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## Research article

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# Five new Palaearctic species of *Docosia* (Diptera: Mycetophilidae), with updated molecular phylogeny of the genus

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**Abstract.** A new phylogenetic hypothesis is proposed for the relationships among the species within the genus *Docosia* Winnertz, 1863, based on a combined analysis of five DNA markers (28S, ITS2, COI, COII and CytB). Five new species are described, *Docosia anatolica* Ševčík sp. nov. from Turkey, *D. japonica* Kurina sp. nov. from Japan, *D. peloponnensis* Ševčík sp. nov. from Greece, *D. svanetica* Kurina sp. nov. from Caucasus and *D. polyspina* Kurina sp. nov. from the Russian Far East. New country records of the following species are presented: *D. diutina* Plassmann, 1996 (Turkey), *D. flavicoxa* Strobl, 1900 (Georgia), *D. gilvipes* (Haliday in Walker, 1856) (Georgia), *D. kerkini* Kurina & Ševčík, 2011 (Bulgaria), *D. moravica* Landrock, 1916 (Georgia), *D. pannonica* Lastovka & Ševčík 2006 (Georgia) and *D. rameli* Kurina & Ševčík, 2011 (Slovakia).

**Keywords.** Bibionomorpha, Sciaroidea, fungus gnats, taxonomy, DNA sequences.

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## Introduction

Fungus gnats of the genus *Docosia* Winnertz, 1863 represent a rather well-defined group within the family Mycetophilidae Newman, 1834 (Diptera Linnaeus, 1758), with a mostly dark body, unmarked wings (Fig. 1) and identification characters present mainly on the male terminalia (Laštovka & Ševčík

2006). It has been demonstrated that characters used to separate species in the keys, other than on male terminalia, do not correspond to the relationships revealed by molecular methods (Ševčík *et al.* 2016a). According to recent molecular studies, the genus *Docosia* belongs to the subfamily Gnoristinae Edwards, 1925, although this subfamily appears as paraphyletic, with respect to Mycetophilinae (Rindal *et al.* 2009; Ševčík *et al.* 2013; Kaspřák *et al.* 2019).

The biology of species of *Docosia* is mostly unknown (Chandler 2010; Ševčík 2010a; Jakovlev 2012), except for *Docosia fumosa* Edwards, 1925 and *Docosia gilvipes* (Haliday in Walker, 1856), which are known to develop in nests of birds (Rulik & Kallweit 2006) and fungi, respectively.

The known distribution of *Docosia* includes the Holarctic Region, with three species reported from the Neotropical Region (Oliveira & Amorim 2011, 2014) and a single species from the Oriental Region (Ševčík 2010b). The common European species *Docosia gilvipes* was also recorded from the Republic of South Africa (Kurina & Ševčík 2012). Altogether, 77 extant species have been described so far from the Holarctic Region including 57 from the Palaearctic and 20 from the Nearctic Regions (reviewed by Zaitzev 2011; Kurina & Ševčík 2012; Taber 2012; 2018; Ševčík *et al.* 2016a). Within the Palaearctic Region, besides the relatively well-studied Europe with 34 known species (Kurina & Ševčík 2012; Ševčík *et al.* 2016a), Central Asia has 11 species recorded (Kurina 2006; Zaitzev 2011; Kurina & Ševčík 2012), while the other areas are rather sporadically sampled and studied.



**Fig. 1.** *Docosia svanetica* Kurina sp. nov., paratype (ZFMK-DIP-00067331), habitus.

The phylogenetic relationships among particular species of *Docosia* are still poorly known, as well as the species spectrum in less-studied areas. The first attempt to reconstruct the phylogeny of several Central European species of *Docosia* was that by Ševčík *et al.* (2016a), based on three mitochondrial gene fragments and the nuclear ITS2 region. Here, we present a new hypothesis for the relationships among the species of *Docosia*, which is a result of an expanded dataset, also including the nuclear 28S gene region, and with more than twice as many species of *Docosia* included.

The opportunity is also taken here to describe five new Palaearctic species, updating the number of Palaearctic species of *Docosia* to 62 (Ševčík & Laštovka 2008; Kurina & Ševčík 2011; Ševčík *et al.* 2016a), and to present new national records of several rare species.

Morphological terminology principally follows that of Söli (1997, 2017).

## Material and methods

The material used for DNA extraction and species descriptions was collected with Malaise traps, light traps or sweep nets at various localities in the years 2012–2019 (see Table 1). The voucher specimens are deposited in the following depositories:

- IUTG = Collection of Ilia State University, Tbilisi, Georgia
- IZBE = Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences (former Institute of Zoology and Botany), Tartu, Estonia
- JSL-OUC = Collection of Jan Ševčík Lab, University of Ostrava, Czech Republic
- NMPC = National Museum, Prague, Czech Republic
- ZFMK = Zoological Research Museum Alexander Koenig, Bonn, Germany

Most of the specimens were collected into 70% ethanol. The type specimens are stored in glycerine medium in a plastic pinned microvial, in ethyl alcohol, or mounted from alcohol, using the chemical method described by Vockeroth (1966), and double-mounted using minute pins. For every studied specimen, the preservation method is indicated in the material section. Illustrations of the terminalia were prepared using a U-DA drawing tube attached to a compound microscope Olympus CX31. The digital images of the general habitus and terminalia were combined using the software LAS ver. 4.1.0. from multiple gradually focused images taken by a Leica DFC 450 camera attached to a Leica 205C stereo microscope or a Leica DM 6000 B compound microscope, respectively (see also Jürgenstein *et al.* 2015). Adobe Photoshop CS5 was used for editing the figures and compiling the plates. Before placing in glycerol, the specimens of several species were treated in proteinase K to extract DNA. The protocol of DNA extraction, amplification, sequencing and phylogenetic analysis is described in Ševčík *et al.* (2016b). The primers used for the PCR amplifications of mitochondrial and nuclear gene fragments are listed in Ševčík *et al.* (2016a, 2016b).

The final molecular dataset consists of 3049 characters: 28S – 924 bp, ITS2 – 488 bp, COI – 658 bp, COII – 546 bp, cytB – 433 bp. All the sequences were deposited in GenBank (see Table 1). The maximum likelihood (ML) analyses of single gene alignments were conducted on the CIPRES computer cluster using RAxML-HPC BlackBox 8.2.10 (Stamatakis 2014) to check potential conflicting topologies and other artefacts. The final concatenated dataset was partitioned by gene and codon position and subsequently analysed using ML method with IQtree (Nguyen *et al.* 2015) on the IQ-TREE web server <http://iqtree.cibiv.univie.ac.at/> (Trifinopoulos *et al.* 2016). Best-fitting substitution models were chosen automatically by the IQ-TREE software (28S – HKY+F+I; ITS2 – TPM2u+F+I+G4; COI\_1 – HKY+F+G4; COI\_2 – TIM2+F+G4; COI\_3 – F81+F+I; COII\_1 – HKY+F+I+G4; COII\_2 – TIM2+F+G4; COII\_3 – TPM2+F+I+G4; cytB\_1 – TPM3u+F+G4; cytB\_2 – TIM3+F+G4; cytB\_3 – TN+F+I+G4), without free-rate heterogeneity. Branch supports were evaluated using 1000 ultrafast

**Table 1** (continued on the next page). List of species included in the molecular analysis, with GenBank accession numbers.

