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Macrocera rohaceki sp. nov. and other interesting records of Keroplatidae (Diptera) from southern and central Europe, with DNA sequence data

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Abstract. A new species of Keroplatidae (Diptera: Sciaroidea), *Macrocera rohaceki* sp. nov., is described from the island of Mallorca (Spain). A further remarkable species, *Paleoplatyura johnsoni* Johannsen, 1910, hitherto only known from the Nearctic Region, is recorded from Sicily (Italy). New records of *Isoneuromyia czernyi* (Strobl, 1909) and *I. pseudochracea* (Landrock, 1925) from Slovakia are provided, including figures of the male terminalia. Their relationships to the widely distributed European species, *I. semirufa* (Meigen, 1818), are reconstructed using three mitochondrial DNA markers (12S, COI, cytb). A key to the European species of *Isoneuromyia* Brunetti, 1912 is also provided.

Key words. Diptera, Sciaroidea, Keroplatidae, Macrocerinae, Orfeliini, fungus gnats, faunistics, taxonomy, DNA barcoding, Palaearctic Region

Introduction

The fungus gnat family Keroplatidae (Diptera: Sciaroidea) currently comprises four subfamilies (Arachnocampinae, Keroplatinae, Macrocerinae and Sciarokeroplatinae), with nearly 1000 described species in 95 genera worldwide (Matile 1990, Papp & Ševčík 2005, Evenhuis 2006, Ševčík et al. 2015, Kurina et al. 2017). Recent molecular and morphological studies indicate a close relationship to the primarily tropical family Lygistorrhinidae (Ševčík et al. 2016a, Kallweit 2014).

Within Macrocerinae, *Macrocera* Meigen, 1803 is the most diverse genus, with more than 200 described species worldwide and about 65 species known from the Palaearctic Region (Evenhuis 2006). The common feature of most *Macrocera* species is their elongated antennae, especially in males, from which the scientific name of the genus is derived; *makros* = big, *keras* = horn, antenna (Greek).

Although many *Macrocera* species can be easily identified according to a characteristic pattern of wing markings, there are some groups with unmarked wings, which are rather uniform in appearance, including the relatively simple male terminalia offering only a limited number of characters. An interesting exception is a group of Mediterranean species with more complex male terminalia in comparison with other members of the genus. At present, there are 9 species described in this group: *Macrocera gemagea* Bechev, 1991 from Bulgaria, *M. sinaitica* Chandler, 1994 from Egypt, *M. aquabellissima* Chandler, 1994, *M. hermonophila* Chandler, 1994 and *M. levantina* Chandler, 1994 from Israel, *M. buskettina* Chandler & Gatt, 2000 from Malta, *M. nuragica* Chandler, 2009 from Sardinia (Italy), and *M. jonica* Martinovský, 2001 and *M. critica* Chandler, Bechev & Caspers, 2006 from the Greek islands Corfu and Crete, respectively (Bechev 1991, Chandler 1994, Martinovský 2001, Chandler & Gatt 2000, Chandler et al. 2006).

The opportunity is also taken here to record an interesting species of Keroplatidae, *Paleoplatyura johnsoni* Johannsen, 1910, hitherto known only from the eastern part of the Nearctic region, and discuss its distribution and possible phylogenetic relationships.

Another keroplatid genus, treated in this contribution, is *Isoneuromyia* Brunetti, 1912. It comprises more than 50 large and colourful species, distributed mostly in the Oriental and Neotropical Regions, with only three known European species – *Isoneuromyia czernyi* (Strobl, 1909), *I. pseudochracea* (Landrock, 1925), and *I. semirufa* (Meigen, 1818), see Evenhuis (2006). Although the most common European species of *Isoneuromyia* apparently is *I. semirufa*, we have recently encountered the other two species relatively more often and they are here recorded as new to Slovakia. For all these three species, the male terminalia are figured and their relationships are reconstructed for the first time by molecular methods.

Material and methods

Most of the specimens were collected using a sweeping net and stored in 70% ethanol. Male terminalia were cleared in a 10% solution of KOH. The holotype of *Macrocera rohaceki* sp. nov. was used for non-destructive DNA extraction and subsequently stored in a microvial filled with glycerol. The morphological terminology principally follows that of Matile (1990). The terminology of wing veins is showed in Fig. 1. The voucher specimens are deposited in the collections of the National Museum, Prague, Czech Republic (NMPC), Silesian Museum, Opava, Czech Republic (SMOC) and in the reference collection of the Jan Ševčík Lab, University of Ostrava, Czech Republic (JSL-UOC).

