

The Lonchaeidae (Diptera) of the GBOL project, with the description of a new *Priscoearomyia* species

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Abstract

The investigation of 331 specimens of the acalypterate dipteran family Lonchaeidae within the GBOL-project resulted in a list of 29 species from which one is new to science, *Priscoearomyia bausenbergensis* **sp. nov.**, and four species represent new records for Germany. For all voucher specimens detailed metadata are provided including validated DNA barcodes. These barcodes build a sound reference basis for future molecular identification of lonchaeid flies and will also allow the inclusion of female specimens in biodiversity studies, when morphological characters for separating these females are not available.

Key Words

COI barcodes, DNA analysis, Europe, faunistics, France, Germany, Lonchaeidae, new records, new species, taxonomy

Introduction

Lonchaeidae is a small family of acalypterate flies, which is distributed worldwide. In Europe, approximately 100 species are known to occur with about 60 in Germany (see Maca 1999, Werner 1995, MacGowan 2004, MacGowan 2015, MacGowan et al. 2007, MacGowan and Rotheray 2008, Reimann and Rulik 2014, Reimann and Stuke 2016, MacGowan and Reimann 2021). The adults are small usually shiny black, sometimes somewhat dulled or slightly green/blue metallic flies. The females always have a strongly sclerotized retractable aculeus. The male genitalia vary in shape and structure in and between the genera. There is a wide range of larval habitats, e.g., under the bark of dead wood, in conifer cones, in rotting plant material, in fruits, in bulbs or in galls in the stems of plants. For more details on the biology see Morge (1963) and MacGowan and Rotheray (2008). In many cases, especially in small dipterans, it has been difficult to proper

identify female specimens. Often there is a group of closely related species, where females look very similar and the morphological assignment to the respective males is very difficult or even impossible. Barcoding provides a tool for solving this problem. In Lonchaeidae the barcode sequences of the different species analyzed so far are very distinct and females can be identified in comparing their barcode with those of the males. Afterwards it is possible to search for additional morphological characters, which can aid in identification keys. Furthermore, barcoding can be used to identify torn or incomplete specimens which are always present in Malaise trap samples, especially when the emptying cycles are longer than one week.

Since 2011 the German Barcode of Life project (GBOL) acts as a national barcoding campaign and builds up a voucher-based barcode reference library (Geiger et al. 2016). So far roughly half of the German animal diversity is documented, while the megadiverse groups of Diptera or Hymenoptera are highly underrepresented. Both

are present with more than 10.000 species in Germany. Therefore, the recent phase – GBOL III: Dark Taxa – is concentrating especially on micro flies and wasps. But even other small dipteran taxa need some serious attention and will reveal more diversity as known before.

In the present study we combine results with reverse identification approaches out of the Global Malaise trap Program (GMP).

Material and methods

The majority of the material examined was caught with three Malaise traps in Rhineland-Palatine in the administrative district Ahrweiler. The remaining samples are from different localities in other federal states of Germany and France (Fig. 1). For a detailed list of localities see Suppl. material 2.

All specimens except the non-GBOL material are stored in purified ethanol at $-20\text{ }^{\circ}\text{C}$. For morphological identification genitalia were dissected, if needed and treated with KOH-solution (5% in aq.) at $70\text{ }^{\circ}\text{C}$ for 30–60 min., then neutralized with acetic acid (5% in aq.) and rinsed in distilled water. The genitalia are stored in small silicon tubes with the respective specimen. From the non GBOL material one specimen is pinned and the genitalia are mounted on a glass slide; the others are stored in de-natured ethanol (70% in aq.).

The lonchaeids were morphologically identified using the keys of Collin (1953), Hackman (1956), MacGowan (2014a, 2014b, 2020), MacGowan and Rotheray (2000, 2008), McAlpine (1982), and Morge (1959a, 1959b, 1959c, 1962, 1963). The nomenclature follows MacGowan (2023). All specimens examined in this study were deposited in the Zoologisches Forschungsmuseum Alexander Koenig, Leibniz-Institut zur Analyse des

Biodiversitätswandels (LIB) in Bonn or the Senckenberg Naturhistorische Sammlungen Dresden, Museum für Tierkunde in Dresden.

After morphological identification most of the specimens were barcoded following the established GBOL procedure:

Tissue was subsampled from each specimen and transferred into 96 well plates for subsequent DNA extraction. For specimens larger than 2 mm 1–3 legs were used for lysis. For very small specimens ($\leq 2\text{ mm}$) the whole body was used non-destructively for lysis (i.e., subsequent voucher recovery). Genomic DNA was extracted using the BioSprint96 magnetic bead extractor and respective kits by Qiagen (Hilden, Germany). Polymerase chain reaction (PCR) was carried out in a total reaction volume of 20 μl , including 2 μl of undiluted DNA template, 0.8 μl of each primer (10 pmol/ μl), 2 μl of ‘Q-Solution’ and 10 μl of ‘Multiplex PCR Master Mix’, containing hot start Taq DNA polymerase and buffers. The latter components are available in the Multiplex PCR kit by Qiagen (Hilden, Germany).

Thermal cycling was performed on GeneAmp PCR System 2700 machines (Life Technologies, Carlsbad, CA, USA) as follows: hot start Taq activation: 15 min at $95\text{ }^{\circ}\text{C}$; first cycle set (15 repeats): 35 s denaturation at $94\text{ }^{\circ}\text{C}$, 90 s annealing at $55\text{ }^{\circ}\text{C}$ ($-1\text{ }^{\circ}\text{C}/\text{cycle}$) and 90 s extension at $72\text{ }^{\circ}\text{C}$. Second cycle set (25 repeats): 35 s denaturation at $94\text{ }^{\circ}\text{C}$, 90 s annealing at $40\text{ }^{\circ}\text{C}$ and 90 s extension at $72\text{ }^{\circ}\text{C}$; final elongation 10 min at $72\text{ }^{\circ}\text{C}$. As established within GBOL at ZFMK the following standard degenerate barcoding primers LCO1490-JJ: 5’-CHACWAAY-CATAAAGATATYGG- 3’ and HCO2198-JJ: 5’-AWACTTCVGGRTGVCCAAARAATCA- 3’ (Astrin and Stüben 2008) LCO1490-JJ2: 5’-CHACWAAY-CAYAARGAYATYGG- 3’ and HCO2198-JJ2: 5’-AN-ACTTCNGGRTGNCCAAARAATCA- 3’ (Astrin et al. 2016) were used. Purification and sequencing were conducted at BGI (Hong Kong, China) by using the amplification primers.

A minor fraction of specimens was processed within the Global Malaise trap Program (GMP) and the CCDB standard barcoding procedures were applied (<https://ccdb.ca/resources/>).

Sequences were semi-automatically edited, assembled using the MUSCLE alignment approach (Edgar 2004) and checked for the occurrence of stop-codons or hints of nuclear mitochondrial DNA segments (NUMTs) in GENEIOUS version 7.1.9 (<http://www.geneious.com>; Kearse et al. 2012). All metadata of specimens treated here were deposited in GBIF (<https://biocase.zfmk.de/ipt/resource?r=gbolonch>); further details like voucher information such as locality data, habitat, collector, identifier, taxonomic classifications, DNA barcode sequences, primer pairs, the sequence data and trace files were deposited in BOLD (<https://doi.org/10.5883/DS-GBO-LONCH>) and subsequently also transferred to GenBank (accession numbers: OP831582–OP831877 and KT781862–KT781863).