Species	Voucher code	Sampling country and year	COI	COII	CYTB	ITS2	28S
<i>Boletina nasuta</i> (Haliday, 1839)	JSGS18	Slovakia, 2013	KT923571	KT923585	KT923598	KT923614	MH114239
<i>Gnoriste bilineata</i> Zetterstedt, 1852	JSGS4	Czech Republic, 2009	KT316839	KT923584	KT923597	KT923613	KP288794
<i>Docosia aceus</i> Garrett, 1925	JSDO23	USA, 2011	MT293016	MT293047	MT293031	MT293064	MT292998
<i>D. anatolica</i> Ševčík sp. nov.	JSDO26	Turkey, 2013	MT293019	MT293050	MT293033	MT293067	MT293001
<i>D. carbonaria</i> Edwards, 1941	JSDO24	Slovakia, 2017	MT293017	MT293048	MT293032	MT293065	MT292999
<i>D. cephaloniae</i> Chandler <i>et al.</i> , 2006	JSDO17	Greece, 2016	MT293012	MT293047	MT293027	MT293060	MT292994
<i>D. dentata</i> Ševčík, Kasprák & Rulík, 2016	JSDO1	Slovakia, 2012	KT923562	KT923575	KT923600	KT923604	MT292983
<i>D. dichroa</i> Loew, 1869	JSDO22b	USA, 2016	MT293015	MT293046	MT293030	MT293063	MT292997
<i>D. diutina</i> Plassmann, 1996	ZFMK-TIS-2516913	Germany, 2013	KU146854	KU146860	KU146858	KU146856	MT292981
<i>D. flavicoxa</i> Strobl, 1900	JSDO9a	Slovakia, 2012	KT923570	KT923583	KT923596	KT923612	MT292991
<i>D. fumosa</i> Edwards, 1925	ZFMK-TIS-2556735	Germany, 2015	KU146855	KU146861	KU146859	KU146857	MT292982
<i>D. fuscipes</i> (Rosier, 1840)	JSDO2	Slovakia, 2015	KT923563	KT923576	KT923590	KT923605	MT292984
<i>D. ghivipes</i> (Walker, 1856)	JSGS29	Slovakia, 2013	KT923572	KT923586	KT923599	KT923615	MH114249
<i>D. japonica</i> Kurina sp. nov.	ZFMK-DIP-00067333	Japan, 2015	n/a	MT293057	MT293040	MT293074	MT293008
<i>D. kerkini</i> Kurina & Ševčík, 2011	JSDO34	Bulgaria, 2018	MT293023	MT293054	MT293037	MT293071	MT293005
<i>D. landrocki</i> Laštovka & Ševčík, 2006	JSDO7	Slovakia, 2014	KT923568	KT923581	KT923594	KT923610	MT292989
<i>D. lastovkai</i> Chandler, 1994	JSDO4	Slovakia, 2013	KT923565	KT923578	KT923591	KT923607	MT292986
<i>D. montana</i> Laštovka & Ševčík, 2006	JSDO5	Slovakia, 2013	KT923566	KT923579	KT923592	KT923608	MT292987
<i>D. moravica</i> Landrock, 1916	JSDO6	Slovakia, 2013	KT923567	KT923580	KT923593	KT923609	MT292988
<i>D. morionella</i> Mik, 1884	JSDO31	Great Britain, 2017	MT293022	MT293053	MT293036	MT293070	MT293004
<i>D. muelleri</i> Plassmann, 1986	JSDO21	Finland, 2015	MT293014	MT293045	MT293029	MT293062	MT292996
<i>D. muranica</i> Kurina & Ševčík, 2011	JSM10	Slovakia, 2013	KC435639	KT923587	KC435683	KC435708	MT293009
<i>D. nigra</i> Landrock, 1928	JSDO25	Slovakia, 2017	MT293018	MT293049	n/a	MT293066	MT293000
<i>D. pannonica</i> Laštovka & Ševčík, 2006	JSDO28	Slovakia, 2017	MT293020	MT293051	MT293034	MT293068	MT293002
<i>D. peloponnensis</i> Ševčík sp. nov.	JSDO15	Greece, 2016	MT293011	MT293042	n/a	MT293059	MT292993
<i>D. polyspina</i> Kurina sp. nov.	IZBE-0200401	Russia, 2019	MT293025	MT293056	MT293039	MT293073	MT293007
<i>D. rameli</i> Kurina & Ševčík, 2011	JSDO29	Slovakia, 2017	MT293021	MT293052	MT293035	MT293069	MT293003

**Table 1** (continued). List of species included in the molecular analysis, with GenBank accession numbers.

<b>Species</b>	<b>Voucher code</b>	<b>Sampling country and year</b>	<b>COI</b>	<b>COII</b>	<b>CYTB</b>	<b>ITS2</b>	<b>28S</b>
<i>D. rohaceki</i> Ševčík, 2006	JSDO12c	Slovakia, 2016	MT293010	MT293041	MT293026	MT293058	MT292992
<i>D. scitarina</i> (Meigen, 1830)	JSDO8	Czech Republic, 2014	KT923569	KT923582	KT923595	KT923611	MT292990
<i>D. setosa</i> Landrock, 1916	JSDO3	Slovakia, 2015	KT923564	KT923577	KT923601	KT923606	MT292985
<i>D. svanetica</i> Kurima sp. nov.	JSDO39	Georgia, 2019	MT293024	MT293055	MT293038	MT293072	MT293006
<i>D. walpurga</i> Taber, 2011	JSDO20	USA, 2016	MT293013	MT293044	MT293028	MT293061	MT292995

bootstrap (Hoang *et al.* 2017). All other settings were left as default. The node support values are given in the form of ultrafast bootstrap (= ufboot). Ultrafast bootstrap values are more unbiased – ufboot of 95 corresponds roughly to a probability of 95% that a clade is true (Minh *et al.* 2013; Hoang *et al.* 2017). As outgroup taxa we selected two representatives of the subfamily Gnoristinae, in concordance with the most recent molecular study (Kaspřák *et al.* 2019). The resulting phylogenetic tree (consensus tree) was visualized using Interactive Tree Of Life (iTOL; Letunic & Bork 2016).

## Results

### *Taxonomic treatments*

Phylum Arthropoda Latreille, 1829  
Class Insecta Linnaeus, 1758  
Order Diptera Linnaeus, 1758  
Infraorder Bibionomorpha Hennig, 1954  
Superfamily Sciaroidea Billberg, 1820  
Family Mycetophilidae Newman, 1834  
Subfamily Gnoristinae Edwards, 1925  
Genus *Docosia* Winnertz, 1863

*Docosia anatolica* Ševčík sp. nov.

[urn:lsid:zoobank.org:act:7E7CA4EE-F77C-4B6F-87FA-A42B0F0B5A35](https://zoobank.org/act:7E7CA4EE-F77C-4B6F-87FA-A42B0F0B5A35)

Figs 2, 7A

### Differential diagnosis

By structure of the male terminalia, *D. anatolica* sp. nov. resembles *D. juxtamontana* Chandler, 2004 and *D. incolamontis* Chandler, 2004. All species have a bifid gonostylus and posteroventral margin of gonocoxites with a prominent medial process surrounded by two small lobes bearing setae. These setae are apically ramified in *D. anatolica* sp. nov., but with an unknown structure in the other two species. Apically modified setae at the posteroventral margin of gonocoxites are frequent in Central-Asian species (cf. Kurina & Ševčík 2012) but they are also observable in other species, e.g., in the European *D. pannonica* Laštovka & Ševčík, 2006. *Docosia anatolica* sp. nov. has the ventral lobe of the gonostylus obtriangular, with spines at both posterior corners, while it is slender and furcated, with medial and apical spines in *D. juxtamonta* and irregularly crescent-shaped with two subapical spines in *D. incolamontis*.

### Etymology

The species name refers to the type locality in Anatolia (Asia Minor).

### Type material

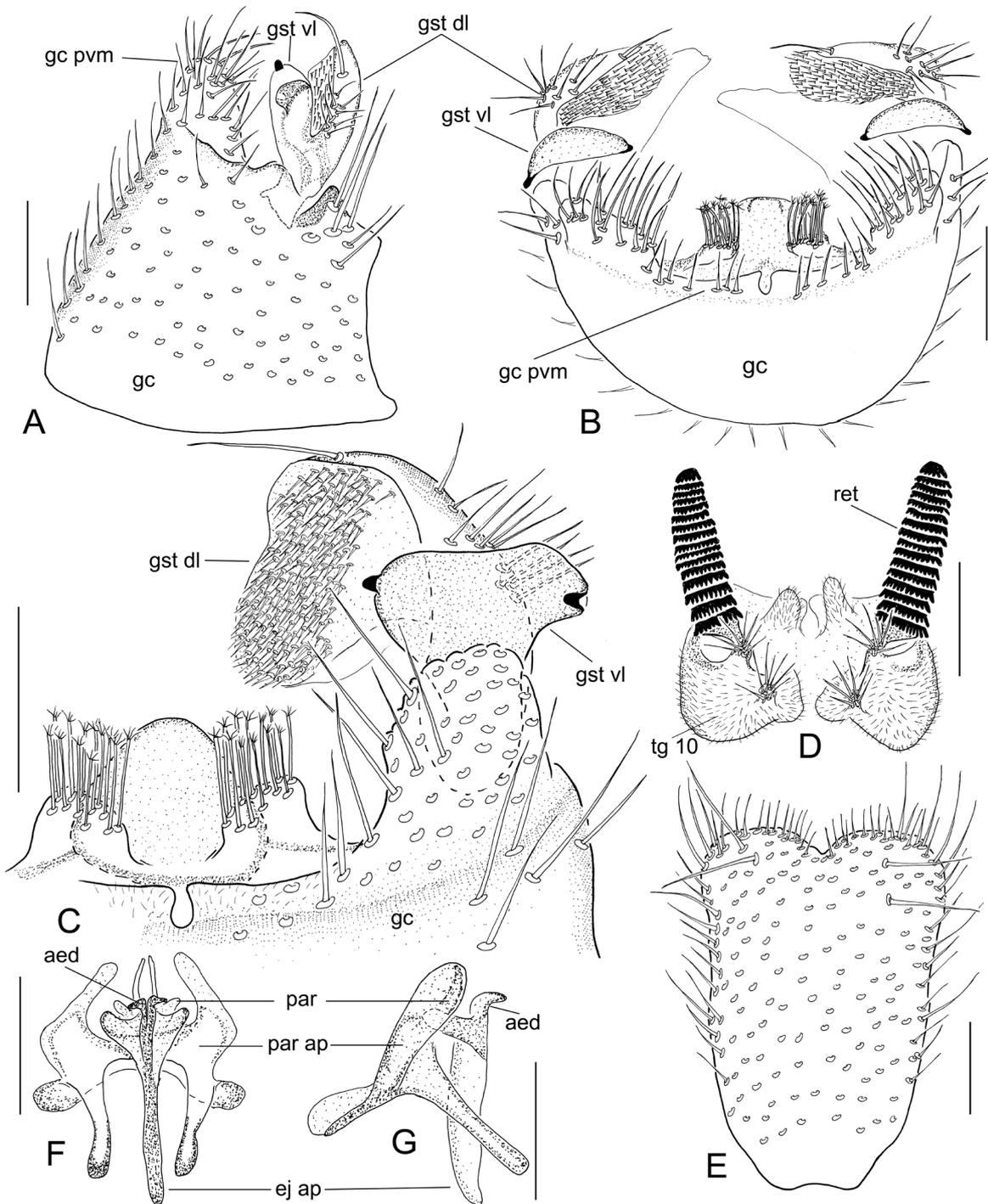
#### Holotype

TURKEY • ♂; Mugla, University Campus; 1100 m a.s.l.; 22 Apr. 2016; M. Barták and Š. Kubík leg.; Malaise trap; in a pinned microvial with glycerol; specimen after DNA extraction; GenBank: MT293019, MT293050, MT293033, MT293067, MT293001; NMPC-JSDO26.