Laboratory methods used to isolate DNA from the specimens of *Macrocera*, *Palaeoplatyura* and *Isoneuromyia* principally follow those described in Ševčík et al. (2016b). Primers used and PCR conditions are summarized in Tables 1 and 2. The species used for phylogenetic analysis are listed in Table 3.

Abbreviations of morphological terms used in text and/or figures:

A_1, A_2 – anal veins
C – costal vein
Cu_{1b}, Cu_2 – cubital veins
frm – radiomedian fusion
h – humeral vein
M, M_1, M_2, M_4 – medial veins
mcu – mediocubital vein
R_1, R_4+_5, R_4, R_5 – radial veins
Rs – radial sector
Sc_1 – subcostal vein
tb – transverse basale
T1–T9 – abdominal terga

Table 1. Primers for PCR amplification and sequencing of the mitochondrial 12S, cytb and COI gene markers.

Gene region	Primer name	Direction	Primer sequences (5'→3')	Source
12S	12Sma	F	CTGGGATTAGATACCCTGTTAT	Koufopanou *et al*. 1999
	12Smb	R	CAGAGAGTGACGGGCGATTTGT	Koufopanou *et al*. 1999
	SR-J-14199	F	TACTATGTTACGACTTAT	Kambhampati & Smith 1995
	MZ-12S-R	R	GCCAGCATTTGCGGTTATAC	Žurovcová lab
COI	LCO1490	F	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. 1994
	HCO2198	R	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*. 1994
cytb	CYTB-F	F	TATGTTTTATGAGGACAAATATC	Su *et al*. 2008
	mCYTB-R	R	ATTACTCCCCCTAATTTATTAGGAAT	Ševčík *et al*. 2016b

Table 2. PCR conditions for DNA amplification.

Cycles	Temp.	Time
	94°C	3:00 min
35×	94°C	1:00 min
	50°C	1:00 min
	72°C	1:30 min
	72°C	7:00 min

Table 3. List of species included in this study with their Accession numbers.

Taxa	12S	COI	CytB	Locality and year
Orfelia nemoralis	KP288681	KT316840	MG049757	Czech Republic, 2013
Isoneuromyia sp.	MG049745	MG049750	MG049762	Peru, 2010
Isoneuromyia sp.	MG049749	MG049751	MG049758	Thailand, 2008
Isoneuromyia pseudochracea	MG049746	MG049753	MG049759	Slovakia, 2014
Isoneuromyia czernyi	MG049747	MG049752	MG049760	Slovakia, 2015
Isoneuromyia semirufa	MG049748	MG049754	MG049761	Czech Republic, 2016
Macrocera rohaceki sp.n.	---	MG049756	---	Spain, 2013
Paleoplatyura johnsoni	---	MG049755	---	Italy, 2016

Results and Discussion

Macrocera rohaceki sp. nov.

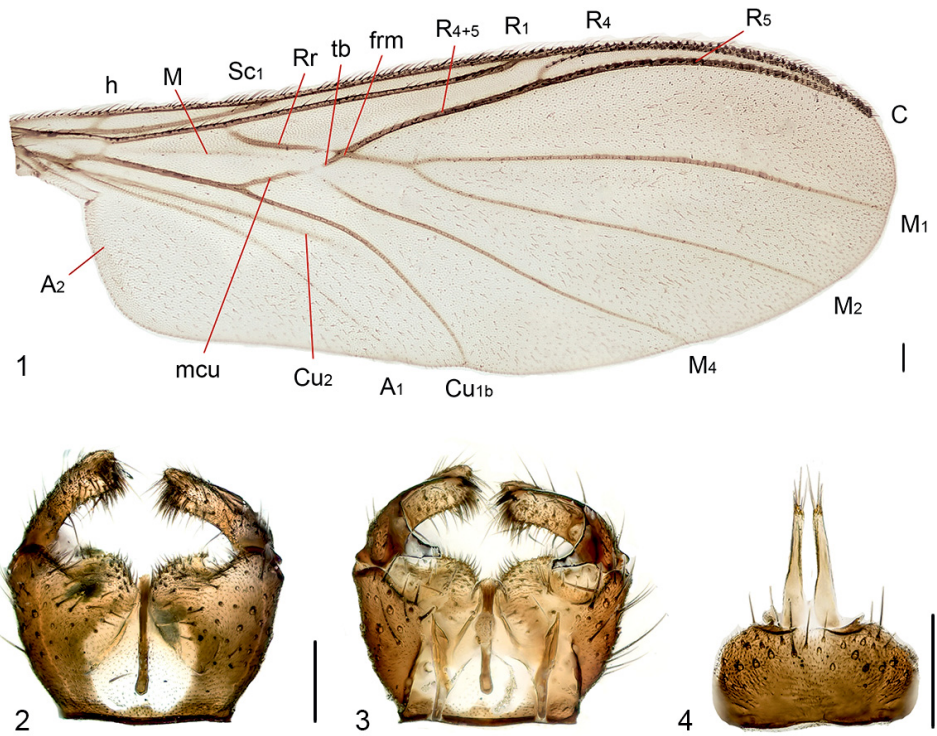
(Figs 1–4)

