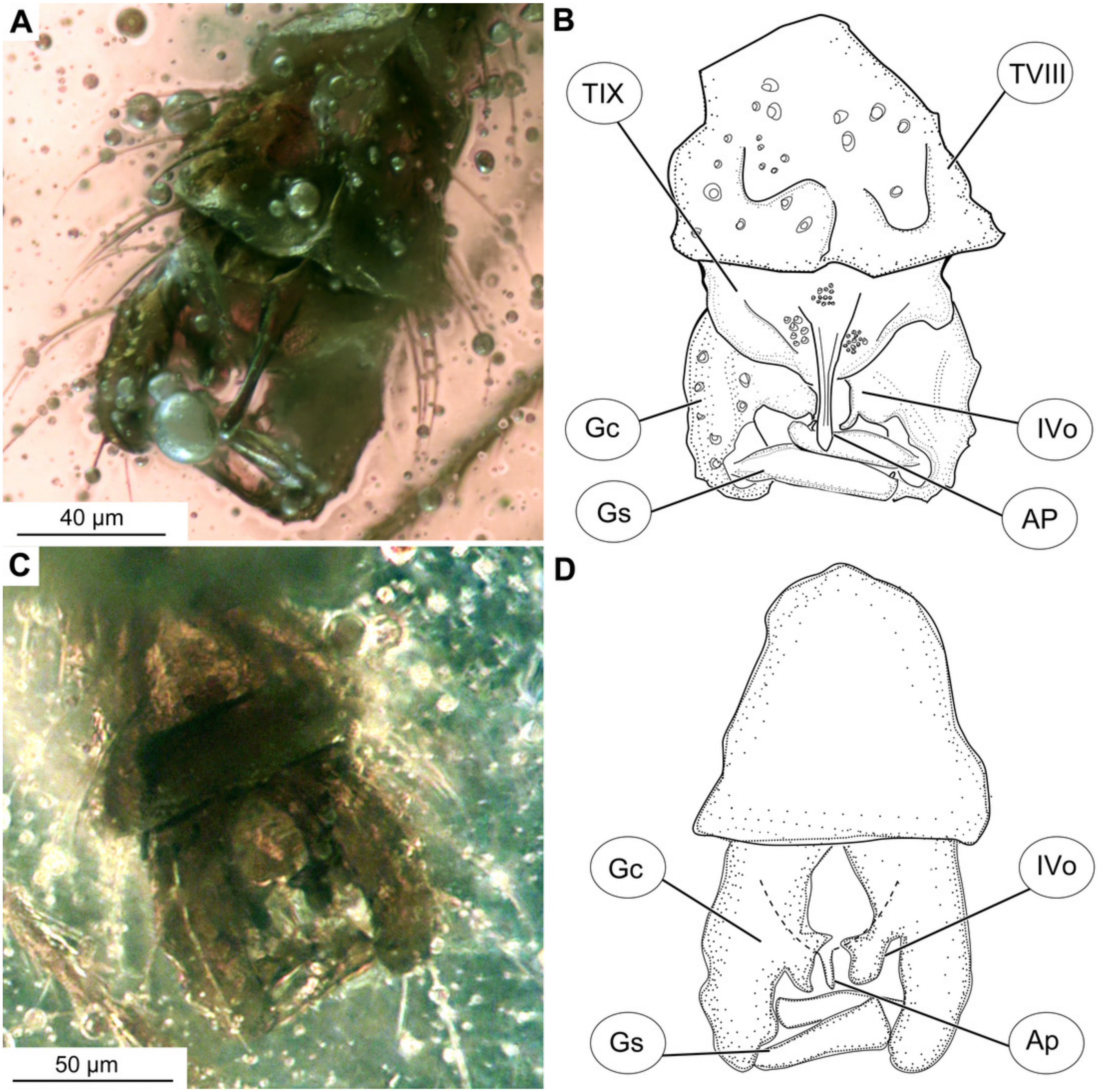
A
40 µm
B
TIX
TVIII
Gc
IVo
Gs
AP
C
50 µm
D
Gc
IVo
Gs
Ap

Figure 6

Figure 6 *Bryophaenocladius zealandiae* sp. nov. Baranov, associated specimen OU47579.

(A) Habitus. (A’) Overview of the amber piece containing the specimen. (B) Habitus, opposite side of the body.