### Description

#### Male (n = 1)

MEASUREMENTS. Length of wing 4.2 mm.



**Fig. 2.** *Docosia anatolica* Ševčík sp. nov., ♂, terminalia. **A.** Lateral view. **B.** Posterior view. **C.** Ventral view of gonostylus. **D.** Dorsal view of cerci. **E.** Dorsal view of tergite 9. **F.** Ventral view of aedeagal complex. **G.** Lateral view of aedeagal complex. Abbreviations: aed = aedeagus; ej ap = ejaculatory apodeme; gc = gonocoxite; gc pvm = posteroventral margin of gonocoxite; gst vl = ventral lobe of gonostylus; gst dl = dorsal lobe of gonostylus; par = paramere; par ap = parameral apodeme; ret = combs of retinacula; tg = tergite. Scale bars = 0.1 mm.

**HEAD.** Blackish brown with numerous pale setae. Three ocelli, lateral ones almost touching compound eyes, separated from eye margins by less than half of their own diameter. Mouthparts light brownish. Palpus brownish yellow, basal segment and apical part of last palpomere darker. Scape, pedicel and all flagellomeres dark brown. Flagellomeres cylindrical, flagellomeres 1 and 2 about twice as long as broad, other flagellomeres (3 to 14) about three times as long as broad.

**THORAX.** All parts of thorax blackish brown, with light setae. Scutellum with numerous setae and several submarginal pale bristles about twice as long as scutellum. Anteprenotum and proepisternum with pale bristles and short darker setae. Laterotergite and other pleural parts bare. Haltere pale yellow.

**LEGS.** All coxae yellow, with basal third darkened. Femora mostly yellow, fore and mid femur dark along ventral margin, more extensively in proximal third; similarly hind femur, which is brownish also around tip. Trochanters blackish brown. Fore tibia brownish yellow, apicomediaally with simple semicircular tibial organ (anteroapical depressed area), without strong setae, only densely covered with fine setulae. Mid tibia darkened dorsally. Hind tibia mostly brownish yellow, with small black dot approximately in middle of dorsal surface.

**WINGS.** Hyaline, unmarked. Radial veins and r-m dark brown, other veins paler, basal parts of m faint, almost not traceable. Sc, Rs and basal third of cu-stem asetose, other veins setose. Costa reaches to about one third of distance between  $R_5$  and  $M_1$ . Sc ends in R before level of beginning of m-stem. Posterior fork begins before anterior fork, approximately at level of basal third of r-m.

**ABDOMEN.** All dark brown.

**TERMINALIA** (Figs 2A–E, 7A). Dark brown except lighter gonostyli and posterior margin of gonocoxites. Tergite 9 subrectangular, about 1.5 times as long as broad, slightly broadening posteriorly, posterior margin with medial incision. Posteroventral margin of gonocoxites with blunt lateral projections and prominent rounded process medioventrally, surrounded by two shorter processes bearing apically ramified thick setae. Gonostylus bifid. Ventral lobe of gonostylus obtriangular in ventral view, with dark tooth at both posterior corners. Dorsal lobe of gonostylus larger than ventral lobe, bent inwards and rounded apically, with large aggregation of setulae along ventral surface anteriorly, prominent subapical seta posteriorly and with about 10 setae posterolaterally. Cercus with 16 combs of retinacula.

#### **Female**

Unknown.

#### **Biology**

Unknown.

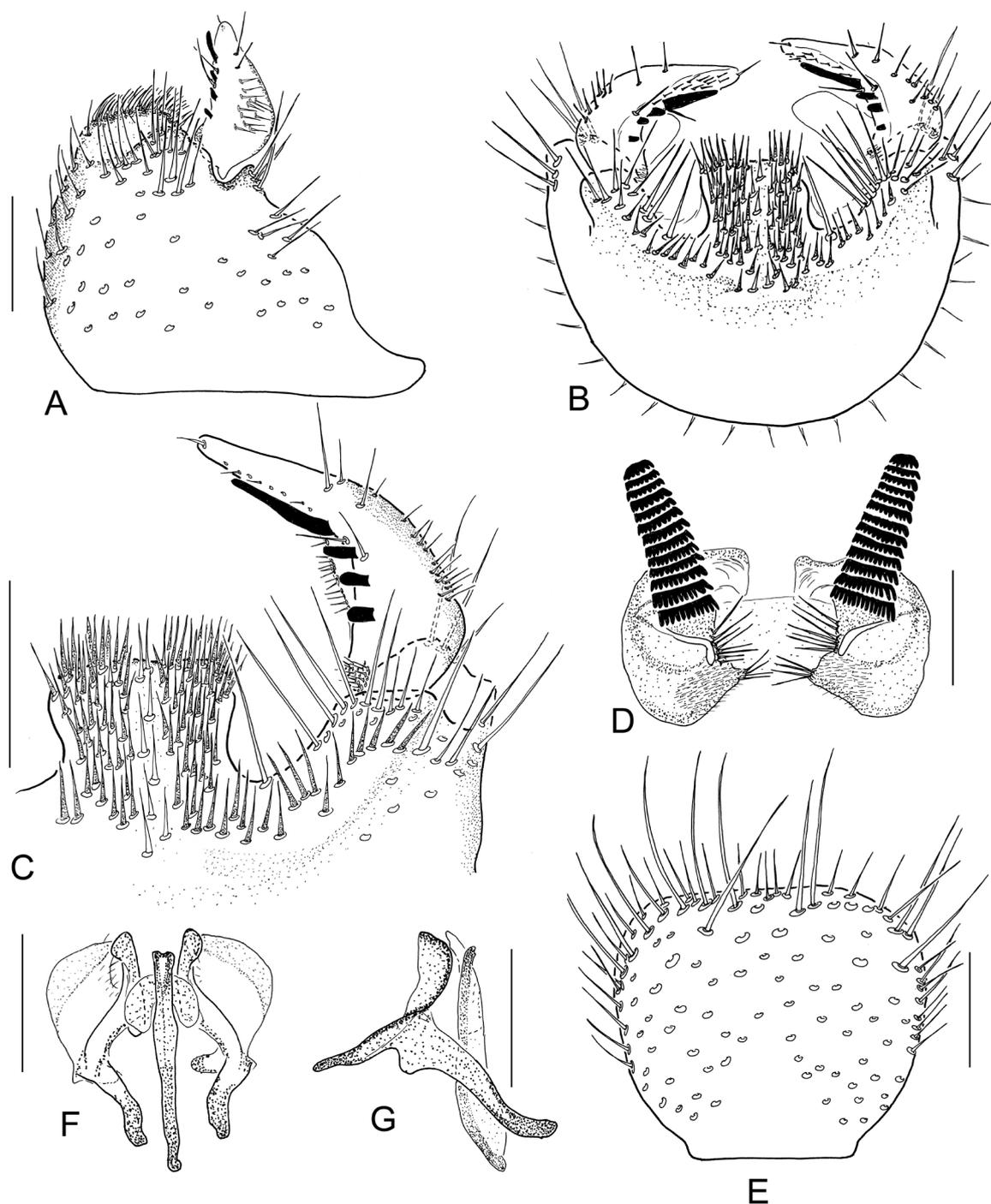
*Docosia japonica* Kurina sp. nov.

[urn:lsid:zoobank.org:act:8D2164B1-0649-41BD-A65A-28434E54E1F4](https://zoobank.org/act:8D2164B1-0649-41BD-A65A-28434E54E1F4)

Figs 3, 7B

#### **Differential diagnosis**

By the structure of the male terminalia, *D. japonica* sp. nov. belongs to a group of Palaearctic species, as defined for *D. polyspina* Kurina sp. nov., and resembles *D. kerkini* Kurina & Ševčík, 2011, *D. polyspina* sp. nov. and *D. svanetica* Kurina sp. nov. All species have a simple sickle- or crescent-shaped gonostylus with a row of spines on the medial margin. However, unlike the other species, *D. japonica* sp. nov. has four medially situated spines with the posteriormost one three times as long as the anterior three (*D. kerkini* has 12 subequal spines, *D. polyspina* sp. nov. has 3 subequal spines and *D. svanetica* sp. nov.