Type material. Holotype: ♂, **SPAIN: Mallorca:** Caimari nr. Selva 0.6 km NW (ravine), 39°46′30″N, 2°53′52″E, 210 m, 5. V. 2013, J. Roháček leg. (NMPC). Paratypes: 8 ♂♂, the same data as holotype (1 in NMPC, 2 in SMOC, 5 in JSL-UOC).

Diagnosis. Brown slender bodied species. No markings on the wing. Wing membrane covered with macrotrichia. Male terminalia with posterior margin of gonostylus forming a rounded C-shaped structure projecting dorsally.

Description. ***Male.*** Wing length 3.4 mm.

Head. Brown. Antennae brown with pedicel and scape paler to yellowish. Antenna approximately as long as the body. Flagellum incomplete in holotype. Palpi yellow. Dark



Figs 1–4. *Macrocera rohaceki* sp. nov., male: 1 – wing; 2 – terminalia, ventral view; 3 – terminalia, dorsal view, tergite 9 removed; 4 – tergite 9 and cerci, external view. Scale bars = 0.1 mm.

setae ventrally on occiput and on face. Short bristles under the mouth.

Thorax. Brownish yellow. Three dark stripes on scutum. Median stripe broadened to the fore margin and starting more apically than the lateral stripes, ending in the middle of scutum, the lateral stripes are identical, ending on the base of scutum, leaving yellow humeral area and also both sides of the scutum are yellowish as well. Pleural sclerites shining brown, bare except for couple of short setae on the upper part of anepisternum. Mediotergite bare, brown, with yellowish side margins. Laterotergite bare, brown. Anepimeron bare, yellowish. Antepronotum yellow with several setae.

Wing (Fig. 1). Transparent, without markings, narrower on basal half. Macrotrichia on the entire wing membrane. Veins brownish. Costal and radial veins covered with setae. Vein Sc_1 ending before the frm. Vein R_4 curved, ending in costa, right beyond the R_1 ending, which is not apically broadened. Costa extending 0.25 of distance from R_5 ending to M_1. Medial veins well developed, ending in wing margin. Frm very short. A_1 vein ending in wing margin. Fusion of mcu, tb and M_4 less pigmented and thus barely visible. Cu_2 straight, ending before meeting with Cu_{1b}. Halteres yellow, covered with short setae, central part darker.

Legs. Long, slender. Coxae and femora yellow. Rest of legs brownish. Covered with setulae. Setae on coxae as long as coxal width. Femoral setae a bit shorter than width of middle femoral region. Setae on tibia shorter than tibial width. Tibial spurs slightly longer than tibial width.

Abdomen. T1–T5 mainly brown with apical third yellowish. T6–T8 darkly brown. All with short dark bristling.

Terminalia. Brown. Cerci narrow and tapered. Gonostylus about three times as long as broad, bearing a subapical tooth and several strong setae (Figs 2–4). Its posterior margin forming a rounded C-shaped structure projecting dorsally. Tergite 9 subrectangular, about twice as broad as long. Cerci narrow and slightly pointed, about 1.2 times as long as tergite 9.

Female. Unknown.

DNA sequence. The DNA sequence of COI barcode region taken from the holotype is deposited in GenBank. Its Accession number is provided in Table 3.

Etymology. The new species is named after Dr. Jindřich Roháček (Slezské zemské muzeum, Opava, Czech Republic), who collected the type material, as well as the specimens of *Paleoplatyura* mentioned below.