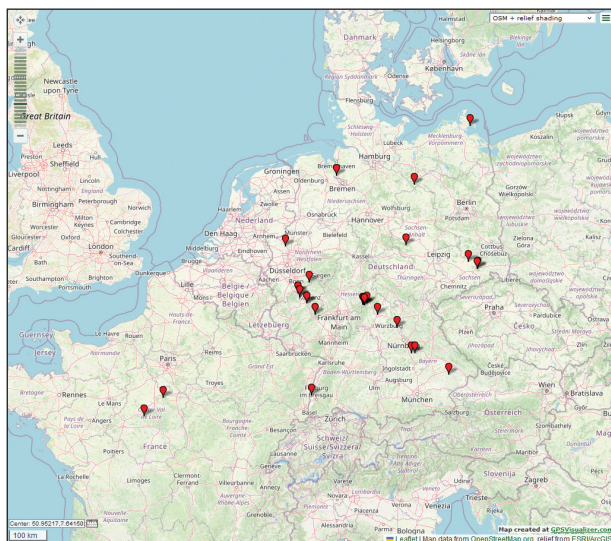


Figure 1. Map with the sample locations for the data presented here. Map created at GPSVisualizer.com. Map data from OpenStreetMap.org under Open Data Commons Open Database License (opendatacommons.org).

A neighbour-joining tree was obtained using the Tamura-Nei model without outgroup (Tamura and Nei 1993). Morphological identifications were checked on taxonomic consistency via the TaxCI script (Rulik et al. 2017). Inconsistent specimens were either morphologically re-identified (males) or assigned by topology within the genetic distance tree (NJ) and BLASTing against reliable barcode compliant entries of BOLD (females). Additionally, the following species delineation methods: Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021) and the BOLD built-in RESL algorithm for Barcode Index Numbers (BINs; Ratnasingham and Hebert 2013) were applied.

Distance statistics were calculated using DiStats (Astrin et al. 2016). For the alignment only barcode sequences with full length of 658 base pairs ($n = 274$) were used.

The following abbreviations and terms are used in the text or in the Suppl. materials 1, 2: Collection codes: **MTD** – Senckenberg Naturhistorische Sammlungen Dresden, Museum für Tierkunde, Dresden, Germany; **ZFMK** – Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany. General abbreviations: **a.s.l.** – (elevation) above sea level; **GBOL** – German Barcode of Life; **LG1–LG4** – Barcode cluster 1 to 4 of the *L. limatula* species group; **MG1–MG3** – Barcode cluster 1 to 3 of the *L. mallochi* species group; **N/A** – not available; **Entomol. Soc. Krefeld** – Entomological Society Krefeld (= Entomologischer Verein Krefeld); **ZFMK leg.** – collected by ZFMK staff; **ZFMK et al. leg.** – collected by ZFMK staff and project co-workers. Morphological abbreviations: **bp** – basiphallus; **ce** – cercus, **dp** – distiphallus; **ea** – ejaculatory apodeme, **ep** – epandrium, **ha** – hypandrial apodeme, **hy** – hypoproct; **pa** – paramere; **ph** – phallus; **su** – surstylus; **ve** – ventral lobe of epandrium. Nomenclatural abbreviations: **L.** – *Lonchaea*; **P.** – *Priscoearomyia*.

Results

Taxonomy

Subfamily Dasiopinae Morge, 1963

Dasiops Rondani, 1856

Dasiops calvus Morge, 1959

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55193°N, 7.16998°E; 194 m a.s.l.; Malaise trap; 1 female (GBOL-223965312), 11 March 2014; 1 male (GBOL-223967397), 01 July 2014; both Rulik leg.; both in ZFMK.

Dasiops mucronatus Morge, 1959

Material. GERMANY • Mecklenburg-Western Pomerania; Putbus; 54.32506°N, 13.53878°E; 27 m a.s.l.; Malaise trap; 6 females (ZFMK-TIS-2578147–ZFMK-TIS-25781472578149, ZFMK-TIS-2578151), 05 June 2016; all Rulik leg.; all in ZFMK.

Dasiops perpropinquus Morge, 1959

Material. GERMANY • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2008888–ZFMK-TIS-2008889), 06 June 2017; both ZFMK et al. leg.; both in ZFMK • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2607649), 30 May 2017; ZFMK leg.; in ZFMK.

Subfamily Lonchaeinae Rondani, 1856

Tribe Earomyini Morge, 1963

Chaetolonchaea Czerny, 1934

Chaetolonchaea dasyops (Meigen, 1826)

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2578121), 02 June 2014; 1 male (ZFMK-TIS-2575253), 27 June 2013; both Rulik leg.; both in ZFMK.

Chaetolonchaea pallipennis (Zetterstedt, 1855)

Material. GERMANY • Hesse; Sinntal; 50.27755°N, 9.61509°E; 356 m a.s.l.; sweep net; 1 male (ZFMK-TIS-2616780), 18 April 2018; Kappert leg.; in ZFMK.

Earomyia Zetterstedt, 1842

Earomyia lonchaeoides Zetterstedt, 1848

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.46541°N, 7.2259°E; 292 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2575284), 09 April 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Winingen; 50.32°N, 7.493°E; 243 m a.s.l.; Malaise trap; 1 male (GBOL-224009772), 04 April 2013; 2 females (GBOL-224009996, GBOL-224010052), 18 April 2013; all Rulik leg.; all in ZFMK. • Saxony; Dresden; 51.13369°N, 13.81147°E; 210 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2516625), 26 March 2014; Reimann leg.; in ZFMK.

Earomyia viridana (Meigen, 1826)

Material. GERMANY • Hesse; Lorch; 50.04912°N, 7.79777°E; 246 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2578120), 17 June 2015; Niehuis leg.; in ZFMK • Hesse; Neuengronau; 50.27454°N, 9.61179°E; 320 m a.s.l.; net sweeping; 1 female (ZFMK-TIS-2595861), 30 April 2017; Kappert leg.; in ZFMK • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2607645, ZFMK-TIS-2607646), 30 May 2017; 1 female (ZFMK-TIS-2607657), 27 June

2017; all ZFMK leg.; all in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2627273), 09 May 2017; 3 females (ZFMK-TIS-2627274–ZFMK-TIS-2627276), 30 May 2017; 1 male (ZFMK-TIS-2606579), 06 June 2017; 1 female (ZFMK-TIS-2627298), 11 July 2017; 1 female (ZFMK-TIS-2627292), 20 July 2017; all ZFMK et al. leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55193°N, 7.16998°E; 194 m a.s.l.; 1 female (GBOL-223958884) 25 July 2013; 6 females (ZFMK-TIS-2574270, ZFMK-TIS-2575258, ZFMK-TIS-2575270–ZFMK-TIS-2575273), 24 April 2014; 7 females (GBOL-223967883, GBOL-223967922, GBOL-223968741, GBOL-223968792, GBOL-223968832, ZFMK-TIS-2575318, ZFMK-TIS-2575319), 16 May 2014; 1 female (ZFMK-TIS-2574264), 13 June 2013; 1 female (GBOL-223969604), 17 June 2014; 4 females (GBOL-223969946, GBOL-223970112, GBOL-223967402, GBOL-223967517), 01 July 2014; 1 female (ZFMK-TIS-2575256), 27 June 2013; 1 female (GBOL-223971280) 15 July 2014; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2578135), 16 May 2014; 3 females (ZFMK-TIS-2578127–ZFMK-TIS-2578129), 02 June 2014; 1 female (ZFMK-TIS-2578144), 15 July 2014; all Rulik leg.; all in ZFMK • Saxony-Anhalt; Harzgerode; 51.66887°N, 11.16425°E; 281 m a.s.l.; Malaise trap, 1 female (ZFMK-TIS-2603069), 28 July 2017; Geiger et al. leg.; in ZFMK.

Priscoearomyia Morge, 1963

Priscoearomyia bausenbergensis sp. nov.

<https://zoobank.org/0FEB4DEE-ED28-4309-BF21-757ABECEB353>
Figs 2A–E, 3A–D, 5

Material examined. *Holotype*: GERMANY • male (ZFMK-TIS-2575291, ZFMK-DIP-00100412); Rhineland-Palatinate; Ahrweiler; Niederzissen; Bausenberg; 50.4647°N, 7.22215°E; 321 m a.s.l.; Malaise trap; 17 June 2014; Rulik leg.; in ZFMK.