**Fig. 3.** *Docosia japonica* Kurina sp. nov., ♂, terminalia. **A.** Lateral view. **B.** Posterior view. **C.** Ventral view of gonostylus. **D.** Dorsal view of cerci. **E.** Dorsal view of tergite 9. **F.** Ventral view of aedeagal complex. **G.** Lateral view of aedeagal complex. Scale bars = 0.1 mm.

has 5 spines with the anteriormost one at the apex of the gonostylus and two times as long as the other). The posteroventral margin of the gonocoxites resembles somewhat *D. selini* Kurina, 2006 described from Kazakhstan and subsequently found in the Chimgan area in Uzbekistan, but both species can be well distinguished by the shape of the gonostylus.

### Etymology

The species is named after its occurrence in Japan.

### Type material

#### Holotype

JAPAN • ♂; Nagano Prefecture, Ueda, Sugadaira Kogen near SMRC; 36°31'18" N, 136°21'03" E; 1330 m a.s.l.; 26 Apr.–3 May 2015; A. Blanke leg.; Malaise trap in grassland; in ethyl alcohol; terminalia in glycerol; one fore leg used for DNA extraction; GenBank: MT293057, MT293040, MT293074, MT293008; ZFMK-DIP-00067333.

### Description

#### Male (n = 1)

MEASUREMENTS. Length of wing 3.8 mm.

HEAD. Black with numerous pale setae. Three ocelli, lateral ones almost touching compound eyes, separated from eye margins by less than half of their own diameter. Mouthparts light brownish. Palpus with basal three segments brown, 4<sup>th</sup> segment light brown, 5<sup>th</sup> segment yellowish. Scape dark brown, pedicel and all flagellomeres somewhat lighter. Flagellomeres cylindrical, about two times as long as broad.

THORAX. All parts of thorax blackish brown to black, with light setae. Scutum somewhat darker than lateral parts of thorax. Scutellum with numerous setae, marginal setae not arranged to distinct pairs. Anteprenotum and proepisternum with pale bristles and short darker setae. Laterotergite and other pleural parts bare. Haltere pale yellow.

LEGS. All coxae, trochanters, femora and tibiae dark brown. Tibial spurs yellow. Tarsi dark yellow but seem brownish because of dense setae. Fore tibia apicomediaally with semicircular tibial organ (anteroapical depressed area), without strong setae, only densely covered with fine yellow setulae.

WINGS. Hyaline, unmarked. Radial veins and r-m dark brown, other veins paler, m-stem faint. Sc, Rs, m-stem, basal part of M<sub>1</sub>, bm-m and basal one third of cu-stem asetose, other veins setose. Costa reaches to about one third of distance between R<sub>5</sub> and M<sub>1</sub>. Sc ends in R at level of beginning of m-stem. Posterior fork begins well before anterior fork, approximately at level of basal fifth of r-m.

ABDOMEN. Dark brown with pale setae, first three segments with tergites laterally and sternites wholly yellowish.

TERMINALIA (Figs 3A–E, 7B). Brown, with gonostyli and posteroventral area of gonocoxites yellow. Tergite 9 subquadrate, about as long as broad, posterior margin straight, with submarginal row of prominent setae about twice as long as other vestiture on tergite. Posteroventral margin of gonocoxites with quadrate, posteriorly slightly widening medial process having lateral aggregations of dark setae, medially separated by stripe of light setae. Gonostylus simple, tapering, apical half bent medially, with (1) long and pointed medial spine at medial margin, (2) row of three more anterior, blunt spines at

medial margin, about three times shorter than posterior spine, (3) medial surface bearing minute setae, and (4) lateral surface bearing about 15 fine setae medially. Cercus with 13 combs of retinacula.

#### **Female**

Unknown.

#### **Biology**

Unknown.

#### ***Docosia peloponnensis* Ševčík sp. nov.**

[urn:lsid:zoobank.org:act:979DF75A-E677-4C63-8B13-BB3F3270115F](https://zoobank.org/act:979DF75A-E677-4C63-8B13-BB3F3270115F)

Figs 4, 7D

#### **Differential diagnosis**

This species is unique among the species of *Docosia* in having a simple gonostylus with a sickle-like posterior projection.

#### **Etymology**

The species name refers to the type locality.

#### **Type material**

##### **Holotype**

GREECE • ♂; Peloponnese, Valtasiniko env.; 1100 m a.s.l.; 22 Apr. 2016; J. Ševčík leg.; sweeping along small brook; in a pinned microvial with glycerol; specimen after DNA extraction; GenBank: MT293011, MT293042, MT293059, MT292993; NMPC- JSDO15.

##### **Paratype**

GREECE • 1 ♂; Peloponnese, Valtasiniko env.; 1100 m a.s.l.; 26 Apr. 2016; M. Tkoč leg.; sweeping along small brook; in a pinned microvial with glycerol; JSL-UOC-JSDO15b.

#### **Description**

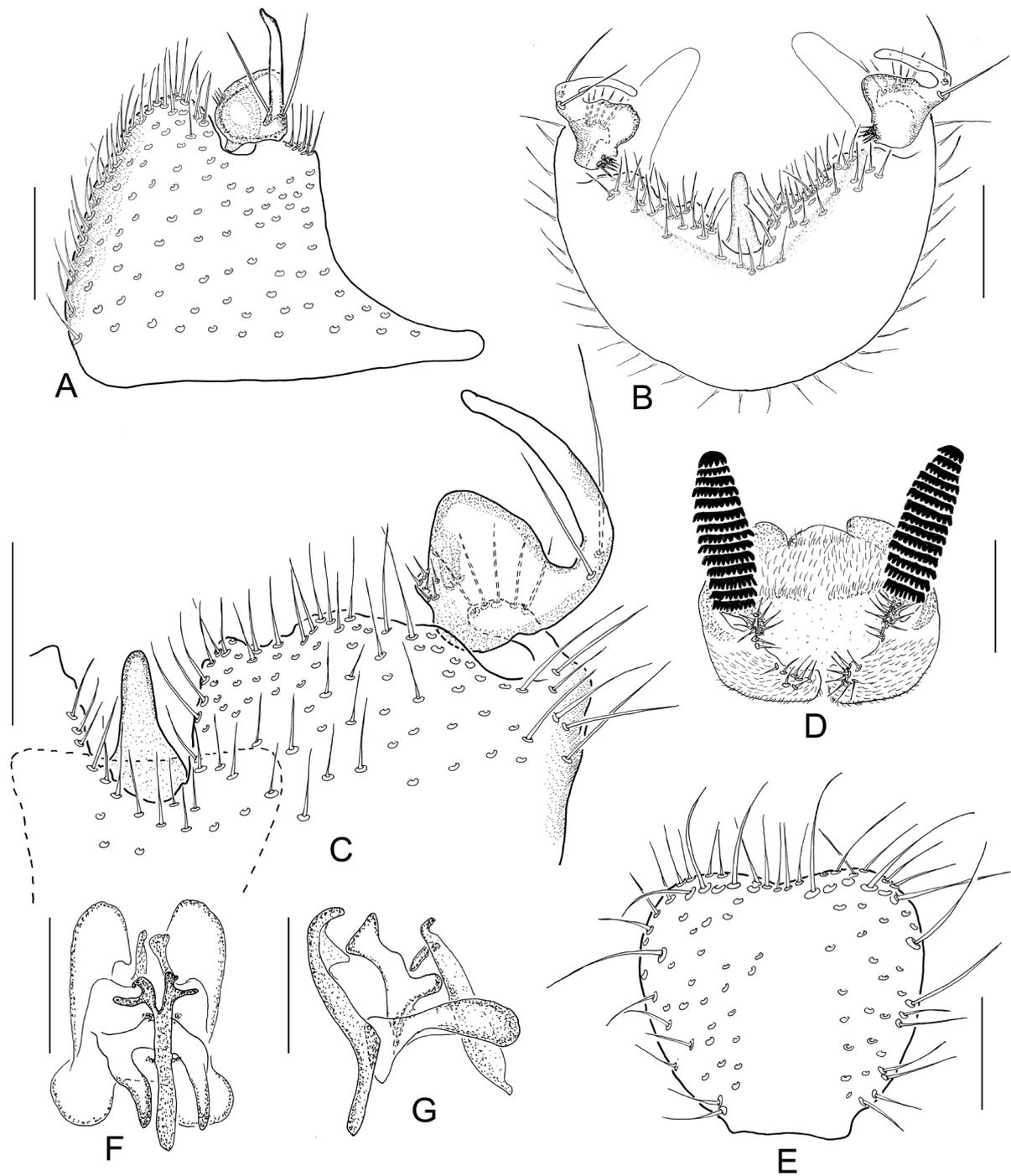
##### **Male (n = 2)**

MEASUREMENTS. Length of wing 4.2 mm.

HEAD. Blackish brown with numerous pale setae. Three ocelli, lateral ones almost touching compound eyes, separated from eye margins by less than half of their own diameter. Clypeus blackish, with setae pale. Mouthparts light brownish. Palpus brownish yellow, basal segment and apical part of last palpomere darker. Scape, pedicel and all flagellomeres dark brown. Flagellomeres cylindrical, flagellomeres 1 to 7 about twice as long as broad, distal flagellomeres (8 to 14) slightly conical, 2.5 to 3 times as long as broad.