Discussion. This species belongs to the well-defined group of Mediterranean species of *Macrocera* characterized by unmarked wings bearing macrotrichia and relatively more complex male terminalia (cf. Chandler 1994). Male terminalia of the new species are somewhat similar to those of *M. sinaitica* Chandler, 1994, especially in the shape of cerci and the structure of the apical part of gonostylus (subapical tooth). The gonostylus in *M. rohaceki* is, however, more elongated and dorsally forming a characteristic C-shaped structure (Fig. 3). Also, the shape of tergite 9 in *M. rohaceki* is different (more rectangular).

New records of Keroplatidae

Isoneuromyia czernyi (Strobl, 1909)

(Figs 5–10, 18–19)

Material examined. SLOVAKIA: Muránska planina National park, Havraní dolina Nature Reserve, 13.vii.2015, 2 ♂♂ 1 ♀, M. Tkoč, J. Roháček & J. Ševčík leg., J. Ševčík det. (JSL-UOC). Muránska planina National park, Šarkanica Nature Reserve, 9.viii.–5. ix.2017, 1 ♂, M. Tkoč, J. Roháček & J. Ševčík leg., J. Ševčík det. (JSL-UOC). Slovenský kras National park, Drieňovec National Nature Reserve, 5.ix.2008, 1 ♂, J. Ševčík leg., det. et coll. (misidentified as *Isoneuromyia semirufa*, see Ševčík & Kurina 2011).

Comments. A rare species, hitherto known only from the Mediterranean region. It was described as a single male from Spain (Strobl 1909), without providing any illustration, and subsequently a female was recorded from Greece by Chandler et al. (2006). This is the first record from Slovakia, as well as from central Europe. The figures of male terminalia, habitus and wings are published here for the first time (Figs 5–10). The DNA sequence of the barcode region (COI) is also provided (Table 3).

This species was observed visiting the inflorescence of *Chaerophyllum aromaticum* (Apiaceae, Figs 18–19), presumably feeding on nectar, indicating that *Isoneuromyia* species can potentially be considered as pollinators, although transfer of pollen from one flower to another has not been observed.

Isoneuromyia pseudochracea (Landrock, 1925)

(Figs 11–17)

Material examined. SLOVAKIA: Muránska planina National park: Kučalach, 15.vii.2014, 1 ♂; Tisovec, Furmanec brook, 12. vii. 2011, 2 ♂♂, all J. Ševčík leg. & det. (JSL-UOC).

Comments. This name was introduced by Landrock (1925) as a replacement name for the pre-occupied name *Platyura ochracea* Dziedzicki, 1925. This species is known from several sparse records in western and central Europe (Evenhuis 2006). This is the first record from Slovakia. A figure of the male terminalia (Figs 14–16), as well as DNA sequence of the barcode region (COI), are provided (Table 3).