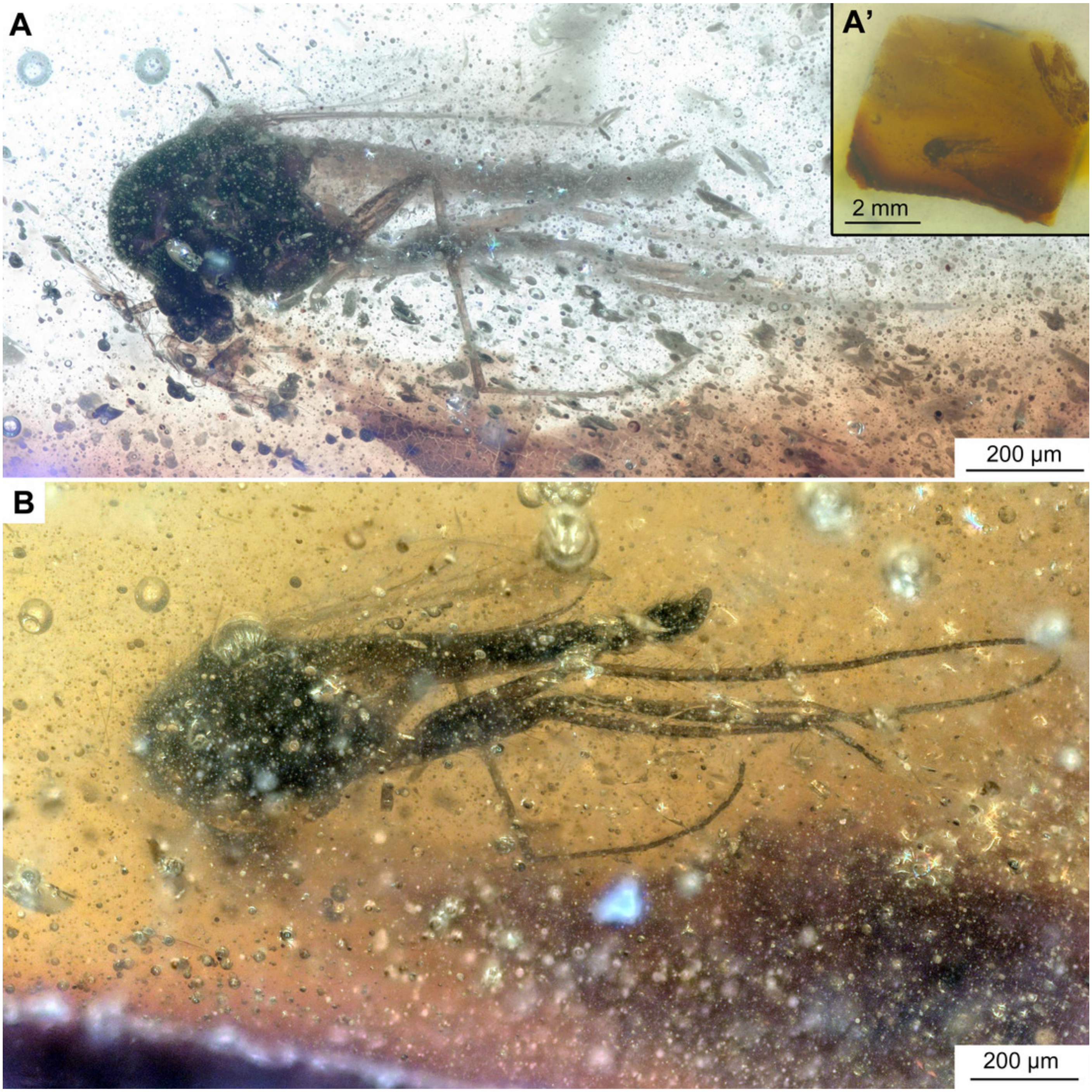
A
A'
2 mm
200 μm
B
200 μm

Figure 7

Figure 7 *Bryophaenocladius zealandiae* sp. nov. Baranov, uCT scans of associated specimens.

(A) Habitus of specimens OU47580 and OU47581 in the same amber piece. (B) Habitus of specimen OU47580, dorso-lateral view. (C) Hypopigium of specimen OU47581. (D) Hypopigium (OU47581), marked. Abbreviations: *AP*, anal point; *Gc*, gonocoxite; *Gs*, gonostylus; *TIX*, abdominal tergite 9. (E) Overview of the amber piece containing specimens OU47580, OU47581 and OU47582.