THORAX. All parts of thorax blackish brown, with light setae. Scutellum with numerous setae and several submarginal pale bristles about twice as long as scutellum. Anteprepronotum and proepisternum with pale bristles and short darker setae. Laterotergite and other pleural parts bare. Haltere pale yellow.

LEGS. All coxae yellow, with basal third darkened. Femora mostly yellow, fore and mid femur dark along ventral margin, more extensively in proximal third; similarly hind femur, brownish also around tip. All trochanters blackish brown. Tibiae and tarsi yellow, tarsal segments seemingly brownish because of dense setulae. Fore tibia apicomediaally with semicircular tibial organ (anteroapical depressed area), without strong setae, only densely covered with fine setulae.



**Fig. 4.** *Docosia peloponnensis* Ševčík sp. nov., ♂, terminalia. **A.** Lateral view. **B.** Posterior view. **C.** Ventral view of gonostylus. **D.** Dorsal view of cerci. **E.** Dorsal view of tergite 9. **F.** Ventral view of aedeagal complex. **G.** Lateral view of aedeagal complex. Scale bars = 0.1 mm.

WINGS. Hyaline, unmarked. Radial veins and r-m dark brown, other veins paler, basal parts of m faint, almost not traceable. Sc, Rs and basal third of cu-stem asetose, other veins setose. Costa reaches to 0.45 of distance between  $R_5$  and  $M_1$ . Sc ends in R slightly beyond level of beginning of m-stem. Posterior fork begins before anterior fork, approximately at level of basal third of r-m.

ABDOMEN. All dark brown.

TERMINALIA (Figs 4A–E, 7D). Dark brown except lighter gonostyli and posterior margin of gonocoxites. Tergite 9 about as long as broad, slightly narrowing towards base, with slightly concave posterior margin. Posteroventral margin of gonocoxites without lateral projections, with distinct U-shaped medial incision and narrow digitiform medioventral process. Gonostylus simple, slightly bulging medially, with aggregation of small setae at mediobasal corner and with characteristic sickle-like posterior projection, with prominent basal setae at its ventral and dorsal sides. Cercus with 14 combs of retinacula.

#### Female

Unknown.

#### Biology

Unknown.

*Docosia polyspina* Kurina sp. nov.

[urn:lsid:zoobank.org:act:53D312AC-5DAB-4B0D-AF1C-819CAC59965F](https://zoobank.org/act:53D312AC-5DAB-4B0D-AF1C-819CAC59965F)

Figs 5, 7C

#### Differential diagnosis

*Docosia polyspina* sp. nov. belongs to a large group of Palearctic species that have a simple, undivided gonostylus, bearing a variable number of black spines in different positions (e.g., *D. flavicoxa* Strobl, 1900, *D. moravica* Landrock, 1916, *D. dentata* Ševčík *et al.*, 2016, *D. matilei* Ševčík & Laštovka, 2008, *D. kerkini*, *D. svanetica* sp. nov., *D. japonica* sp. nov.). *Docosia polyspina* sp. nov. markedly differs from the other species in having the gonostylus with three long spines at the medial margin and the internal flange of the posteroventral margin of the gonocoxites with about 10 simple blunt spines laterally on both sides.

#### Etymology

The name is combined from the Greek ‘πολύ’ (polú) ‘many’ and Latin ‘spīna’ ‘spine’, referring to the number of black spines on internal flange of the gonocoxites posteroventrally.

#### Type material

##### Holotype

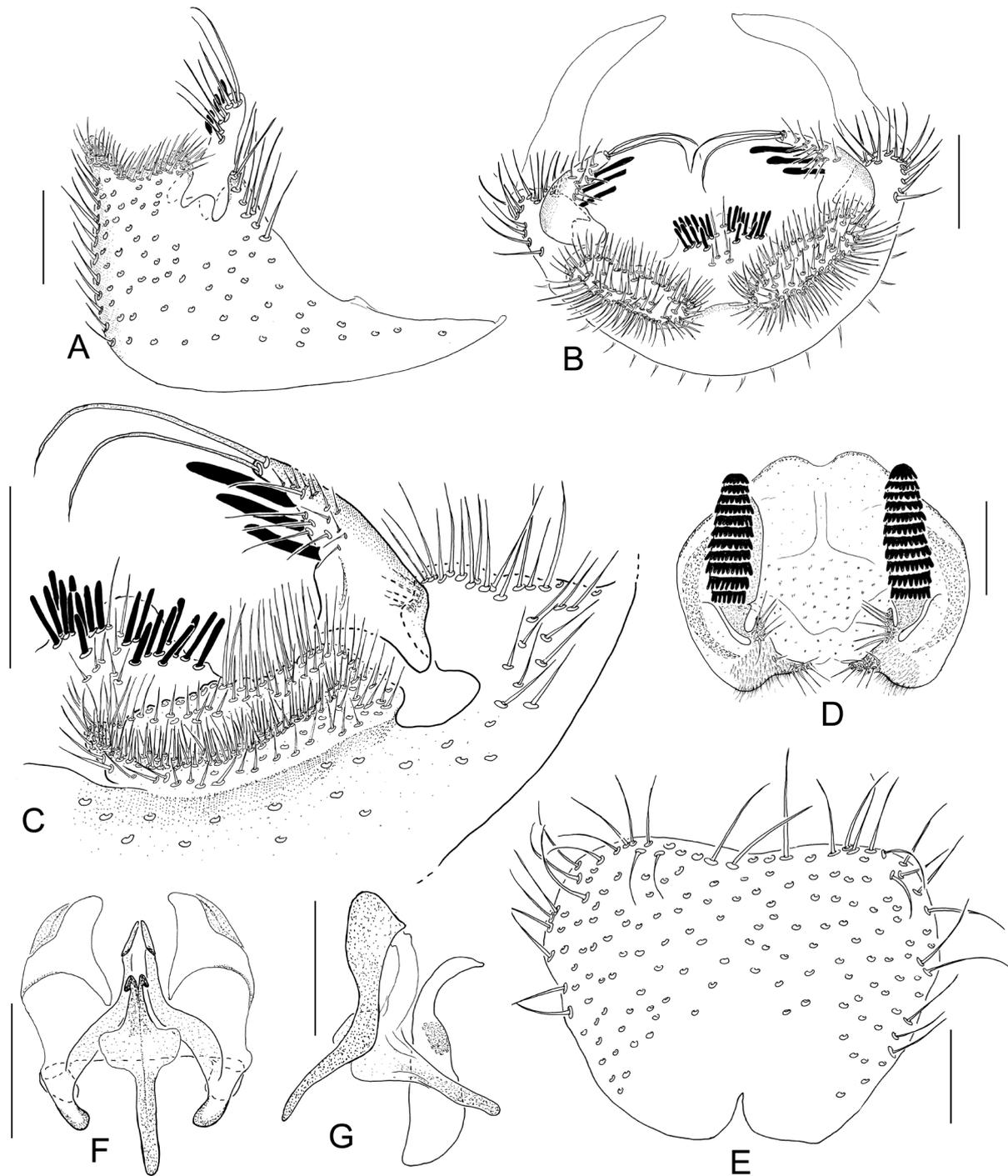
RUSSIA • ♂; Primorsky Krai, 3 km NW of Lukyanovka; 43°11'26.1" N, 132°40'52.1" E; 146 m a.s.l.; 9 May 2019; O. Kurina leg.; sweep net; mounted from ethyl alcohol; terminalia in glycerol; one fore leg used for DNA extraction; GenBank: MT293025, MT293056, MT293039, MT293073, MT293007; IZBE-0200401.

#### Description

##### Male (n = 1)

MEASUREMENTS. Length of wing 3.0 mm.

HEAD. Blackish with numerous pale setae. Three ocelli, lateral ones almost touching compound eyes, separated from eye margins by less than half of their own diameter. Mouthparts yellow to light brownish.



**Fig. 5.** *Docosia polyspina* Kurina sp. nov., ♂, terminalia. **A.** Lateral view. **B.** Posterior view. **C.** Ventral view of gonostylus. **D.** Dorsal view of cerci. **E.** Dorsal view of tergite 9. **F.** Ventral view of aedeagal complex. **G.** Lateral view of aedeagal complex. Scale bars = 0.1 mm.

Palpus yellowish, basal segment somewhat darker. Scape dark brown, pedicel and basal half of first flagellomere dark yellow, rest of flagellomeres brown, lighter than scape. First three flagellomeres cylindrical, about 1.5 times as long as broad, rest of flagellomeres moniliform, about as long as broad.

THORAX. All parts of thorax blackish brown, with light setae. Scutellum with numerous setae and pair of submarginal pale bristles about twice as long as scutellum. Antepronotum and proepisternum with pale bristles and short darker setae. Laterotergite and other pleural parts bare. Haltere pale yellow.

LEGS. All coxae yellow, with basal fifth of fore- and midcoxae, and basal third of hind coxa darkened. Trochanters brownish. All femora and tibiae yellow. Fore tibia apicomediaally with semicircular tibial organ (anteroapical depressed area), without strong setae, only densely covered with fine setulae. Tarsi seem brownish because of dense setae.