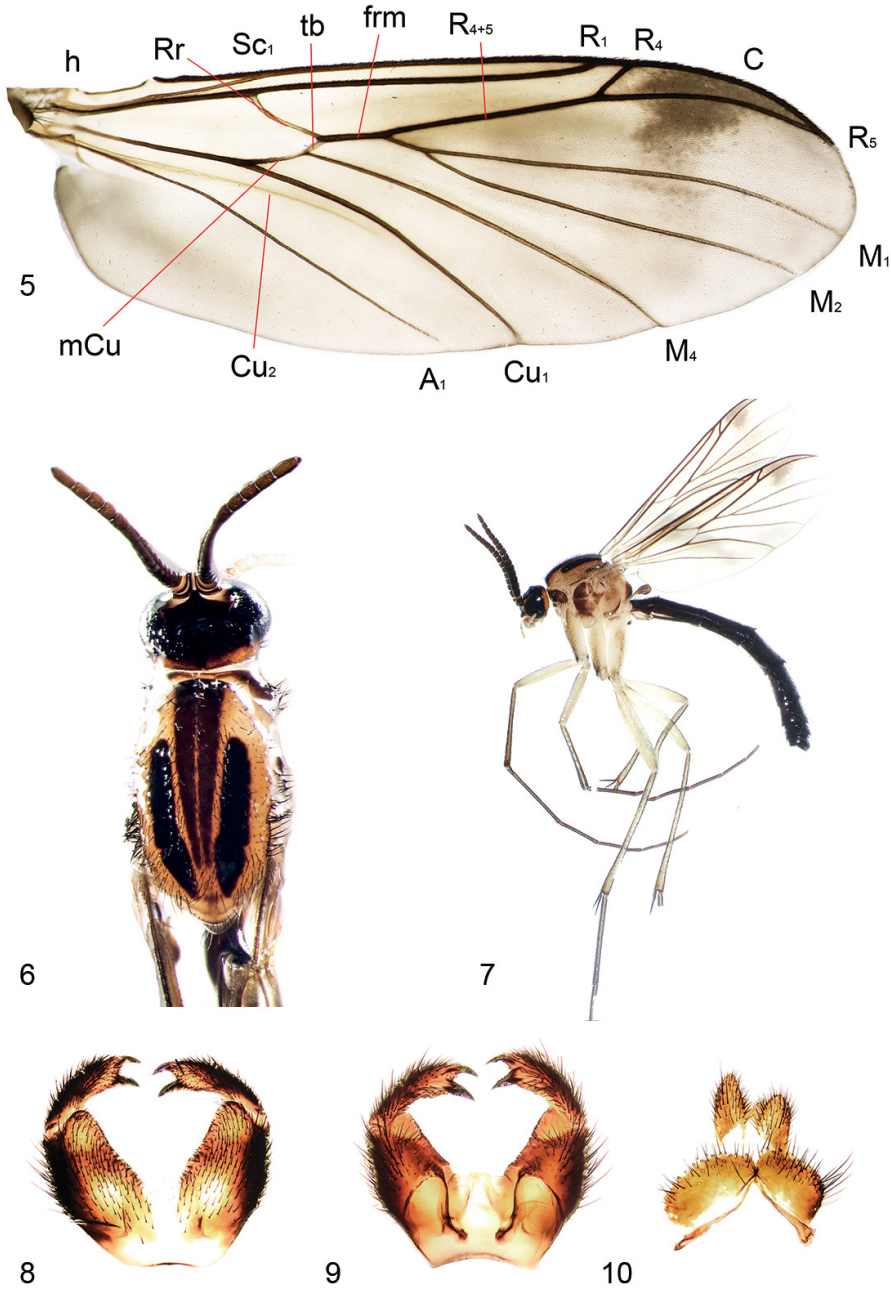
Isoneuromyia semirufa (Meigen, 1818)

(Figs 20–25)

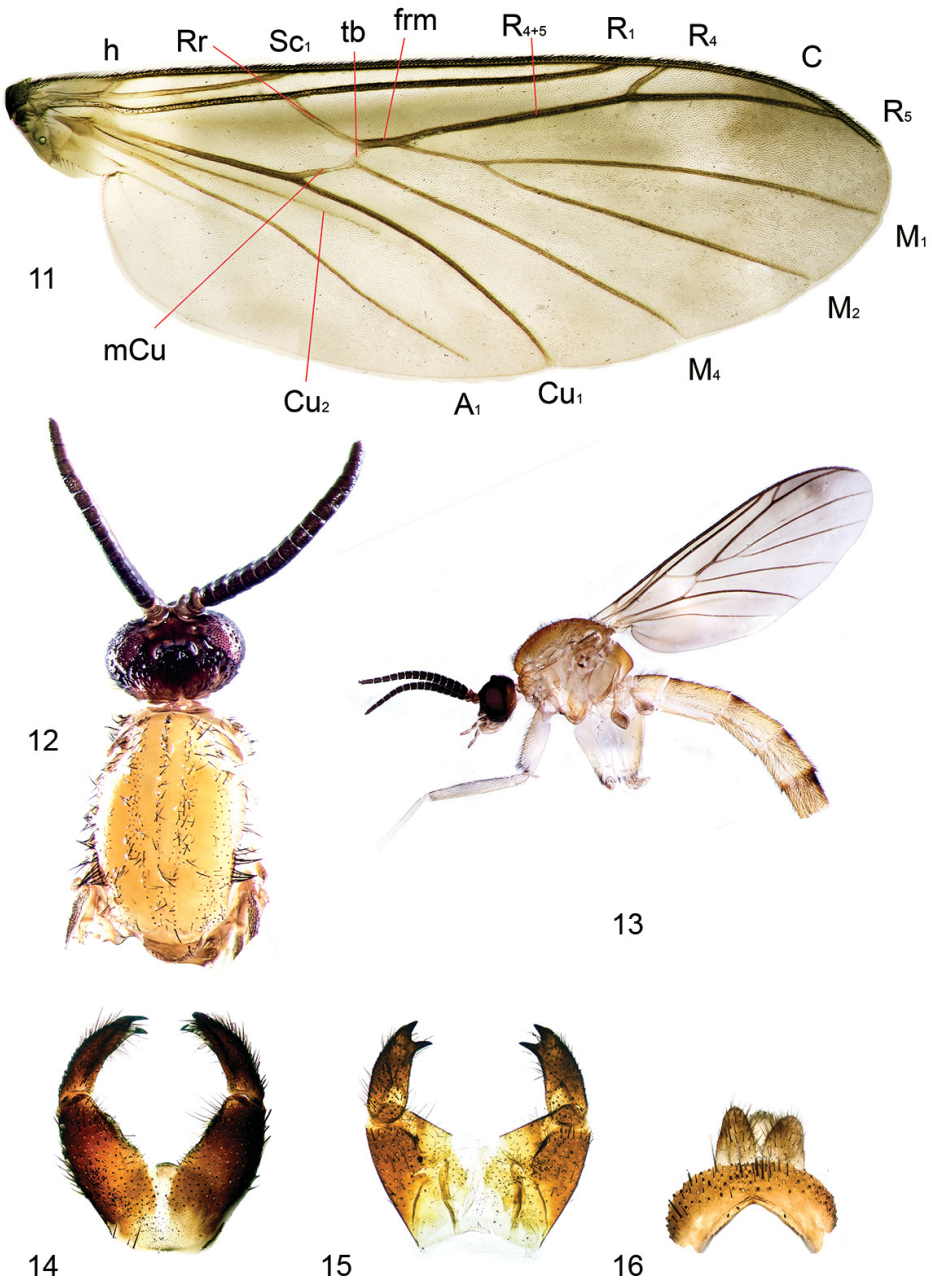
Material examined. CZECH REPUBLIC: Bohemia: Jizerské hory Mts., several localities (see Ševčík & Vonička 2008); Krkonoše Mts., Kotelní jámy, 30.vii.2008, 3 ♂♂, M. Barták leg. (Malaise trap). **Moravia:** Moravskoslezské Beskydy Mts., Černá Ostravice, 24.viii.2016, 1 ♂; Hrubý Jeseník Mts., Rejvíz, 57 ♂♂ 12 ♀♀, 2.vii.–28.ix.2005, J. Roháček & J. Ševčík leg. (Malaise traps), J. Ševčík det. (JSL-UOC).