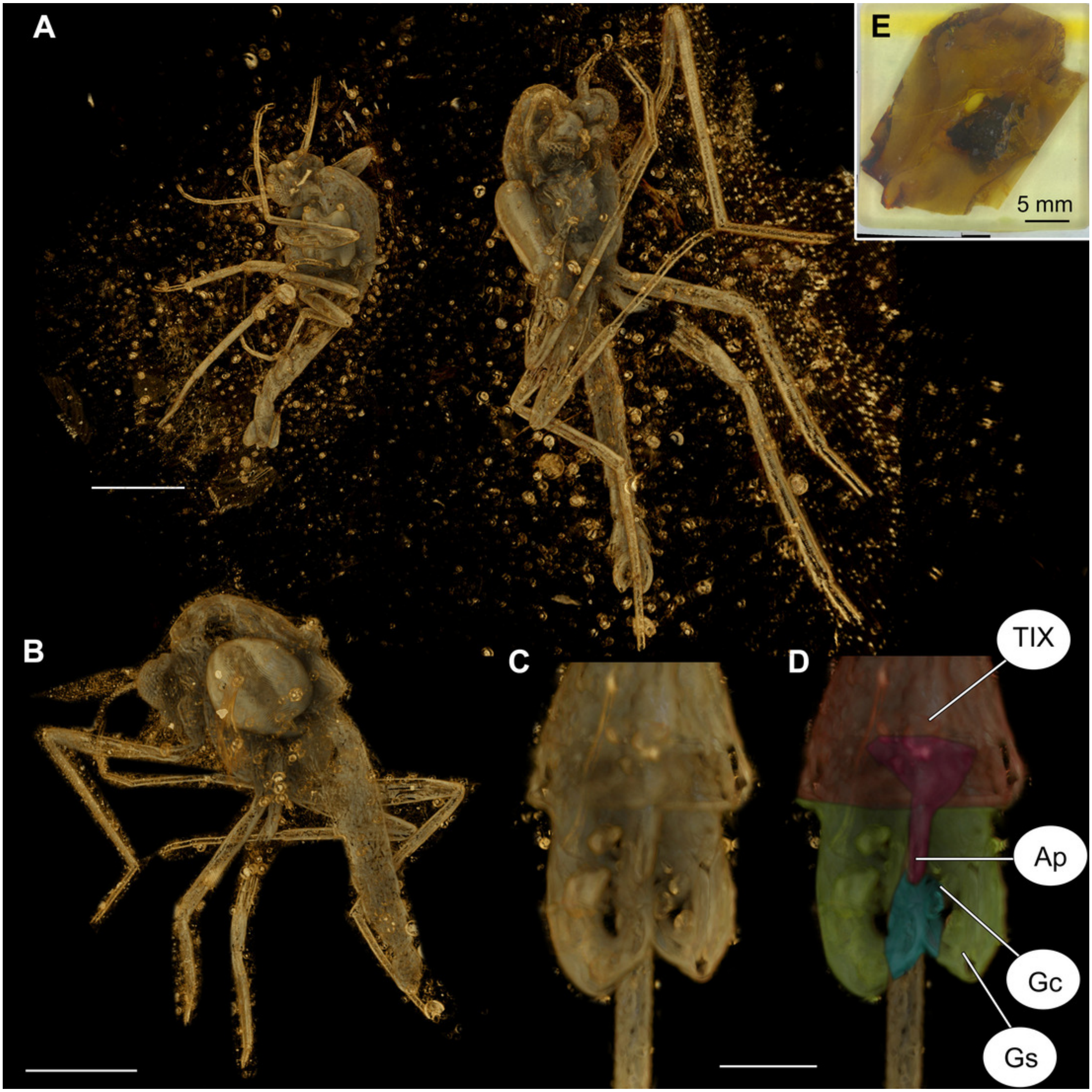
A
E
5 mm
B
C
D
TIX
Ap
Gc
Gs

Figure 8

Figure 8 Morphotype 1 cf. *Bryophaenocladius zealandiae*.

(A) Habitus of specimen OU47574. (B) Habitus of specimen OU47573. (C) Palpomere 3 (OU47574), arrow marks distal protrusion. (D) Hypopigium, lateral view (OU47574).