Paratypes: GERMANY • 1 female (ZFMK-TIS-2578106, ZFMK-DIP-00100413); Hesse; Lorch am Rhein; castle Nollig; 50.04912°N, 7.79777°E; 246 m a.s.l.; Malaise trap; 17 June 2015; Niehuis leg.; in ZFMK • 2 males (MTD-Dip-8223, MTD-Dip-8660); Bavaria; Berching; Rappersdorf; Aspen; 49.12318°N, 11.4916°E; 526 m a.s.l.; Malaise trap; 22 May 2020; Hable leg.; in MTD • 2 males (MTD-Dip-8222, MTD-Dip-8659); Bavaria; Limbach; Stephaniter Weiher; Aspen; 49.74018°N, 10.82520°E; 297 m a.s.l.; Malaise trap; 21 May 2020; Grasse and Schmidl leg.; in MTD • 2 males (MTD-Dip-8224, MTD-Dip-8225); Bavaria; Freystadt; mixed forest S of Burggriesbach; Aspen; 49.12375°N, 11.36218°E; 445 m a.s.l.; Malaise trap; 06 June 2021; Hable leg.; in MTD • 1 male (MTD-Dip-8226); Hesse; Oberzell (Sinntal); swampy area with beech and ash

trees; 50.31837°N, 9.70374°E; 538 m a.s.l.; hand collecting; 31 May 2021; Kappert leg.; in MTD.

FRANCE • 1 male (MTD-Dip-8021); Centre-Val de Loire; Huisseau-sur-Cosson; 47.57927°N, 1.47151°E; 86 m a.s.l.; Lindgren funnel trap; 19 May 2021; Sallé and Paupe leg.; in MTD • 1 male (MTD-Dip-8052); Centre-Val de Loire; Loury; 48.03878°N, 2.18281°E; 136 m a.s.l.; Lindgren funnel trap; 19 May 2021; Sallé and Paupe leg.; in MTD.

Description. Male: Head. Eye with sparse very short hairs. Frons and face completely greyish brown dusted. 9 to 10 frontal setulae in a row along the eye margins, 16 scattered interfrontal setulae. Orbital seta strong and long (0.35 mm), as long as outer vertical seta. Inner vertical seta slightly longer than outer vertical seta. Orbital plate dusted, with a shining central streak, bare apart from orbital seta. Ocellar seta strong, as long as orbital seta. Few scattered ocellar setulae, 0.25 times as long as ocellar seta. Lunula bare. Four anterior genal setulae in a single row. Postpedicel short, oval, not reaching mouth edge, 1.5 times as long as wide, distinct orange spot on the basal medial surface, which occupies approximately one third of the inner surface. Arista two times as long as postpedicel, short pubescent.

Thorax. Thoracic dorsum black, dusted greyish brown, covered in setulae one third length of orbital seta. Scutellum completely dusted greyish brown, no obvious contrast to thoracic dorsum, bare apart from the 4 marginal setae. Lateral sclerites completely dusted. Anepisternum with anterodorsal seta absent, three strong posterior setae in a dorsoventral row, approximately 12–16 scattered setulae on disc. Katepisternum with two strong posterior seta and one to two weaker anterior setae in an irregular row close to the dorsal margin, central part bare, approximately 4–7 setulae ventrally. One seta on proepimeron and one on proepisternum. Calypter yellow-white with light brown fringe. Wings yellowish in anterior half, turning slightly brownish towards wing tip, posterior half whitish yellow. Veins yellowish basally turning brownish towards wing tip. All legs entirely black.

Abdomen. Tergite 5 2.2 times the length of tergite 4, funnel-shaped in dorsal view, strongly narrowing in posterior half, with a closely spaced group of 6 stiff setulae on each side before the apex. A small triangular membranous area on central anterior margin (Fig. 3C). In lateral view anteriorly bulged, abruptly narrowing in posterior half, beak-shaped (Fig. 3D).

Male terminalia. Epandrium (ep) short, belt like, three times as high as long (Figs 2A, 3A), with a terminal fringe of setulae on each side, gradually increasing in length from ventral to dorsal. Ventral lobes (ve) not sclerotized, only present as a membranous strip not exceeding the width of the epandrium, covered in small setulae. Fused cerci (ce) not sclerotized, hardly visible, with a row of setae. Surstyli (su) extending ventromedially from the shell of the epandrium as two lightly sclerotized processes covered in very short thick-based setulae (Fig. 3B). No prenisetae visible. Hypoproct (hy) a cap-shaped plate,

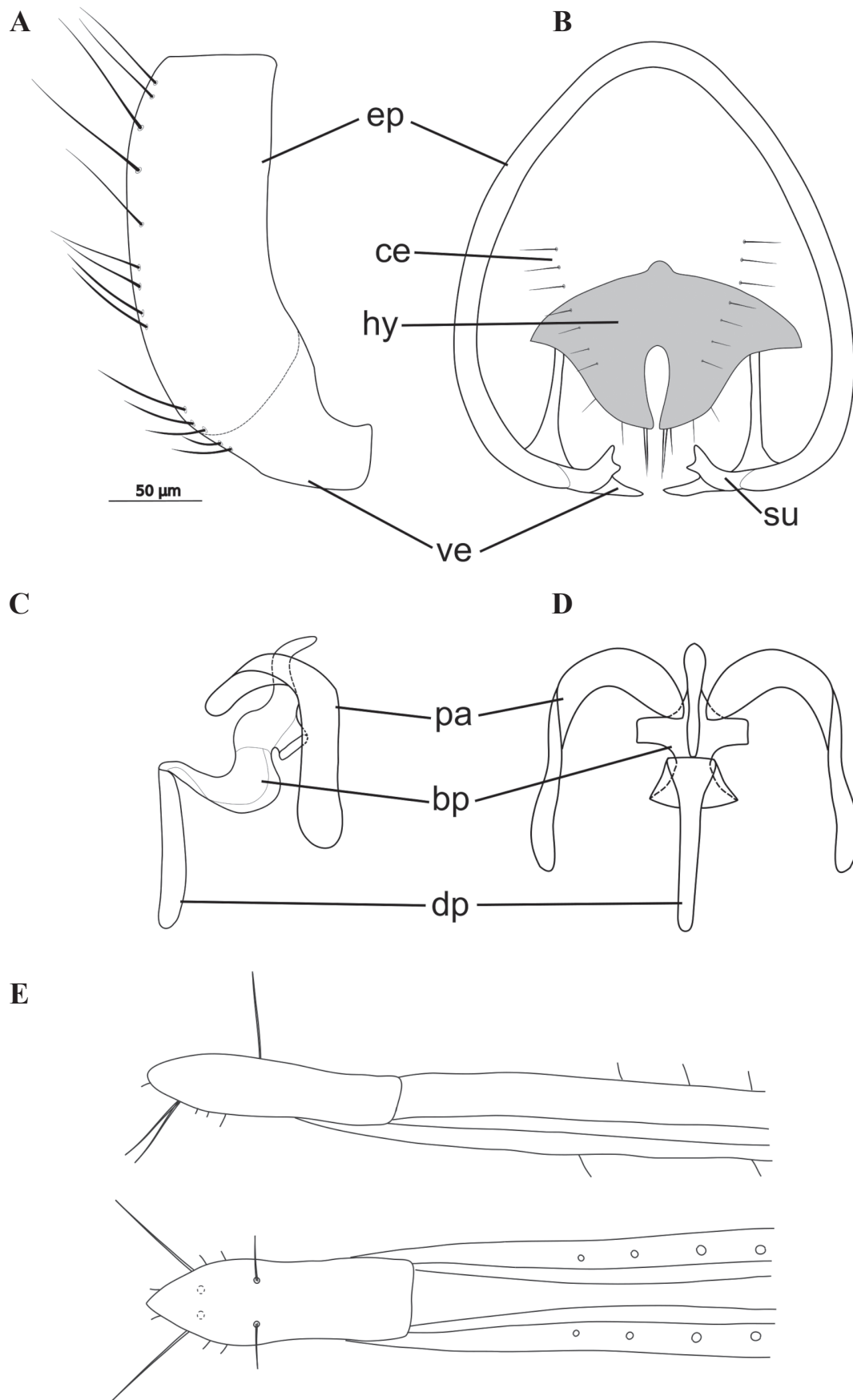


Figure 2. Drawings of male (A–D) and female (E) genitalia of *Priscoearomyia bausenbergensis* sp. nov.: A. Lateral view of epandrium; B. Posterior view of epandrium and associated structures; C. Lateral view of phallus and parameres; D. Ventral view of phallus and parameres; E. Lateral (above) and dorsal (below) view of female ovipositor.