WINGS. Hyaline, unmarked. Radial veins and r-m dark brown, other veins paler, m-stem faint, almost not traceable. Sc, Rs, m-stem, bm-m and basal two thirds of cu-stem asetose, other veins setose. Costa reaches to about one third of distance between  $R_5$  and  $M_1$ . Sc ends in R at level of beginning of m-stem. Posterior fork begins well before anterior fork, approximately at level of basal fifth of r-m.

ABDOMEN. All dark brown with pale setae.

TERMINALIA (Figs 5A–E, 7C). Light brown, with gonostyli slightly lighter. Tergite 9 widening posteriorly, about 1.3 times as long as broad, posterior margin slightly concave, anterior margin with V-shaped medial cleft. Posteroventral margin of gonocoxites with lateral extensions, covered with dense aggregation of fine setae, setae longer at posterior margin. Internal flange of posteroventral margin drawn out to large, posteriorly rounded medial process, with about 10 simple blunt spines laterally on both sides and about 10 setae medially. Gonostylus simple, tapering, with (1) row of three blunt medially directed spines at medial margin, about twice as long as spines on internal flange of posteroventral margin of gonocoxites, (2) two extremely long apically curved setae, clearly deviating from other setae of terminalia, and (3) about 15 setae on apical half ventrally. Cercus with 11 combs of retinacula.

#### **Female**

Unknown.

#### **Biology**

Unknown.

*Docosia svanetica* Kurina sp. nov.

[urn:lsid:zoobank.org:act:51A54E2C-274B-4DB9-880B-0FDF2020E34A](https://zoobank.org/act:51A54E2C-274B-4DB9-880B-0FDF2020E34A)

Figs 1, 6, 7E

#### **Differential diagnosis**

By the structure of the male terminalia, *D. svanetica* sp. nov. belongs to a group of Palaearctic species as defined for *D. polyspina* sp. nov. and resembles *D. kerkini*. Both species have a simple sickle- or crescent-shaped gonostylus with a row of medial spines and a posteroventral margin of gonocoxites with submedial humps, bearing a comb of short setae. The subsimilar combs of the setae at the posteroventral margins of the gonocoxites are represented in several Palaearctic species (e.g., *D. dentata*, *D. lastovkai* Chandler, 1994, *D. melita* Chandler *et al.*, 2006), which have an otherwise different structure of the gonostylus and aedeagal complex. *Docosia svanetica* sp. nov. differs markedly from *D. kerkini* in the shape of the ninth tergite, which is subcircular, while it is apically very wide and broadly concave in *D. kerkini*. Moreover, *D. svanetica* sp. nov. has a gonostylus with a prominent apical and four smaller

medial spines in spite of about 12 medial subequal spines in *D. kerkini*. The laterotergite is setose in *D. svanetica*, while it is bare in *D. kerkini*.

### Etymology

The species name refers to the type locality in Svanetia, a historic province of Georgia, in the north-western part of the country.

### Type material

#### Holotype

GEORGIA • ♂; Samegrelo-Zemo Svaneti, near Ushguli, path to glacier; 42°56'37.4" N, 43°3'14.2" E; 2220 m a.s.l.; 15–17 Jun. 2019; X. Mengual leg.; Malaise trap; in ethyl alcohol; ZFMK-DIP-00067332.

#### Paratypes

GEORGIA • 1 ♂; same collection data as for holotype; IZBE-0200402 • 2 ♂♂; same collection data as for holotype; ZFMK-DIP-00067331, ZFMK-DIP-00067330 • 1 ♂; same collection data as for holotype; IUTG ZFMK-DIP-00067329 • 1 ♂; same collection data as for holotype; after DNA extraction; GenBank: MT293024, MT293055, MT293038, MT293072, MT293006; JSL-UOC-JSDO39 • 1 ♂; Samegrelo-Zemo Svaneti, S of Lakhushdi, meadow; 42°59'55.8" N, 42°39'00" E; 1270 m a.s.l.; 13–14 Jun. 2019; X. Mengual leg.; Malaise trap; in alcohol; IZBE-0200403 • 1 ♂; Samegrelo-Zemo Svaneti, S of Lakhushdi, meadow; 42°59'56.3" N, 42°39'07" E; 1270 m a.s.l.; 13–14 Jun. 2019; X. Mengual leg.; double Malaise trap; in alcohol; IZBE-0200404 • 1 ♂; Samtskhe-Javakheti, road from Abastumani to Saime, near river; 41°46'38.2" N, 42°50'14" E; 1366 m a.s.l.; 10–11 Jun. 2019; X. Mengual leg.; double Malaise trap; in alcohol with terminalia in glycerol; IZBE-0200405.

### Description

#### Male (n = 9)

MEASUREMENTS. Length of wing 3.0–3.4 mm (holotype = 3.2 mm).

HEAD. Blackish brown with numerous pale setae. Three ocelli, the lateral ones almost touching compound eyes, separated from eye margins by less than half of their own diameter. Mouthparts light brownish. Palpus with two basal segments brownish and three apical segments yellow. Scape, pedicel and all flagellomeres dark brown. Flagellomeres cylindrical, flagellomeres 1 and 2 about 1.3 times as long as broad, other flagellomeres 2–2.5 times as long as broad.

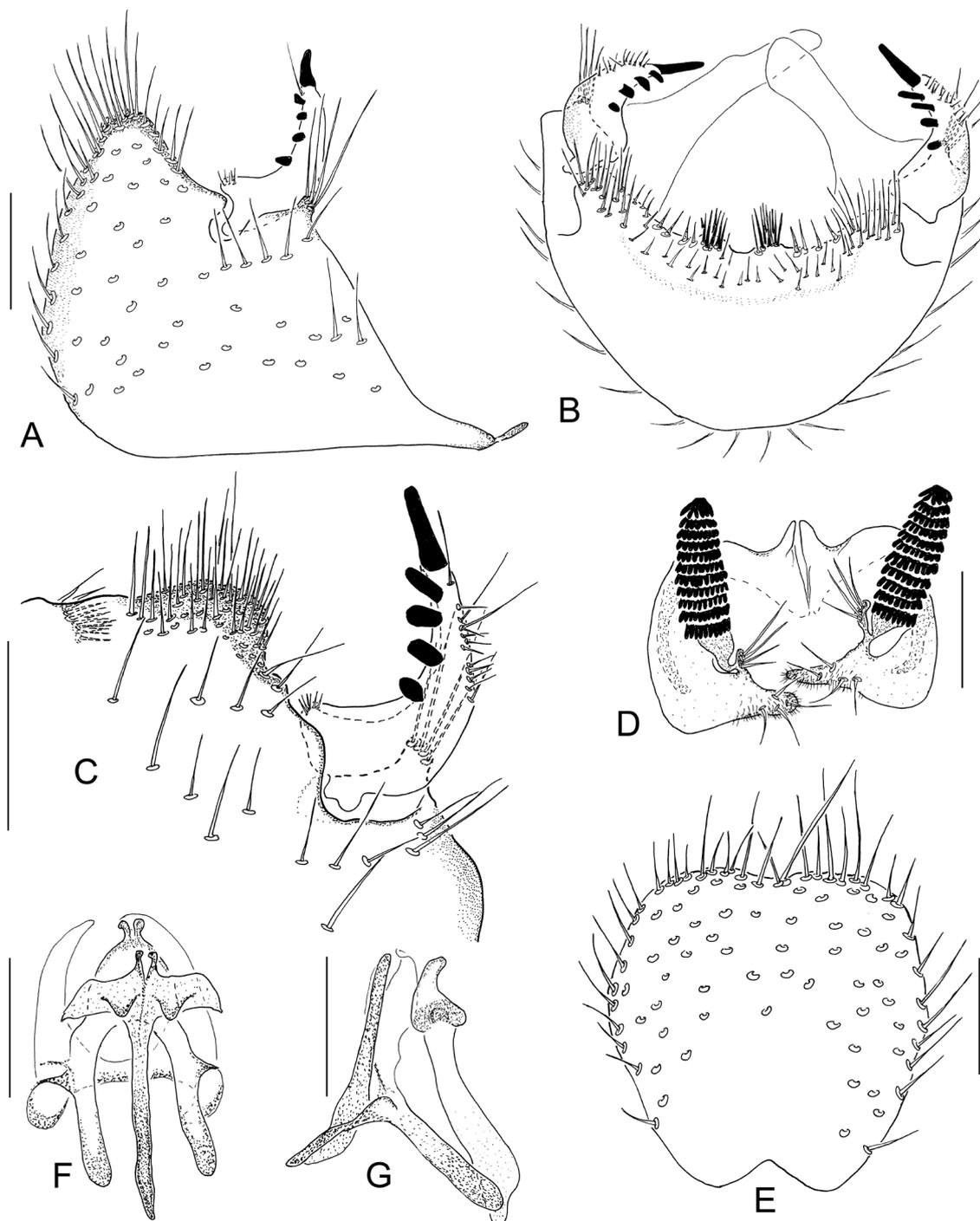
THORAX. All parts of thorax dark brown to blackish, with light setae. Scutellum with numerous pale setae and several submarginal pale bristles about twice as long as scutellum. Anteprenotum and proepisternum with pale bristles and short darker setae. Laterotergite with 6–8 light bristles on posterior half, other pleural parts bare. Haltere pale yellow.