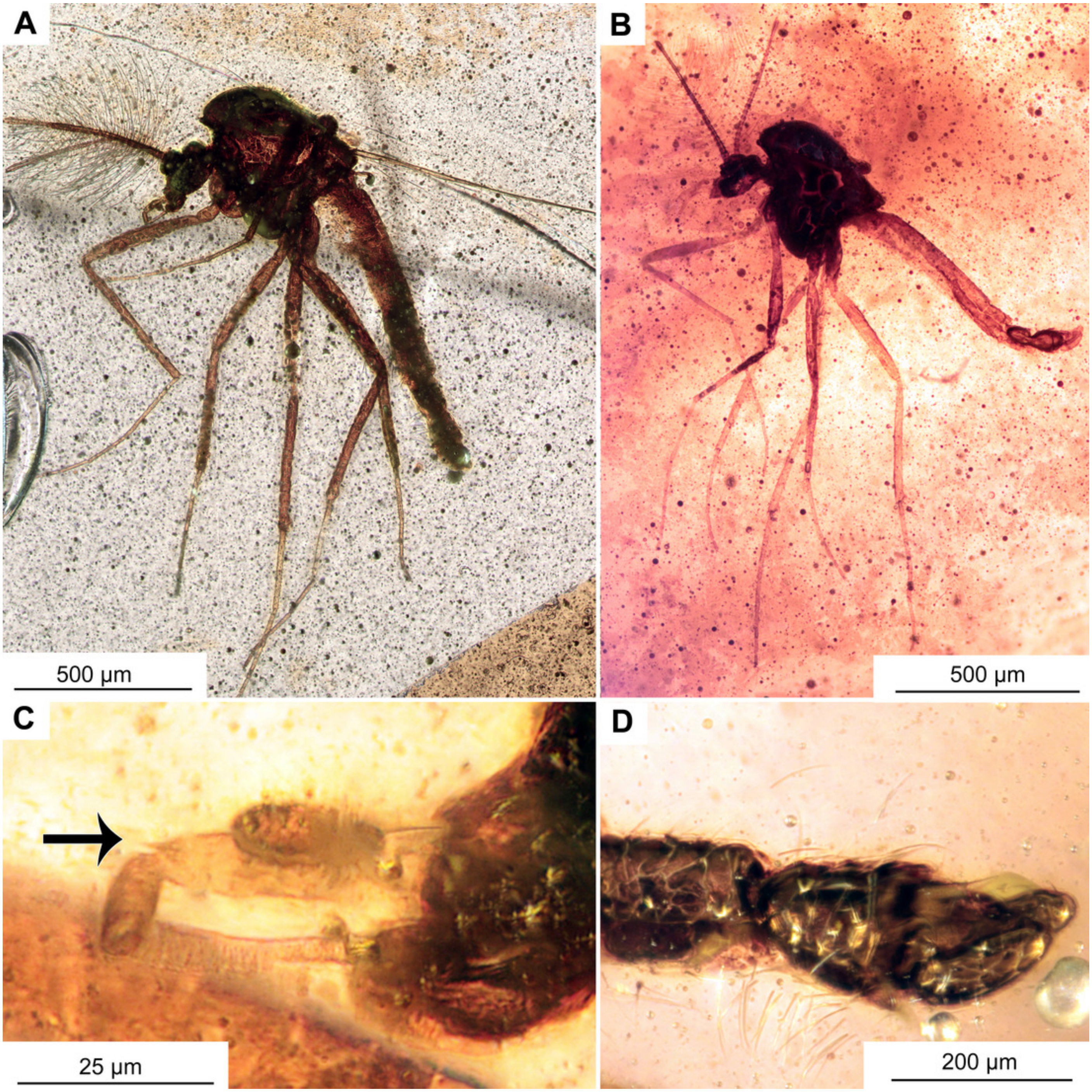
A
500 µm
B
500 µm
C
25 µm
D
200 µm

Figure 9

Figure 9 *Gymnometriocnemus extinctus* sp. nov. Baranov, holotype OU47546, male.

(A) Habitus. (B) Head. (C) Last flagellomere apical setae marked by arrow. (D) Hypopigium, ventral view.