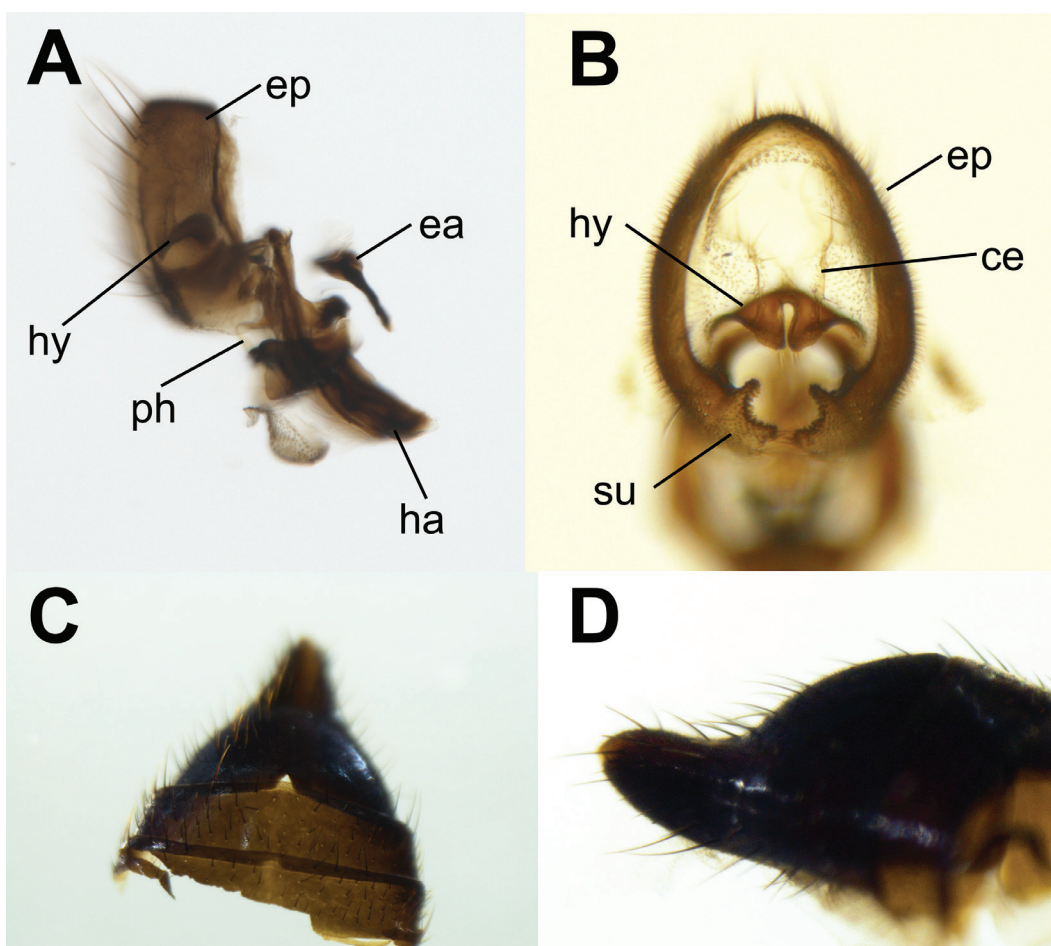


Figure 3. Abdomen and genital capsule of male holotype of *Priscoearomyia bausenbergensis* sp. nov.: **A.** Lateral view of male genital; **B.** Apicoventral view of male genital; **C.** Dorsal view of tip of abdomen; **D.** Lateral view of tip of abdomen.

ventral margin with two broad triangular apical lobes, almost touching in midline, leaving a narrow oval incision (Figs 2B, 3B), both lobes with a fringe of hairs increasing in length towards the middle. Phallus Z-shaped with a massive basiphallus (bp) attached to a cross shaped plate anteriorly. Distiphallus (dp) long and straight, less sclerotized (Fig. 2C). Parameres (pa) belt like, not well sclerotized, strongly curved before ending close to base of phallus, not fused in midline (Fig. 2C, D). Hypandrial apodeme (ha) strongly sclerotized.

Measurements: Body length 3.74 mm. Wing length 3.60 mm.

Female: Head. As in male. Postpedicel oval, reaching mouth edge, 1.6 times as long as wide. Distinct orange spot at the basal inner surface. Arista 1.75 times as long as postpedicel, short pubescent.

Thorax. Thoracic dorsum sub-shining black, completely dusted greyish brown, anteriorly covered in setulae one third length of orbital seta, posterior to the suture some setulae reaching almost half the length of orbital seta. Scutellum completely dusted greyish brown, dusting stronger than on thoracic dorsum, with 4 marginal setae (only bases visible in paratype). Lateral sclerites completely dusted. Anepisternum with no anterodorsal seta and three strong posterior setae in a dorsoventral

row. With a few scattered setulae on disc. Katepisternum with two strong posterior setae and two weaker (half the length of the posterior) in an irregular row close to the dorsal margin, central part bare, 7 setulae ventrally. One seta on proepimeron and one on proepisternum. Calypter yellow-white with light brown fringe. Wings yellowish in anterior half, turning slightly brownish towards wing tip, posterior half yellowish white. Veins yellowish basally turning brownish towards wing tip. Only hind legs present in female paratype, entirely black.

Abdomen. Tergites completely brownish dusted. Sternites completely dusted. Aculeus black with yellow tip. Apical segment as in Fig. 2E. In dorsal view slightly constricted in the middle. In side view a long ventro-apical seta with three very small setulae anterior to these on each side and a tiny setula close to the tip. Dorsal surface with a pair of long setae at position 0.6 of the total length measured from the base of the segment. Apical dorsal setae missing in paratype female specimen.

Measurements: Body length 3.26 mm. Wing length 3.43 mm.

Etymology. This species is named after the collecting site of the male holotype.

Biology. Unknown.

Distribution. Only known from Europe (Germany, France).

Differential diagnosis. With the plate-like hypoproct and epandrium without well sclerotized ventral lobes, this species belongs to a group with *P. greciana* (McAlpine, 1983) and *P. hermoensis* MacGowan & Freidberg, 2008. It differs from both mentioned species by the shape of the phallus, which is more or less U-shaped in *P. greciana* and *P. hermoensis* (see MacGowan 2014a, figs 22–31), but Z-shaped in *P. bausenbergensis*. Furthermore, the shape of the hypoproct is quite different. In *P. greciana* the hypoproct is a dome-shaped plate with two very short apical lobes. In *P. hermoensis* these lobes are broader and longer and have numerous finger-like processes on the outer margin (MacGowan 2014a). In *P. bausenbergensis* the

apical lobes are very broad with smooth outer margins and enclosing a narrow oval incision. The extremely narrowed and elongated tip of the abdomen separates the new species from all other known species of *Priscoearomyia*, except *P. greciana*, which shows this character to a lesser extent. The small triangular membranous area on the anterior margin of tergite 5 is not present in *P. greciana* and clearly separates *P. bausenbergensis* from the latter species. The large distinct orange spot at the base of postpedicel separates the described species from all others, except *P. withersi* MacGowan, 2014, which can have a small spot present. The new species can be included in the key to the *Priscoearomyia* species by MacGowan (2014a) as follows:

- | | | |
|----|---|---|
| 1 | Epandrium without an obvious ventral lobe, hypoproct wider than long, in form of a disc, prenisetae absent (<i>greciana</i> group) | 2 |
| – | Epandrium with a square or rectangular ventral lobe, hypoproct not disc shaped, prenisetae present (other <i>Priscoearomyia</i> species) | 3 |
| 2 | Abdominal tergite 5 elongated, strongly narrowing before tip | 2a |
| – | Abdominal tergite 5 not elongated. Apical lobes of hypoproct with finger-like projections laterally, outer margin of surstyli obviously serrated. Phallus U-shaped, thickened with basal processes | |
| | <i>P. hermonensis</i> MacGowan & Freidberg | |
| 2a | Abdominal tergite 5 without median triangular membranous area. Apical lobes of hypoproct rounded, without processes, surstyli with multiserial rows of denticles along outer margin and extending over posterior surface. Phallus narrow and sinuous | <i>P. greciana</i> McAlpine |
| – | Abdominal tergite 5 with median triangular membranous area. Apical lobes of hypoproct very broad, without processes, medially enclosing oval incision. Surstyli extending ventromedially from the shell of the epandrium as two lightly sclerotized processes covered in very short thick-based setulae. Phallus Z-shaped | <i>P. bausenbergensis</i> sp. nov. |

***Priscoearomyia greciana* (McAlpine, 1983)**

Fig. 5

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.4647°N, 7.22215°E; 321 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2574198, ZFMK-TIS-2575292), 17 June 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2575242), 27 June 2013; Rulik leg.; in ZFMK.