LEGS. All coxae yellow, with basal fifth of fore- and midcoxae, and basal third of hind coxa darkened. Trochanters brownish. Femora mostly yellow, fore and mid femora dark along ventral margin in proximal half, hind femur apically darkened. All tibiae yellow. Fore tibia apicomediaally with semicircular tibial organ (anteroapical depressed area), without strong setae, only densely covered with fine setulae. Tarsi seem darker because of dense setae.

WINGS. Hyaline, unmarked. Radial veins and r-m dark brown, other veins paler, m-stem and basal parts of  $M_1$  and  $M_2$  faint, almost not traceable. Sc, Rs, bm-m, m-stem and basal third of cu-stem asetose, other veins setose. Costa reaches to about one third of the distance between  $R_5$  and  $M_1$ . Sc ends in R at level of beginning of m-stem. Posterior fork begins before anterior fork, approximately at level of basal third of r-m.

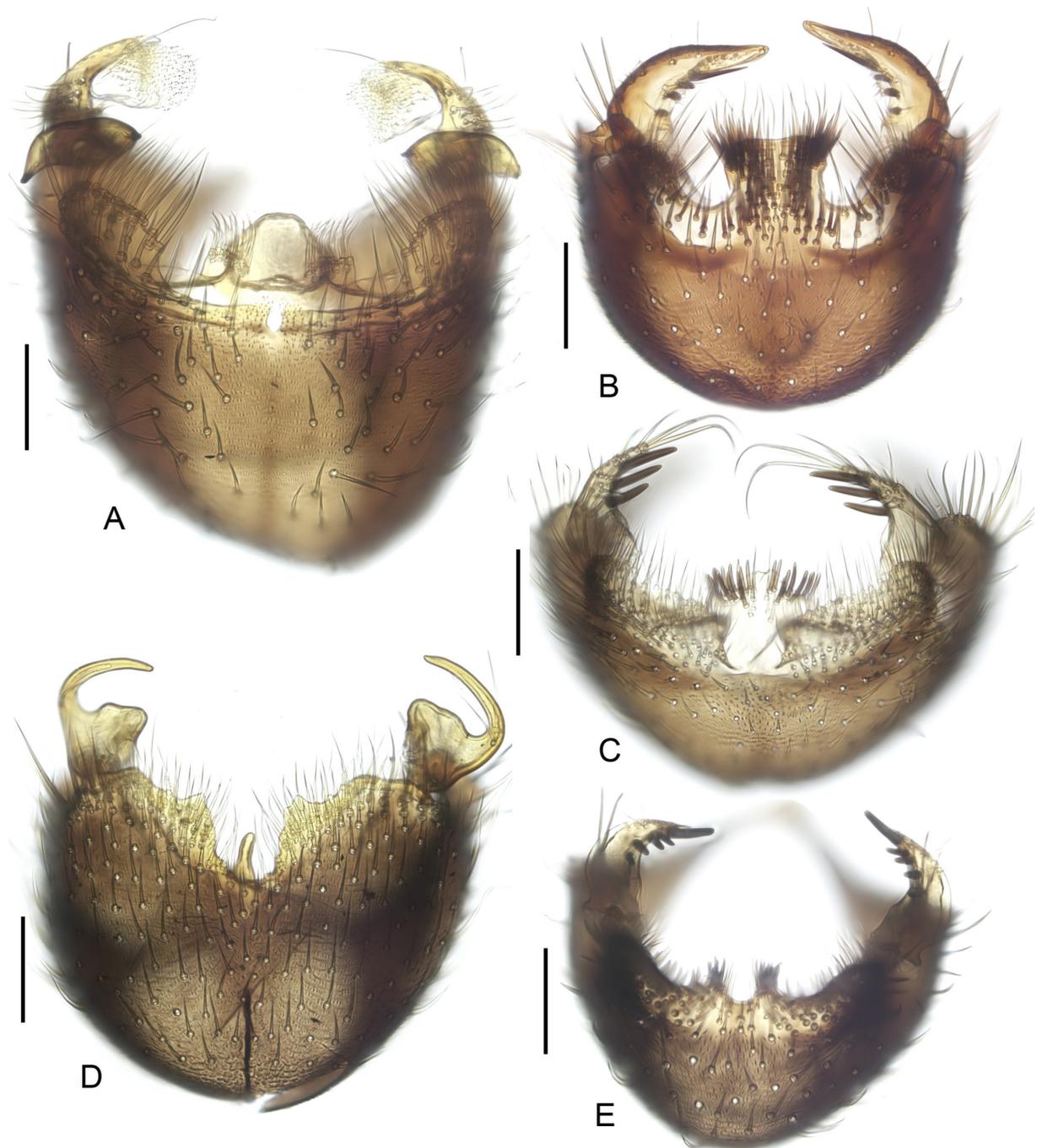
ABDOMEN. Dark brown to blackish, with second and third segments light brown.

TERMINALIA (Figs 6A–E, 7E). Dark brown except lighter gonostyli. Tergite 9 subcircular, about as long as broad, posterior margin slightly concave, anterior margin with wide V-shaped incision medially.



**Fig. 6.** *Docosia svanetica* Kurina sp. nov., ♂, terminalia. **A.** Lateral view. **B.** Posterior view. **C.** Ventral view of gonostylus. **D.** Dorsal view of cerci. **E.** Dorsal view of tergite 9. **F.** Ventral view of aedeagal complex. **G.** Lateral view of aedeagal complex. Scale bars = 0.1 mm.

Posteroventral margin of gonocoxites with extended flange, with submedial humps bearing combs of strong short setae. Gonostylus simple, sickle-shaped, tapering, with (1) apical blunt spine, (2) row of four blunt spines medially, about twice shorter than the apical one, and (3) about 15 setae laterally on apical half. Cercus with 11 combs of retinacula.



**Fig. 7.** Male terminalia, ventroposterior view. **A.** *Docosia anatolica* Ševčík sp. nov. **B.** *D. japonica* Kurina sp. nov. **C.** *D. polyspina* Kurina sp. nov. **D.** *D. peloponnensis* Ševčík sp. nov. **E.** *D. svanetica* Kurina sp. nov. Scale bars = 0.1 mm.

**Female**

Unknown.

**Biology**

Unknown.

***Molecular phylogeny of Docosia***

The phylogenetic tree for the concatenated dataset based on five gene fragments is presented in Fig. 8. The genus *Docosia* was found to be monophyletic with a maximum ultrafast bootstrap support value (ufboot = 100).

A clade containing two species, *D. fumosa* Edwards, 1925 and *D. morionella* Mik, 1884, branched most basally, as a sister group to all the other species of *Docosia* included in this analysis, followed by two species of the *D. gilvipes* (Walker, 1856) group and two species of the *D. dichroa* Loew, 1869 group. All the other species of *Docosia* still form a monophyletic group, though with moderate support (ufboot = 93). Within this group, the following pairs of species grouped together: *D. carbonaria* Edwards, 1941 + *D. rameli* Kurina & Ševčík, 2011 (ufboot = 100), followed by *D. cephaloniae* Chandler, Bechev & Caspers, 2006 + *D. lastovkai* (ufboot = 100) and *D. muranica* Kurina & Ševčík, 2011 + *D. fuscipes* (Roser, 1840), ufboot = 93.

Most of the relationships among the rest of the species included to this dataset are less supported (ufboot < 80), except for the group of six species related to *D. nigra* Landrock, 1928, which apparently constitute a monophyletic group (ufboot = 85).

***New national records***

The opportunity is taken here to record the following species as new to regional faunas.

***Docosia diutina* Plassmann, 1996**

**Material examined**

TURKEY • 1 ♂; Mugla, 11 km east, pine wood and meadow; 1310 m a.s.l.; 1 May 2013; M. Barták and Š. Kubík leg.; in ethanol; JSL-OUC-DOD.

***Docosia flavicoxa* Strobl, 1900**

**Material examined**

GEORGIA • 3 ♂♂; Lagodekhi Nature Reserve, Matsimi river gorge; 17–27 Jun. 2011; G. Japoshvili leg.; Malaise trap; in ethanol; IZBE-0200406.