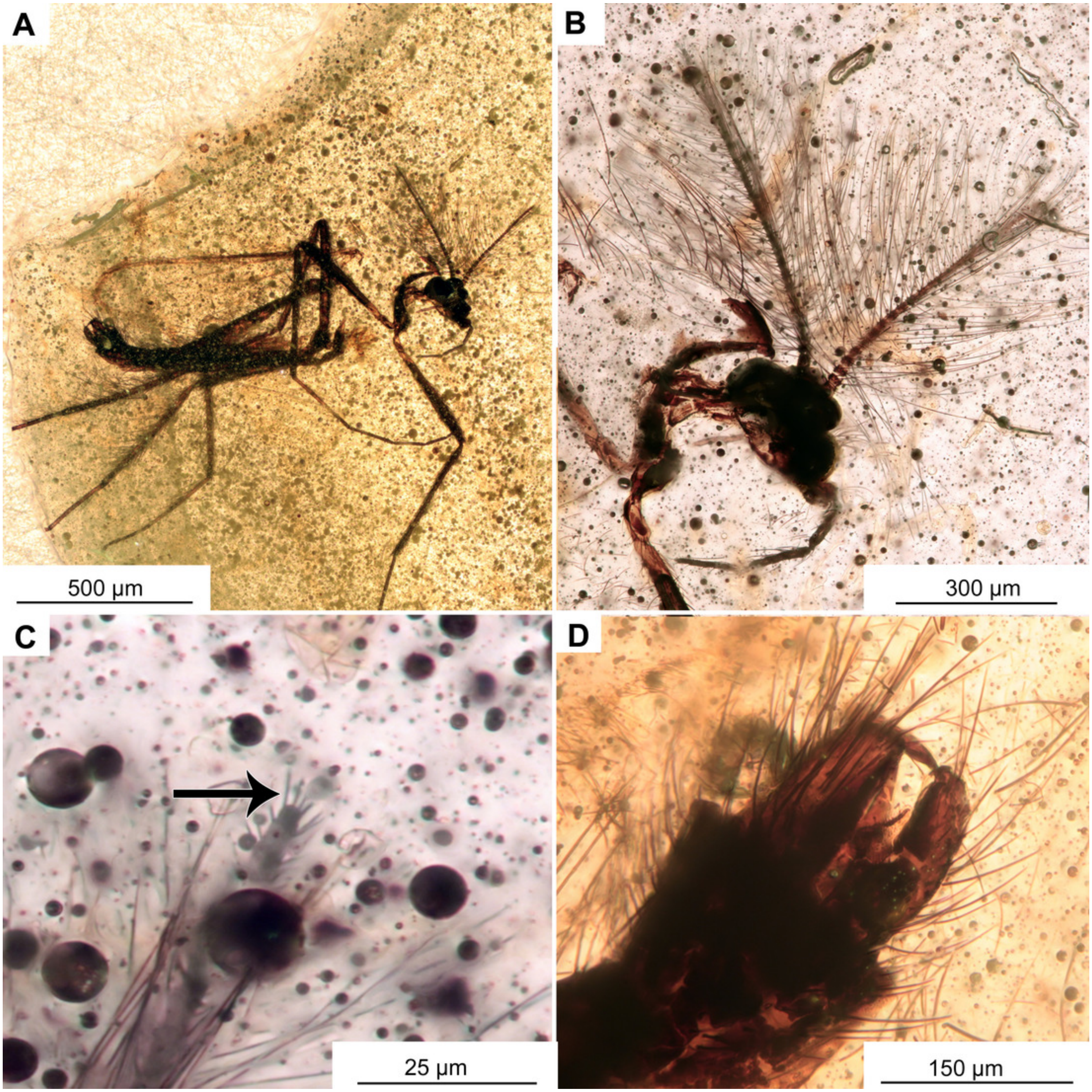
A
500 µm
B
300 µm
C
25 µm
D
150 µm

Figure 10

Figure 10 *Gymnometriocnemus extinctus* sp. nov. Baranov, holotype OU47546.

(A) Photomicrograph of wing. (B) Line drawing of wing. (C) Photomicrograph of hypopigium, dorsal view. (D) Line drawing of hypopigium, dorsal view. Abbreviations: *AP*, anal point; *Gc*, gonocoxite; *Gs*, gonostylus; *IVo*, inferior volsella; *TIX*, abdominal tergite 9; *C*, costal vein; *Cu1*, cubital vein 1; *M1+2*, medial vein 1+2; *M3+4*, medial vein 3+4; *R1*, radial vein 1; *R2+3*, radial vein 2+3; *R4+5*, radial vein 4+5.