***Priscoearomyia nigra* (Meigen, 1826)**

Fig. 5

Material. GERMANY • Hesse; Lorch; 50.04912°N, 7.79777°E; 246 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2578107), 17 June 2015; Niehuis leg.; in ZFMK • North Rhine-Westphalia; Wesel; 51.64820°N, 6.70026°E; 27 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2603019); 3 females (ZFMK-TIS-2603006, ZFMK-TIS-2603008, ZFMK-TIS-2603013), 09 June 2016; all Entomol. Soc. Krefeld leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2575246), 27 June 2013; Rulik leg.; in ZFMK • Saxony-Anhalt; Harzgerode; 51.66887°N,

11.16425°E; 281 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2603070), 28 July 2017; Geiger et al. leg.; in ZFMK.

***Priscoearomyia rameli* (MacGowan, 2014)**

Fig. 5

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2575245), 1 female (ZFMK-TIS-2575250), 27 June 2013; both Rulik leg.; both in ZFMK • Saxony-Anhalt; Harzgerode; 51.66887°N, 11.16425°E; 281 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2603071), 2 females (ZFMK-TIS-2603072, ZFMK-TIS-2603073), 28 July 2017; all Geiger et al. leg.; all in ZFMK.

***Priscoearomyia withersi* (MacGowan, 2014)**

Fig. 5

Material. GERMANY • Hesse; Lorch; 50.04912°N, 7.79777°E; 246 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2578105), 17 June 2015; Niehuis leg.; in ZFMK • Mecklenburg-Western Pomerania; Putbus; 54.32506°N, 13.53878°E; 27 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2578153), 1 female (ZFMK-TIS-2578152), 05

June 2016; both Rulik leg.; both in ZFMK • North Rhine-Westphalia; Wesel; 51.64820°N, 6.70026°E; 27 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2603018), 12 females (ZFMK-TIS-2603003–ZFMK-TIS-2603005, ZFMK-TIS-2603007, ZFMK-TIS-2603009–ZFMK-TIS-2603012, ZFMK-TIS-2603014–ZFMK-TIS-2603017), 09 June 2016; all Entomol. Soc. Krefeld leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55193°N, 7.16998°E; 194 m a.s.l.; Malaise trap; 1 female (GBOL-22397235829), July 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.46541°N, 7.2259°E; 292 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2575321), 16 May 2014; 1 female (ZFMK-TIS-2574252), 01 July 2014; 2 females (ZFMK-TIS-2574255, ZFMK-TIS-2574256), 2 males (ZFMK-TIS-2574257, ZFMK-TIS-2574258), 11 July 2013; 1 male (ZFMK-TIS-2574216), 25 July 2013; 2 females (ZFMK-TIS-2574217, ZFMK-TIS-2574218), 25 July 2013; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2574263), 13 June 2013; 3 males (ZFMK-TIS-2574281, ZFMK-TIS-2575243, ZFMK-TIS-2575244), 9 females (ZFMK-TIS-257428, ZFMK-TIS-2574283–ZFMK-TIS-2574285, ZFMK-TIS-2575247–ZFMK-TIS-2575249, ZFMK-TIS-2575251, ZFMK-TIS-2575252), 27 June 2013; 1 female (ZFMK-TIS-2578145), 15 July 2014; 3 females (ZFMK-TIS-2574238, ZFMK-TIS-2574243, ZFMK-TIS-2574244), 08 August 2013; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.4647°N, 7.22215°E; 321 m a.s.l.; Malaise trap; 4 males (ZFMK-TIS-2575293–ZFMK-TIS-2575296), 13 females (ZFMK-TIS-2575297–ZFMK-TIS-2575309), 17 June 2014; 1 female (ZFMK-TIS-2574195), 15 July 2014; 2 females (ZFMK-TIS-2574196, ZFMK-TIS-2574197), 17 June 2014; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Winnigen; 50.32°N, 7.493°E; 243 m a.s.l.; Malaise trap; 1 female (GBOL-224008773), 13 June 2013; 1 female (GBOL-224010268), 11 July 2013; 1 female (GBOL-224010704), 01 August 2012; all Rulik leg.; all in ZFMK.

Tribe Lonchaeini Rondani, 1856

Lonchaea Fallén, 1820

Lonchaea chorea (Fabricius, 1781)

Material. GERMANY • Mecklenburg-Western Pomerania; Zartwitz; car-net; 1 male (ZFMK-TIS-2584167), 12 June 2015; Kleeberg leg.; in ZFMK • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2607650), 30 May 2017; 1 male (ZFMK-TIS-2607636), 9 females (ZFMK-TIS-2607637–ZFMK-TIS-2607641, ZFMK-TIS-2607693–ZFMK-TIS-2607696), 13 June 2017; all ZFMK leg.; all in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2627290), 1 female (ZFMK-TIS-2627291),

23 May 2017; 2 females (ZFMK-TIS-2606587, ZFMK-TIS-2606588), 06 June 2017; 1 male (ZFMK-TIS-2627293), 20 June 2017; 3 females (ZFMK-TIS-2606571, ZFMK-TIS-2606573, ZFMK-TIS-2606574), 18 July 2017; all ZFMK et al. leg.; all in ZFMK • North Rhine-Westphalia; Wesel; 51.64820°N, 6.70026°E; 27 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2602997), 19 May 2016; Entomol. Soc. Krefeld leg.; in ZFMK • Saxony; Dresden; 51.12879°N, 13.78985°E; 203 m a.s.l.; hand collecting; 1 female (ZFMK-TIS-2584169), 01 July 2008; Reimann leg.; in ZFMK • Saxony; Großenhain; 51.2929°N, 13.4721°E; 117 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2584168), 23 June 2011; Reimann leg.; in ZFMK.

Lonchaea fraxina MacGowan & Rotheray, 2000

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 3 females (ZFMK-TIS-2574266, ZFMK-TIS-2575265, ZFMK-TIS-2575266), 24 April 2014; Rulik leg.; in ZFMK • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2607672), 09 May 2017; 1 male (ZFMK-TIS-2607652), 30 May 2017; ZFMK leg.; in ZFMK.

Remark. New to Germany.

Lonchaea fugax Becker, 1895

Material. GERMANY • Lower Saxony; Lüchow-Dannenberg; 53.04133°N, 11.47986°E; 19 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2602984), 20 August 2013; Schütte et al. leg.; in ZFMK • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2607642, ZFMK-TIS-2607643), 30 May 2017; 1 female (ZFMK-TIS-2607682), 13 June 2017; 1 female (ZFMK-TIS-2607656), 11 July 2017; all ZFMK leg.; all in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2606575), 18 July 2017; ZFMK et al. leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2574288), 1 female (ZFMK-TIS-2575255), 27 June 2013; 2 females (ZFMK-TIS-2574245, ZFMK-TIS-2574246), 25 July 2013; 2 females (ZFMK-TIS-2574230, ZFMK-TIS-2574236), 08 August 2013; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2578124), 02 June 2014; Rulik leg.; in ZFMK.