Comments. A widely distributed European species. It may have been confused in the collections with the very similar *I. czernyi*. In the Czech and Slovak Republics, this species has mostly been found in mountains, being relatively common in Malaise trap samples from spruce forests and peat-bogs (e.g. Ševčík & Vonička 2008).

However, the question of the identity of *I. semirufa* remains still open, because Morge (1975) illustrated under this name a slightly different form, with a mostly reddish abdomen. Similarly, a form with orange thorax and three dark lateral stripes on scutum, corresponding to *I. czernyi*, was illustrated under the name *I. signata* (Meigen, 1818), which poses a question over their possible synonymy and validity of the latter name. These questions could be definitely solved after examination of the relevant types in the Meigen's collection and after their association with recent material, suitable for DNA extraction.

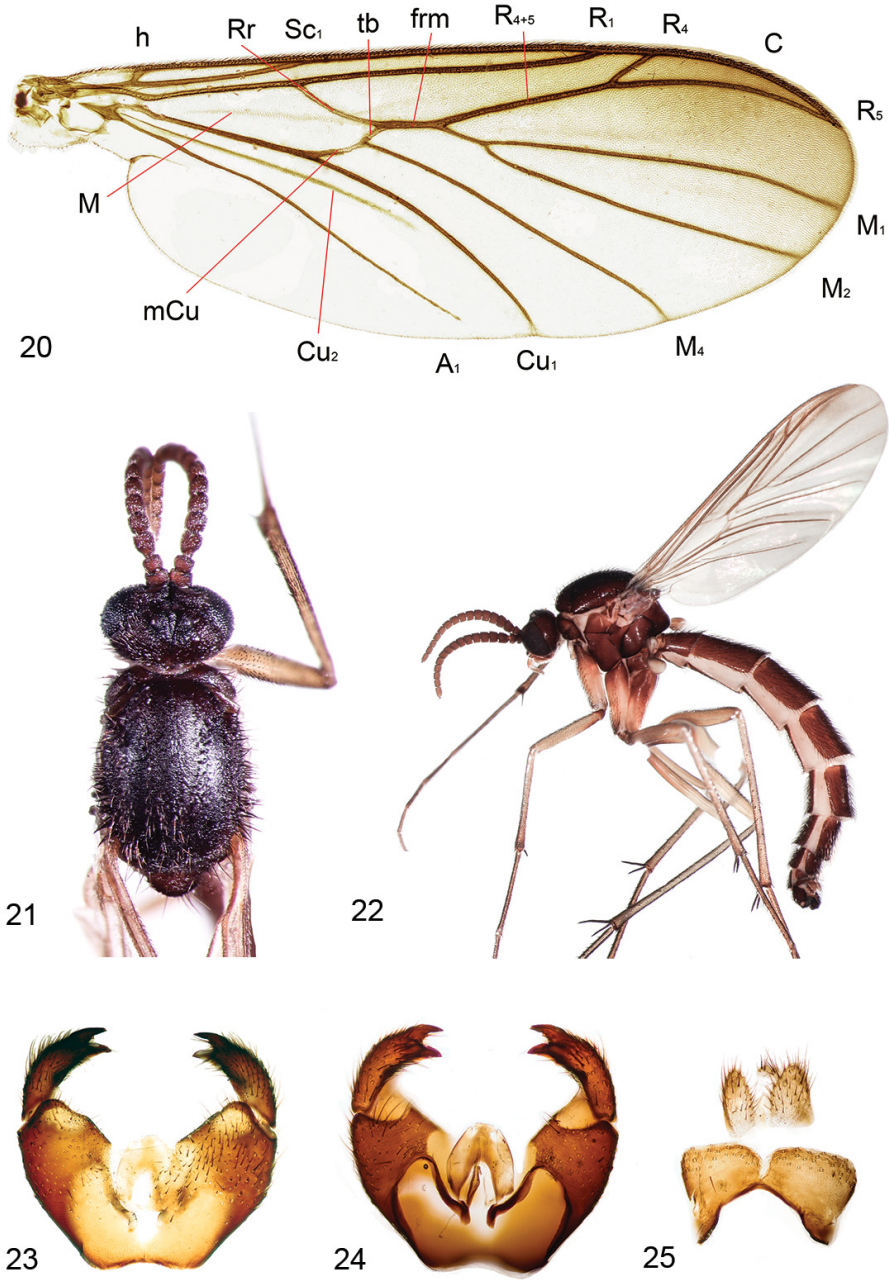


Figs 5–10. *Isoneuromyia czernyi* (Strobl, 1909), male: 5 – wing; 6 – thorax, dorsal view; 7 – habitus; 8 – terminalia, ventral view; 9 – terminalia, dorsal view, tergite 9 removed; 10 – tergite 9 and cerci, external view.



Figs 11–16. *Isoneuromyia pseudochracea* (Landrock, 1925), male: 11 – wing; 12 – thorax dorsally; 13 – habitus; 14 – terminalia, ventral view; 15 – terminalia, dorsal view, tergite 9 removed; 16 – tergite 9 and cerci, external view.

Figs 17–19. 17 – habitus of *Isoneuromyia pseudochracea* (Landrock, 1925); 18 – male of *Isoneuromyia czernyi* (Strobl, 1909) visiting flower of *Chaerophyllum aromaticum* (Apiaceae); 19 – female of *Isoneuromyia czernyi* (Strobl, 1909). (Photos by Jan Ševčík and Michal Tkoč).



Figs 20–25. *Isoneuromyia semirufa* (Meigen, 1818), male: 20 – wing; 21 – thorax dorsally; 22 – habitus; 23 – terminalia, ventral view; 24 – terminalia, dorsal view, tergite 9 removed; 25 – tergite 9 and cerci, external view.

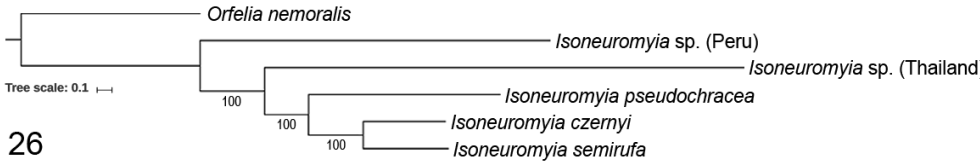


Fig. 26. Phylogenetic tree based on 3 mitochondrial gene markers (12S, COI, cytb) outlining relationships among all three European species of *Isoneuromyia* Brunetti, 1912.

Phylogenetic relationships of the European *Isoneuromyia*

Phylogenetic relationships among the European species of *Isoneuromyia* were reconstructed, based on the combined dataset of 12S, COI and cytb gene sequences (Fig. 26). The most common species, *I. semirufa*, was found to be closely related to *I. czernyi*, together forming a sister group to *I. pseudochracea*. These relationships are well in agreement with morphological characters.