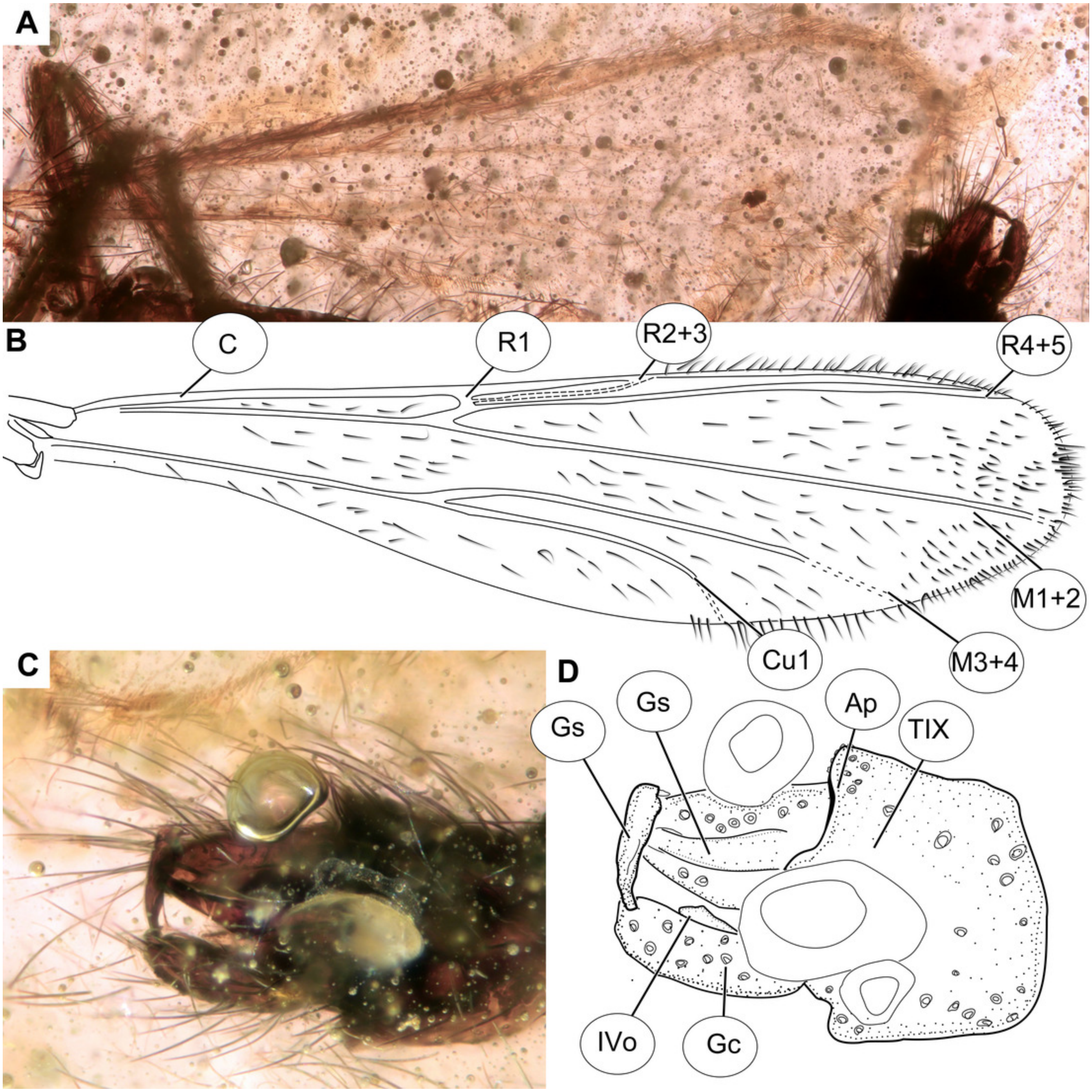
A
B
C
R1
R2+3
R4+5
M1+2
Cu1
M3+4
C
D
Gs
Gs
Ap
TIX
IVo
Gc

Figure 11

Figure 11 *Gymnometriocnemus extinctus* sp. nov. Baranov, female paratypes.

(A, B) Habitus and wing of specimen OU47577. (C) Habitus of specimen OU47578.