Lonchaea iona MacGowan, 2001

Material. GERMANY • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2607680), 08 May 2017; ZFMK leg.; in

ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55193°N, 7.16998°E; 194 m a.s.l.; Malaise trap; 1 female (GBOL-223966645), 24 April 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.46541°N, 7.2259°E; 292 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2574274, ZFMK-TIS-2574275), 24 April 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 8 females (ZFMK-TIS-2574265, ZFMK-TIS-2575259–ZFMK-TIS-2575264, ZFMK-TIS-2575267), 24 April 2014; 6 females (ZFMK-TIS-2575313–ZFMK-TIS-2575315, ZFMK-TIS-2575329, ZFMK-TIS-2578136, ZFMK-TIS-2578156), 16 May 2014; 1 male (ZFMK-TIS-2578126), 1 female (ZFMK-TIS-2578125), 02 June 2014; all Rulik leg.; all in ZFMK.

Remark. New to Germany.

Lonchaea kapperti MacGowan, 2020

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 3 males (ZFMK-TIS-2574267–ZFMK-TIS-2575269), 24 April 2014; 1 male (ZFMK-TIS-2575316), 16 May 2014; all Rulik leg.; all in ZFMK.

Lonchaea krivosheinae Kovalev, 1973

Material. GERMANY • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2607654), 11 July 2017; ZFMK leg.; in ZFMK • North Rhine-Westphalia; Wesel; 51.64820°N, 6.70026°E; 27 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2602999), 09 June 2016; Entomol. Soc. Krefeld leg.; in ZFMK.

Remark. New to Germany.

Lonchaea lateralis MacGowan, 2016

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2574289), 27 June 2013; 1 female (ZFMK-TIS-2578155), 15 July 2014; 1 female (ZFMK-TIS-2574237), 08 August 2013; 1 female (ZFMK-TIS-2578100), 16 September 2013; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Winningen; 50.32°N, 7.493°E; 243 m a.s.l.; Malaise trap; 1 male (GBOL-224008221), 20 September 2012; Rulik leg.; in ZFMK.

Remark. New to Germany.

Lonchaea nitens (Bigot, 1885)

Material. GERMANY • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2606578), 06 June 2017; ZFMK et al. leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.;

Malaise trap; 3 females (ZFMK-TIS-2574268, ZFMK-TIS-2574269, ZFMK-TIS-2575274); 1 female (ZFMK-TIS-2575317), 24 April 2014; all Rulik leg.; all in ZFMK.

Lonchaea palposa Zetterstedt, 1847

Material. GERMANY • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2607635), 13 June 2017; ZFMK leg.; in ZFMK • North Rhine-Westphalia; Wesel; 51.64820°N, 6.70026°E; 27 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2602995), 19 May 2016; 1 female (ZFMK-TIS-2603001), 09 June 2016; both Entomol. Soc. Krefeld leg.; both in ZFMK.

Lonchaea patens Collin, 1953

Material. GERMANY • Saxony-Anhalt; Harzgerode; 51.66887°N, 11.16425°E; 281 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2603064), 28 July 2017; Geiger et al. leg.; in ZFMK.

Lonchaea peregrina Becker, 1895

Material. GERMANY • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2607653), 11 July 2017; ZFMK leg.; in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2606576), 18 July 2017; ZFMK et al. leg.; in ZFMK • Saxony-Anhalt; Harzgerode; 51.66887°N, 11.16425°E; 281 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2603063), 28 July 2017; Geiger et al. leg.; in ZFMK.

Lonchaea postica Collin, 1953

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2575328), 16 May 2013; 1 female (ZFMK-TIS-2574286), 27 June 2013; both Rulik leg.; both in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55193°N, 7.16998°E; 194 m a.s.l.; Malaise trap; 1 female (GBOL-223960563), 08 August 2013; Rulik leg.; in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2627287), 30 May 2017; 1 female (ZFMK-TIS-2606580), 06 June 2017; both ZFMK et al. leg.; both in ZFMK.

Lonchaea scutellaris Rondani, 1875

Material. GERMANY • Hesse; Sinntal; 50.27645°N, 9.61269°E; 5 m a.s.l.; net sweeping; 1 female (ZFMK-TIS-2562240), 01 August 2015; Kappert leg.; in ZFMK

• Lower Saxony; Lüchow-Dannenberg; 53.04133°N, 11.47986°E; 19 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2602981), 1 male (ZFMK-TIS-2602980), 20 August 2013; both Schütte et al. leg.; both in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2627299), 25 July 2017; ZFMK et al. leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55193°N, 7.16998°E; 194 m a.s.l.; Malaise trap; 2 females (GBOL-223959525, GBOL-223972393), 26 August 2013; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.46541°N, 7.2259°E; 292 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2574219), 25 July 2013; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2575257), 27 June 2013; 1 female (ZFMK-TIS-2574247), 11 July 2013; 1 female (ZFMK-TIS-2575277), 25 July 2013; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Dieblich; 50.31387°N, 7.49413°E; 230 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2578101), 29 July 2014; Rulik leg.; in ZFMK • Saxony-Anhalt; Harzgerode; 51.66887°N, 11.16425°E; 281 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2603067), 28 July 2017; Geiger et al. leg.; in ZFMK.

Lonchaea sylvatica Beling, 1873

Material. GERMANY • Lower Saxony; Lüchow-Dannenberg; 53.04133°N, 11.47986°E; 19 m a.s.l.; Malaise trap; 3 females (ZFMK-TIS-2602986–ZFMK-TIS-2602988), 20 August 2013; Schütte et al. leg.; in ZFMK • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2607647), 30 May 2017; ZFMK leg.; in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2627283, ZFMK-TIS-2627284), 30 May 2017; ZFMK et al. leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.46541°N, 7.2259°E; 292 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2574260), 11 July 2013; Rulik leg.; in ZFMK • Saxony-Anhalt; Harzgerode; 51.66887°N, 11.16425°E; 281 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2603068), 28 July 2017; Geiger et al. leg.; in ZFMK.

Lonchaea tarsata Fallén, 1820

Material. GERMANY • Hesse; Lorch; 50.04912°N, 7.79777°E; 246 m a.s.l.; Malaise trap; 6 females (ZFMK-TIS-2578114–ZFMK-TIS-2578119), 17 June 2015; Niehuis leg.; in ZFMK.

Lonchaea ultima Collin, 1953

Material. GERMANY • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2607644), 30 May 2017; ZFMK leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N,

7.17226°E; 271 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2574271), 24 April 2014; Rulik leg.; in ZFMK.

Unclarified species and unnamed clusters in *Lonchaea*

Lonchaea cf. *spicata* MacGowan, 2008

Material. GERMANY • Hesse; Sinntal; 50.29290°N, 9.55060°E; 374 m a.s.l.; net sweeping; 1 male (ZFMK-TIS-2616851), 27 April 2018; Kappert leg.; in ZFMK.

Remarks. This specimen could not be assigned to *L. spicata* with confidence and holotype comparison was not possible due to unavailability. Therefore, we could only assign the specimen to *L. cf. spicata*.

L. limatula Collin, 1953 - group

Note. There are four barcode clusters (LG1–LG4) with specimens that show morphological similarities to *L. limatula*.

Cluster LG1

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2574229), 08 August 2013; Rulik leg.; in ZFMK.

Remarks. There was no respective male in our sampling. Therefore, this female could not be assigned to species level by barcoding.

Cluster LG2

Material. GERMANY • Saxony-Anhalt; Harzgerode; 51.66887°N, 11.16425°E; 281 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2603065), 28 July 2017; Geiger et al. leg.; in ZFMK.

Remarks. There was no respective male in our sampling. Therefore, this female could not be assigned to species level by barcoding.

Cluster LG3

Material. GERMANY • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2578146), 15 July 2014; Rulik leg.; in ZFMK.

Remarks. There was no respective male in our sampling. Therefore, this female could not be assigned to species level by barcoding.