Key to the European species of *Isoneuromyia*

1 Thorax all orange yellow (Figs. 12, 13), stem of veins M_1+M_2 longer than radio-medial fusion (frm), costa well produced beyond the tip of R5. (Fig. 11). .... ***I. pseudochracea*** (Landrock, 1925)

– Thorax mostly dark brown, stem of M_1+M_2 shorter than frm, C ending at the tip of R_5 or slightly produced. 2

2 Scutum brownish yellow with 3 longitudinal dark stripes (Fig. 6). Wing with distinct subapical dark band. Costa slightly produced (Fig. 5). .... ***I. czernyi*** (Strobl, 1909)

– Thorax all dark brown (Figs 21, 22). Wings clear or slightly clouded, without distinct dark markings. Costa ending at R_5 (Fig. 20). .... ***I. semirufa*** (Meigen, 1818)

Paleoplatyura johnsoni Johannsen, 1910

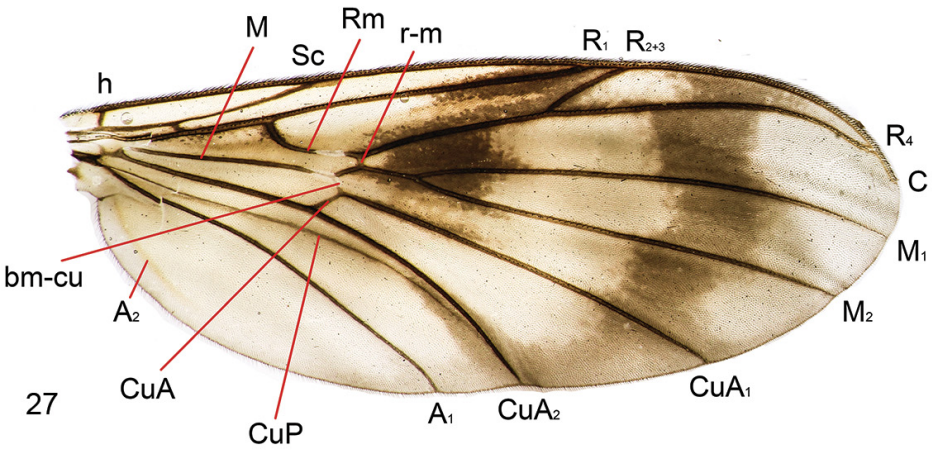
(Figs 27–31)

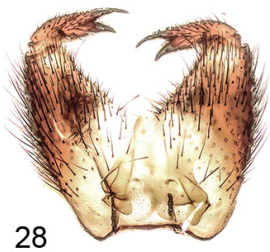
Material examined. ITALY: Sicily: N Sicily, Raccuia, 0.9 km W, Fiumara di Sinagra, 450 m, sweeping vegetation along small creek, 19.iv.2016, 2 ♂♂, 1 ♀, J. Roháček leg., J. Ševčík det. (JSL-UOC).

Comments. A rare species, only known from several localities in the eastern part of Nearctic Region (Shaw 1952). This is the first record of this remarkable species from the Palaearctic Region. DNA sequence of the barcode region of COI is also provided (Table 3).

This species possibly represents one of the most primitive and ancient forms of Keroplatidae and can be considered (together with its two extant congeners) as a "living fossil". Three additional species of *Palaeoplatyura* were described from the Eocene and Oligocene fossils (Evenhuis 2006). The exact phylogenetic position of this genus is unknown but preliminary data based on the multigene molecular analysis (Mantič et al., in prep.) suggest that the clade containing *Platyura* and *Paleoplatyura* branched rather basally, forming a sister group to all the other taxa of Keroplatidae, except Arachnocampinae.

This finding also represents a very rare occasion, when a species of Keroplatidae is found in both the Nearctic and Palaearctic Regions. The only known example in the extant keroplatid





Figs 27–31. *Palaeoplatyura johnsoni* Johannsen, 1910, male: 27 – wing; 28 – terminalia, ventral view; 29 – terminalia, dorsal view, tergite 9 removed; 30 – tergite 9 and cerci, external view; 31 – habitus.

fauna is *Orfelia discoloria* (Meigen, 1818), but this may change as soon as a detailed revision of that species group is made. There is also a possibility of a recent introduction by human activities as was demonstrated for some other species of Keroplatidae (Chandler & Pijnakker 2009). Although the type material of *P. johnsoni* has not been studied, the identity of the species was established according to the detailed redescription given by Shaw (1952).

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