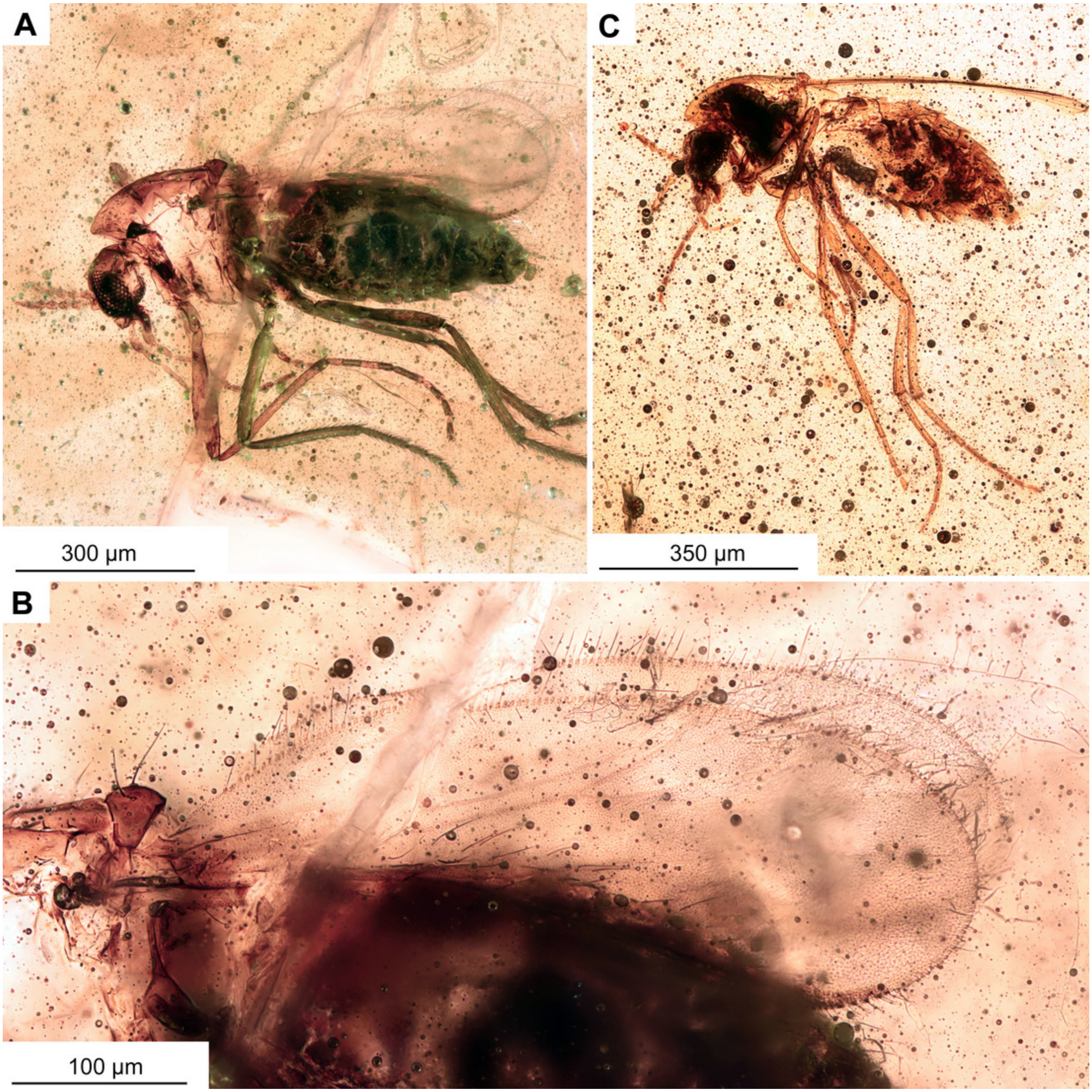

Figure 12

Figure 12 *Gymnometriocnemus extinctus* sp. nov. Baranov, female paratypes.

(A) Head of specimen OU47577. (B) Head of specimen OU47578; b’ close-up of last flagellomere with apical setae. (C) Female genitalia (OU47578). (D) Female genitalia, marked (OU47578). Abbreviations: *A8*, abdominal segment 8; *Ce*, cerci; *Gca*, gonocoxite apodem; *Gc*, gonocoxite (8); *Gp8*, gonapophysis 8; *TIX*, tergite 9.