Cluster LG4

Material. GERMANY • Mecklenburg-Western Pomerania; Putbus; 54.32506°N, 13.53878°E; 27 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2578150), 05 June 2016;

Rulik leg.; in ZFMK • Lower Saxony; Lüchow-Danzenberg; 53.04133°N, 11.47986°E; 19 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2602985), 20 August 2013; Schütte et al. leg.; in ZFMK • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2607655), 11 July 2017; ZFMK leg.; in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2606577), 18 July 2017; ZFMK et al. leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2574239), 08 August 2013; Rulik leg.; in ZFMK.

Remarks. There was no respective male in our sampling. Therefore, these females could not be assigned to species level by barcoding. Comparison to data on BOLD indicates, that this is most probably *Lonchaea contigua* Collin, 1953.

L. mallochii MacGowan & Rotheray, 2000 - group

Note. There are three barcode clusters (MG1–MG3) with specimens that show morphological similarities to *L. mallochii*.

Cluster MG1

Material. GERMANY • North Rhine-Westphalia; Wesel; 51.64820°N, 6.70026°E; 27 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2602993), 19 May 2016; Entomol. Soc. Krefeld leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55193°N, 7.16998°E; 194 m a.s.l.; Malaise trap; 1 female (GBOL-223971591), 15 July 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.4647°N, 7.22215°E; 321 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2574233), 29 July 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2574262), 29 July 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.46541°N, 7.2259°E; 292 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2574259, ZFMK-TIS-2575334), 11 July 2013; Rulik leg.; in ZFMK • Saxony-Anhalt; Harzgerode; 51.66887°N, 11.16425°E; 281 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2603066), 28 July 2017; Geiger et al. leg.; in ZFMK.

Remarks. There was no respective male in our sampling. Therefore, these females could not be assigned to species level by barcoding.

Cluster MG2

Material. GERMANY • Hesse; Lorch; 50.04912°N, 7.79777°E; 246 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2578113), 17 June 2015; Niehuis leg.; in ZFMK.

Remarks. There was no respective male in our sampling. Therefore, this female could not be assigned to species level by barcoding.

Cluster MG3

Material. GERMANY • North Rhine-Westphalia; Wesel; 51.64820°N, 6.70026°E; 27 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2602994, ZFMK-TIS-2603000), 19 May 2016; Entomol. Soc. Krefeld leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2575254), 27 June 2013; Rulik leg.; in ZFMK.

Remarks. There was no respective male in our sampling. Therefore, these females could not be assigned to species level by barcoding.

L. peregrina Becker, 1895 - group

Note. There is one barcode from a specimen that shows morphological similarities to *L. peregrina*.

Material. GERMANY • Lower Saxony; Lüchow-Danzenberg; 53.04133°N, 11.47986°E; 19 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2602983), 20 August 2013; Schütte et al. leg.; in ZFMK.

Remarks. There was no respective male in our sampling. Therefore, this female could not be assigned to species level by barcoding.

Silba Macquart, 1851

Silba fumosa (Egger, 1862)

Material. GERMANY • Baden-Württemberg; Oberbergen; 48.09677°N, 7.67622°E; 387 m a.s.l.; pitfall trap; 1 female (ZFMK-TIS-2593340), October 2011; Gack leg.; in ZFMK • Bavaria; Euerbach; 50.05242°N, 10.11571°E; 243 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2549003, ZFMK-TIS-2549005), 30 August 2011; Wagner leg.; in ZFMK • Bavaria; Landau; 48.60401°N, 12.73976°E; 421 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2548999, ZFMK-TIS-2549001), 20 June 2011; Wagner leg.; in ZFMK • Lower Saxony; Schwanewede; 53.23498°N, 8.59133°E; 7 m a.s.l.; yellow pan trap; 2 females (ZFMK-TIS-2549011, ZFMK-TIS-2549015), 27 June 2001; Rudzinski leg.; in ZFMK • North Rhine-Westphalia; Hennef; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2607681), 08 May 2017; 1 female (ZFMK-TIS-2607648), 30 May 2017; both ZFMK leg.; both in ZFMK • North Rhine-Westphalia; Schladern near Windeck; 50.8°N, 7.585°E; 124 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2627280, ZFMK-TIS-2627281), 30 May 2017; 1 female (ZFMK-TIS-2606586); all ZFMK et al. leg.; all in ZFMK • North Rhine-Westphalia; Wesel; 51.64820°N, 6.70026°E; 27 m a.s.l.; Malaise trap; 1 male (ZFMK-TIS-2602996), 19 May 2016; 1 female (ZFMK-TIS-2602998), 09 June 2016; both Entomol. Soc. Krefeld leg.; both in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55193°N, 7.16998°E; 194 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-1800146), 25 July 2013; 1 male (ZFMK-TIS-1800756), 08 August 2013;

1 male (GBOL-223959994), 26 August 2013; 1 female (ZFMK-TIS-1801372), 11 February 2014; 1 female (GBOL-223967436), 01 July 2014; 1 male (GBOL-223970864), 15 July 2014; 1 male (GBOL-223971662), 29 July 2014; 1 female (ZFMK-TIS-1803982), 29 July 2014; 1 female (GBOL-223971994), 29 July 2014; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.55175°N, 7.17226°E; 271 m a.s.l.; Malaise trap; 1 female (ZFMK-TIS-2574287), 27 June 2013; 1 male (ZFMK-TIS-2578093), 2 females (ZFMK-TIS-2574228, ZFMK-TIS-2578094), 08 August 2013; 1 female (ZFMK-TIS-2578095), 16 September 2013; 1 female (ZFMK-TIS-2578133), 16 May 2014; 2 females (ZFMK-TIS-2578122, ZFMK-TIS-2578123), 02 June 2014; 2 females (ZFMK-TIS-2578143, ZFMK-TIS-2578154), 15 July 2014; all Rulik leg.; all in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.4647°N, 7.22215°E; 321 m a.s.l.; Malaise trap; 2 females (ZFMK-TIS-2574232, ZFMK-TIS-2575290), 29 July 2014; Rulik leg.; in ZFMK • Rhineland-Palatinate; Ahrweiler; 50.46541°N, 7.2259°E; 292 m a.s.l.; Malaise trap; 3 females (ZFMK-TIS-2575331–ZFMK-TIS-2575333), 16 September 2013; Rulik leg.; in ZFMK • Rhineland-Palatinate; Winingen; 50.32°N, 7.493°E; 243 m a.s.l.; Malaise trap; 1 male (GBOL-224008644), 25 July 2013; Rulik leg.; in ZFMK.

Genetic analysis

Our sampling includes 331 specimens of which 68 are males and 263 are females. Barcoding resulted in COI-sequences

of 298 specimens, which allowed us to identify most of the female specimens which could not be identified by morphology. All specimens and the according BOLD and GenBank accession numbers for successfully sequenced material are listed in Suppl. material 2.

Average COI sequence length for the 298 sequences was 653 bp of 658 bp full barcode length, including twenty-four shorter sequences composed from 561 to 648 residues, respectively.

Among nucleotides, there was a compositional bias towards AT: 66.6% especially at third codon positions (average 90.1%) which is close to levels previously reported for other Diptera groups (e.g., Bernasconi et al. 2000; Cywinska et al. 2010; Rivera and Currie 2009). In detail, overall base composition was: A 28.5, C 16.7, G 16.7, T 38.1%.

Altogether, 37,401 pairwise distances were computed for our dataset: of these, 6,560 were intraspecific distances.

Maximum intraspecific distances averaged 0.52% (range 0–2.13%) while the nearest neighbor distance averaged 6.93% (range 2.28–13.07% – 14.3%), roughly 13-fold higher than the maximum intraspecific distance (Fig. 4) indicating an existing barcode gap for our dataset.

Our combined analysis resulted in a list of 29 species from which one, *Priscoearomyia bausenbergensis* is new to science. Furthermore, it includes eight well separated female only molecular clusters (see Suppl. material 1). Four species are new to the list of German Lonchaeidae. Included in the list are also those clusters of female specimens which could not be identified to species level. If possible, a closer relation to known species is given. One specimen could only be assigned with cf. due to taxonomic issues.