***Docosia gilvipes* (Haliday in Walker 1856)**

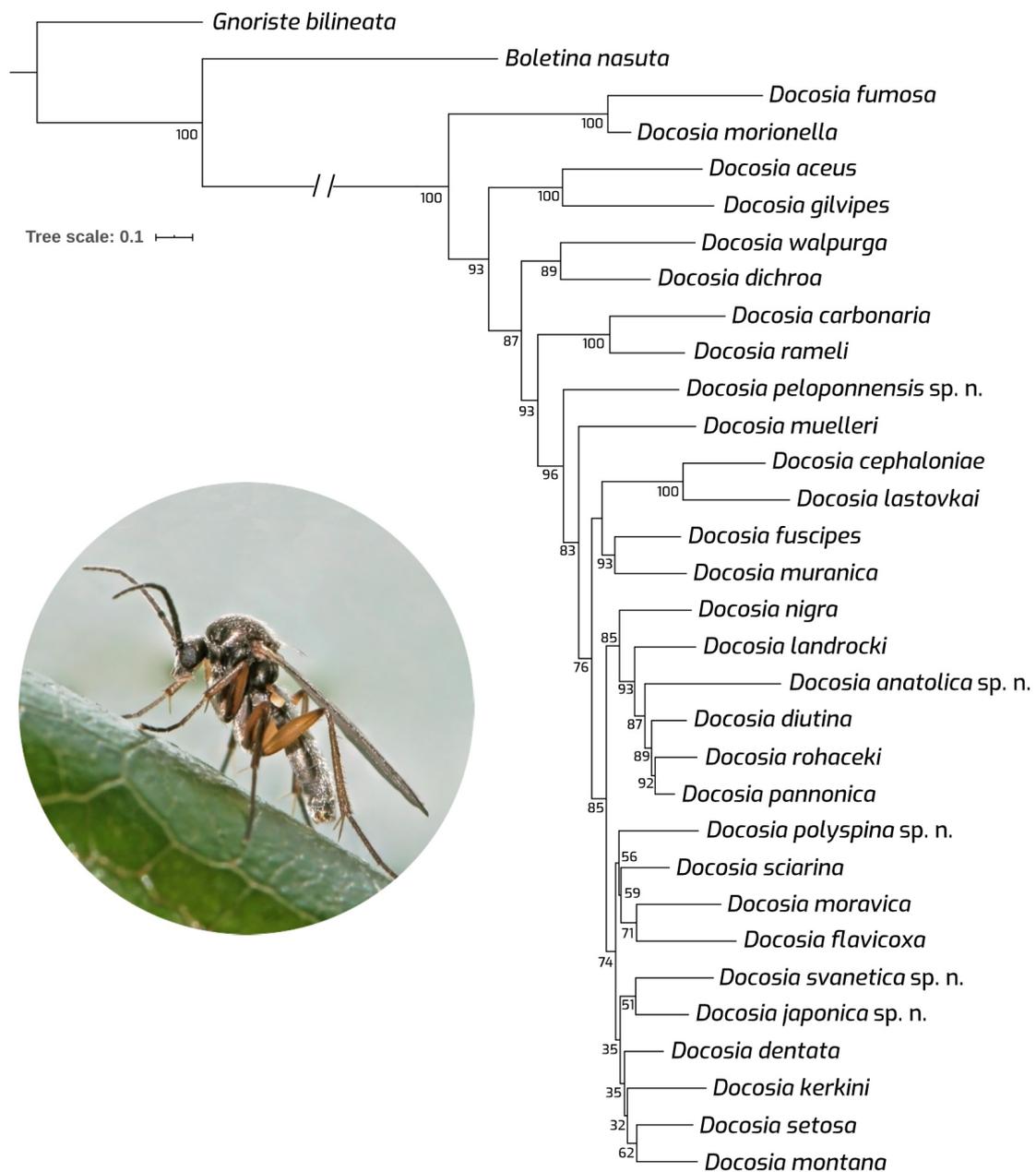
**Material examined**

GEORGIA • 1 ♀; Marelisi southeast of Surami; 41°57'56" N, 43°17'20.7" E; 412 m a.s.l.; 19 May 2012; O. Kurina leg.; sweep net; in ethanol; IZBE-0200407 • 1 ♂; Samegrelo-Zemo Svaneti, S of Lakhushdi, meadow; 42°59'56.3" N, 42°39'0.7" E; 1270 m a.s.l.; 13–14 Jun. 2019; X. Mengual leg.; double Malaise trap; in ethanol; ZFMK-DIP-00067328.

*Docosia kerkini* Kurina & Ševčík, 2011

**Material examined**

BULGARIA • 1 ♂; Pavel Banya, 6 km S; 5–6 May 2018; M. Barták and Š. Kubík leg.; sweep net; in ethanol; JSL-OUC- JSDO34.



**Fig. 8.** Maximum likelihood hypothesis for relationships among selected species of *Docosia* Winnertz, 1863 based on DNA sequence data (28S, ITS2, COI, COII and CytB), 3049 characters. Above node number = ultrafast bootstrap values (ufboot). The stem of the *Docosia* clade has been shortened to half its original length.

*Docosia moravica* Landrock, 1916

**Material examined**

GEORGIA • 1 ♂; Samtskhe-Javakheti, road from Abastumani to Saime; 41°47'48.8" N, 42°50'38" E; 1725 m a.s.l.; 10–11 May 2019; X. Mengual leg.; Malaise trap; in ethanol; ZFMK-DIP-00067327.

*Docosia pannonica* Laštovka & Ševčík, 2006

**Material examined**

GEORGIA • 1 ♂; Gvelethi near Kazbegi; 42°42'17.3" N, 44°37'15.7" E; 1644 m a.s.l.; 16 May 2012; O. Kurina leg.; light trap; in ethanol; IZBE-0200408.

*Docosia rameli* Kurina & Ševčík, 2011

**Material examined**

SLOVAKIA • 1 ♂; Horša, Horšianska dolina; 48°15'07.88" N, 18°41'57.30" E; 230 m a.s.l.; 26 Apr.–5 May 2017; Ľ. Vidlička and O. Majzlan leg.; Malaise trap; in ethanol; JSL-OUC- JSDO29.

**Discussion**

If we compare the tree in Fig. 8 with the previous molecular phylogeny of *Docosia* by Ševčík *et al.* (2016a), it is evident that more extensive taxon sampling and the addition of one gene fragment have not increased the resolution substantially. Although we used a different algorithm (IQ-Tree vs RAxML in the previous paper), the node support values are in both cases relatively low, indicating that new and more variable molecular markers should be tested to recover the interrelationships among particular species of *Docosia*.

Several pairs of closely related species can be recognized in the molecular tree (Fig. 8), although a close relationship between relevant species in a pair is not always clear from a morphological point of view. The first example is the well-supported clade comprising *Docosia fumosa* and *D. morionella*. Although the former species is widely distributed, relatively well known, and its terminalia were figured, e.g., by Laštovka & Ševčík (2006), the latter species is extremely rare, with only several records available and with male terminalia figured by Chandler & Blasco-Zumeta (2001). It is difficult to find distinct similarities between these two species. The laterotergite is pubescent in *D. fumosa* but bare in *D. morionella*, showing that pubescence of laterotergite is apparently a homoplastic character, evolved in parallel in different lineages (see also Ševčík *et al.* 2016a). Concerning the colouration of the legs, both *D. fumosa* and *D. morionella* have a black hind femur, but this character state is also present in *D. carbonaria* and some other species. On the other hand, the male terminalia of *D. morionella* are rather similar to those of *D. gilvipes*, especially in the overall shape of the gonocoxites and the structure of the cerci, as noted by Chandler & Blasco-Zumeta (2001). However, *D. gilvipes* differs in having Sc setose and ending free. The latter character has been widely used to separate *D. gilvipes* from the other Palearctic species. However, another very similar species, *D. pseudogilvipes* Kurina, 2008 recorded from the Italian Alps, has Sc bare and ending in R (Kurina 2008). Moreover, other species considered as related to *D. gilvipes*, e.g., the Nearctic *D. aceus* Garrett, 1925 (and probably also *D. mcgrawi* Taber, 2018) have Sc ending in R, while the recently described Neotropical species *D. adusta* Oliveira & Amorim, 2011 has Sc ending free and bare (Oliveira & Amorim 2011). Such a variation in wing venation shows that morphological characters other than on male terminalia should be used with caution in phylogenetic reconstructions of *Docosia*, and possibly also within other genera of fungus gnats. However, a careful case-by-case study is required before any general conclusion can be drawn.

Two species of the *D. dichroa* Loew, 1869 group are easily recognizable thanks to their rufous abdomen, a unique feature among all species of *Docosia*. We follow here the interpretation of these two species by Taber (2011, 2012), although Petr Laštovka in his unpublished material (original figures plus hand-made notes available to the first author) came to the conclusion that the species recently named by Taber (2011) as *D. walpurga* is actually true *D. dichroa*, and *D. dichroa* sensu Johannsen (1909) and Taber (2011, 2012) needs a new name. However, we leave this issue here as open, until a re-examination of the type material is made (see also discussion in Taber 2018).

Another well-supported clade is *D. carbonaria* + *D. rameli*. Both species share the pubescence of the laterotergites, the similar shape of gonostylus and the overall structure of the posterior margin of the gonocoxites. Also, *D. cephaloniae* and *D. lastovkai* share many synapomorphies, mainly on the male terminalia, and several other species belong to this group of species as well (cf. Chandler *et al.* 2006). Both *D. carbonaria* and *D. lastovkai* apparently represent complexes of closely related species, especially in the Mediterranean region and eastern Europe, and other species may be discovered in the future.

Three southeastern European species (*D. diutina*, *D. pannonica* and *D. rohaceki*) represent a specific and well-defined group, based on their male terminalia. Especially the longitudinal tergite 9, distinct posterolateral processes of gonocoxites and the apically modified (ramifying) setae at the posteroventral margin of the gonocoxites are very characteristic. These species also share some colour characteristics, e.g., a dark basal half of the hind coxa. Most of these features are also present in *D. anatolica* sp. nov. and *D. nigra*. All these species constitute a monophyletic group in the molecular tree (Fig. 8). In addition, several species not included in our molecular analysis certainly belong to this group, e.g., *D. juxtamontana* Chandler, 2004.

## Acknowledgements

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