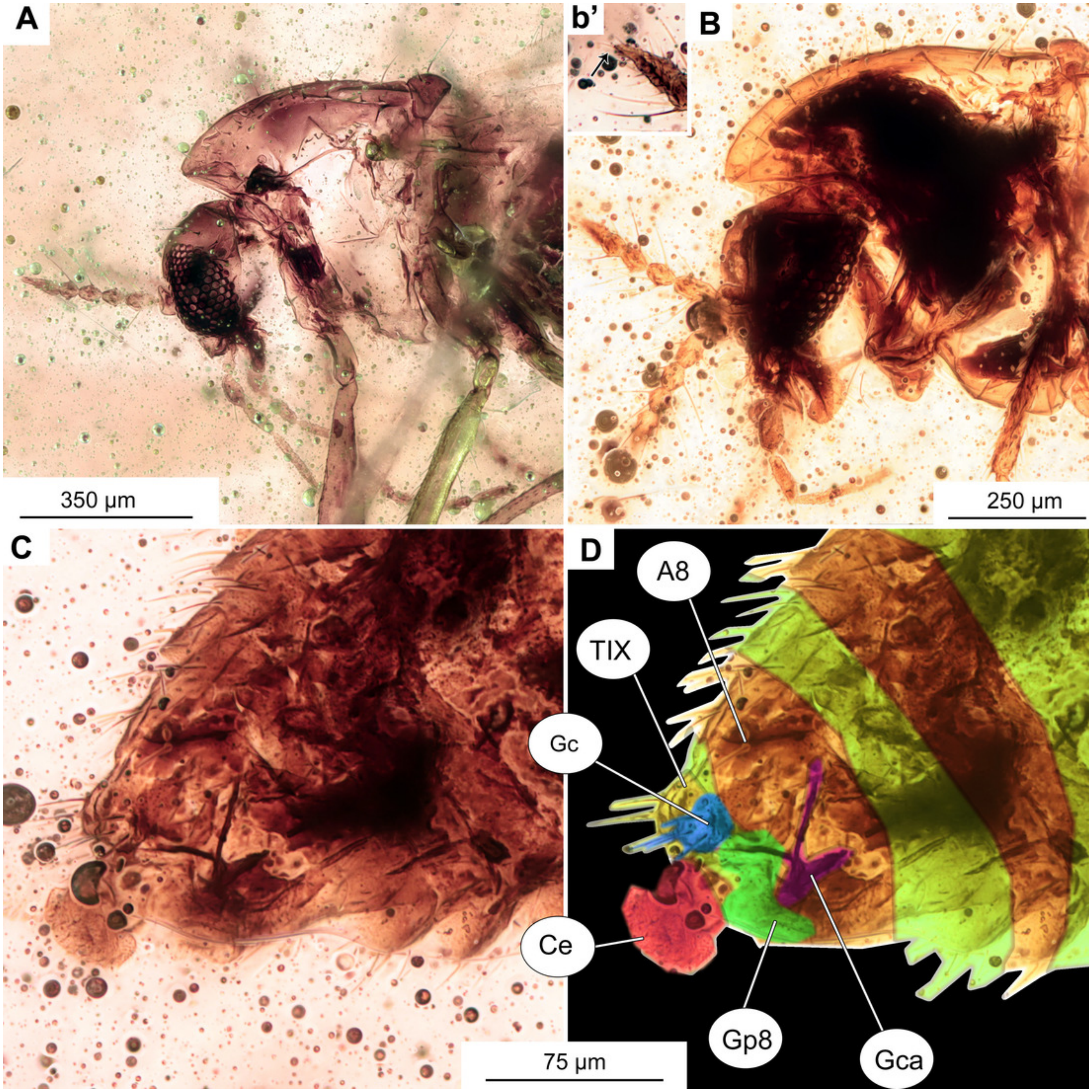
A
350 µm
b'
B
250 µm
C
75 µm
D
A8
TIX
Gc
Ce
Gp8
Gca

Figure 13

Figure 13. Holotype (adult male, number NZAC02044947) of *Kuschelius dentifer* Sublette and Wirth, 1980.

(A). Wing. (B) Head, arrow marks a apical protrusion of the 3d palpomere. (C) Hypopigium. (D) Midtibia with spurs and the comb. (E) Hindtibia with the spurs and comb. All photos in this plate are made by Dr. Leanne Elder, licensed under CC BY 4.0 and used with the photographer’s explicit permission.