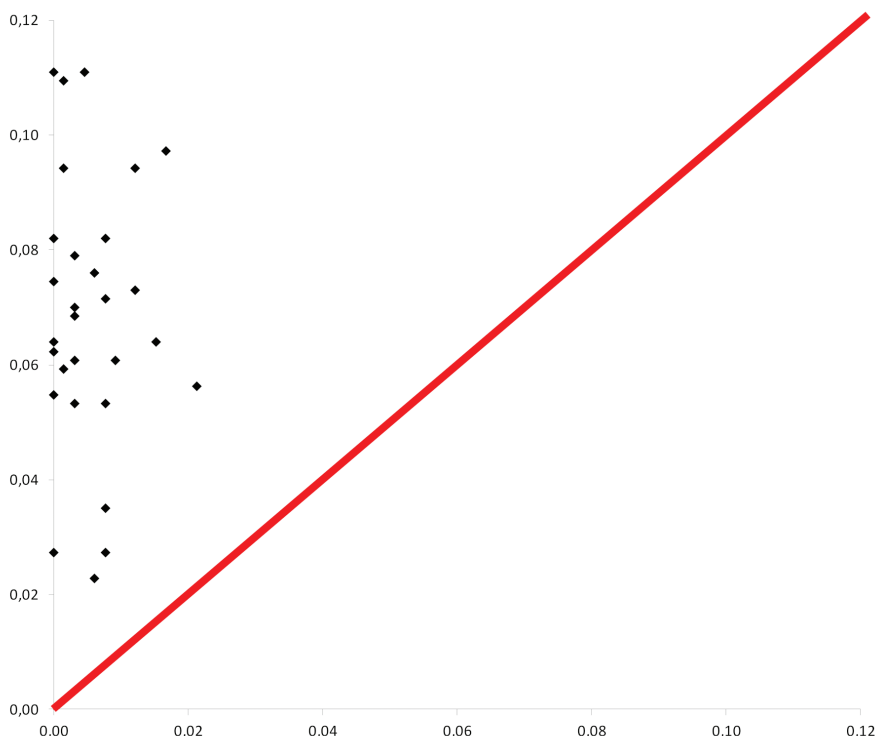


Figure 4. Barcode gap analysis. Relationship between maximum intraspecific and nearest neighbor p-distances. Points above the diagonal line indicate species with a barcode gap. Molecular species delimitation by ASAP and BINs suggests 38 putative species, which fits well with our morphological findings (Fig. 5 and Suppl. material 1).

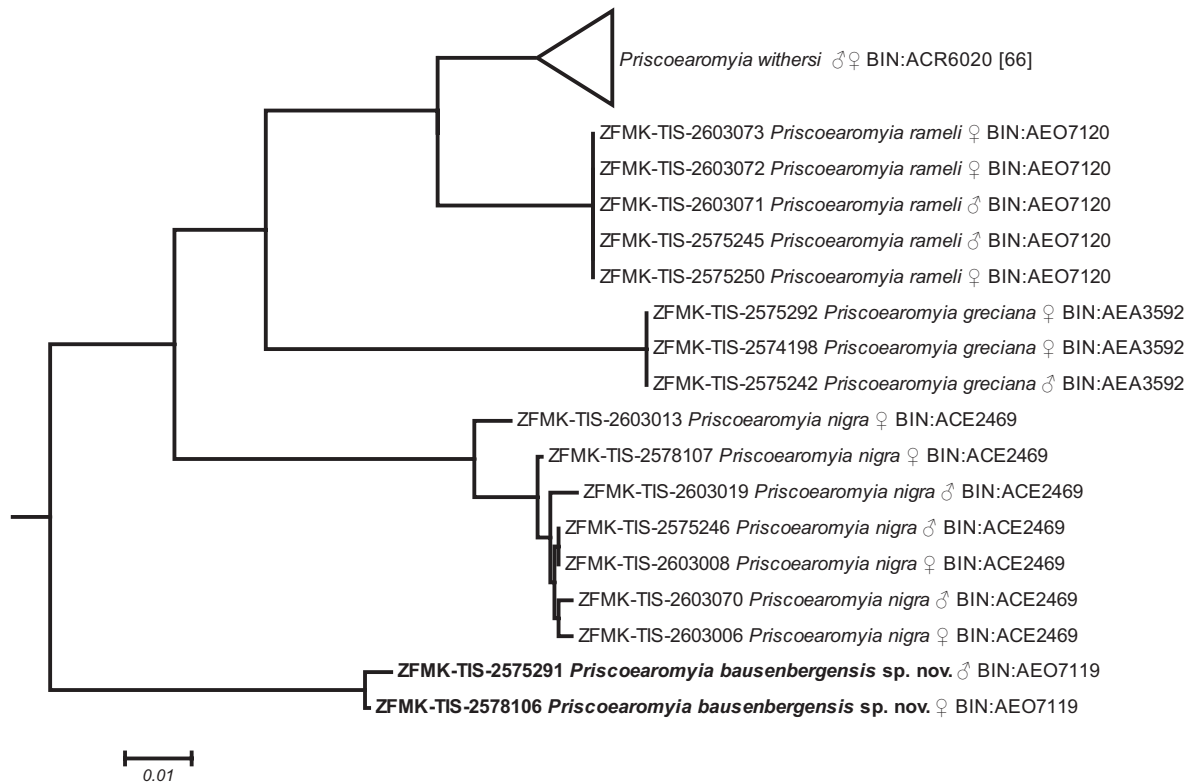


Figure 5. Neighbor joining tree of *Priscoearomyia* species. Data taken from the German Barcode of Life project (GBOL) and identification verified by morphology; cluster of *P. withersi* collapsed, with the number of sequences indicated in brackets thereafter.

Discussion

In our sampling there were many more females than males. For most genera in the Lonchaeidae aerial swarming behavior of the males is known (McAlpine and Munroe 1968). This might be a more general habit in this taxon as already mentioned in MacGowan and Rothery (2008) and would partly explain why fewer males are obtained by Malaise trap sampling at ground level. The females are actively searching for substrates suitable for egg laying and may therefore be more often encountered by this method. As already mentioned, it was possible to identify most of the female specimens by barcode comparison or group females based on clustering. This is especially important in those species groups where the females are extremely similar and a morphological identification is impossible at the moment, but as far as barcodes of the corresponding males become available this may change immediately. Otherwise, we would not have been able to identify all the female specimens of the genus *Priscoearomyia* and the female specimens of e.g. the *fraxina* group of species within the genus *Lonchaea* (*L. fraxina* and *L. iona* in our sampling). Additionally, the assignment of the female paratype of *P. bausenbergensis* with certainty was only possible based on the barcoding results. The finding of a new species in our sampling is not that surprising. As already mentioned by MacGowan and Reimann (2021), there were several new species descriptions in the genus *Priscoearomyia* in the last decades. At the moment the species can only be separated by means of the male genitalia. This indicates that there may be many

more distinct species in collections, which are not yet recognized. Another reason, of course, is the lack of taxonomic expertise in Lonchaeidae and in a broader sense in many other acalypterate dipteran taxa. Here barcoding of trap samples or other material can aid in finding new species. If there are clusters of specimens which cannot be assigned to known species, these clusters are worthy of deeper morphological investigation. But, at first, there is the need of barcode references for as many species as possible. Our study provides a sound reference basis for future molecular identification of lonchaeid specimens. This can significantly influence the data accessible for biogeographic studies, if all data of female records can be included.

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Supplementary material 1

Results of species delimitation analysis

Authors: André Reimann, Björn Rulik

Data type: pdf

Explanation note: fig. S1. Results of species delimitation analysis (ASAP, BINs and morphology) plotted on the COI genetic distance tree.

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Supplementary material 2

Metadata of all specimens used in this study including locality details and BOLD and GenBank references

Authors: André Reimann, Björn Rulik

Data type: xlsx

Explanation note: The table includes all metadata of the specimens used in the study. The locality data are given in detail with all information including locality description, date, method and collector. For those specimens with barcode the BOLD-ID and GenBank accession numbers are given..

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