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A new species of *Dasyhelea* Kieffer, 1911 (Diptera: Ceratopogonidae) visiting flowers of *Aristolochia* in France

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Abstract: Both sexes of *Dasyhelea porczynskii* Szadziewski, Blatrix et Dominiak, **n. sp.** collected from flowers of *Aristolochia clematitis* L. in southern France are described and illustrated. The new species is closely related to *D. leptoclada* Remm, 1967 female of which is briefly diagnosed for the first time.

Keywords: subgenus *Pseudoculicoides*, saline habitats, *Aristolochia clematitis* L., European birthwort, COI, DNA barcoding

Introduction

Dasyhelea Kieffer, 1911 is a large and complex genus of biting midges (Diptera: Ceratopogonidae) containing more than 600 extant species worldwide (Borkent & Dominiak 2020, Borkent *et al.* 2022). From Europe, including the Canaries, circa 65 species were reported so far (Dominiak & Salmela 2023). The genus includes biting midges of diverse morphology and biology. Unlike other ceratopogonids, there are no predaceous or ectoparasitic species among *Dasyhelea* as females have reduced, vestigial mandibles and maxillae that lack teeth. However, like other ceratopogonids, adult males and females use honeydew and nectar as sources of energy. There is still a lot of gaps in knowledge about the biology and faunistics of species belonging to this group of ceratopogonids. The immature stages are aquatic or semiaquatic and many species of *Dasyhelea* live in algae, mosses, or liverworts and among plants on water margins. Other inhabit puddles, springs, swamps, peat-bogs and moist soil or phytotelmata, which are small waterbodies formed, for example, in tree hollows, damaged tree trunks and leaf axils (Dominiak 2012).

The purpose of this paper is to describe another species of the genus *Dasyhelea* from Europe, based on specimens found in birthwort's flowers.

Material and methods

The specimens were collected together with flowers of *Aristolochia clematitis* L. in the protected areas *Site Naturel Protégé des Salines de Villeneuve* (hereafter 'Salines de Villeneuve') and *Réserve Naturelle Nationale de l'Estagnol* (hereafter 'Estagnol'), Hérault, Occitania, southern France. Collections in Salines de Villeneuve were authorized by the *Conservatoire du Littoral*, owner of the site, and followed the recommendations of the *CEN Occitanie*, co-manager of the site. Collections in Estagnol followed the requirements of the ten-year management plan of the reserve, approved by the Regional Scientific Council for Natural Heritage (CSRPN) and the Ministry of Ecological Transition.

All specimens examined for this study are housed in the collection of extant invertebrates at the Museum of Amber Inclusions (CEIG), Department of Invertebrate Zoology and Parasitology, University of

Gdańsk, Poland. The specialized traditional morphological terms and abbreviations used in the paper are explained in the papers by Szadziewski (1986) and Dominiak (2012).

One specimen of the new species was collected at the Salines de Villeneuve (22 April 2021) and three at the Estagnol (3 May 2021), and two specimens of *Dasyhelea leptoclada* Remm, 1967 were collected at the Estagnol (3 and 20 May 2021). For each specimen, DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the protocol by Cruaud *et al.* (2019) on whole specimens without damaging the integument, with a digestion step of 2 hours instead of overnight. The mitochondrial marker COI (658 bp), coding for part of the cytochrome c oxidase subunit 1, was amplified by polymerase chain reaction (PCR) using the primer pair LCO-1490 (5'-GGTCAACAAATCATA AAGATAT TGG-3') and HCO-2198 (5'-TAAACTC AGGGTGACCAAAAATCA-3) (Folmer *et al.* 1994). Sanger dideoxy sequencing of PCR amplicons was performed by Eurofins Genomics (Germany) in both directions using the same primers as those used for the initial amplification. Sequences were edited using CodonCode Aligner (CodonCode Corporation, Dedham, MA, USA), and contigs were built from forward and reverse sequences generated for each gene. Pairwise genetic distance was estimated using the Kimura 2-parameter model (K2p).

The COI sequences were deposited on the GenBank under the following accession numbers: OR960968 (*Dasyhelea porczynskii* Szadziewski, Blatrix et Dominiak, **n. sp.**, RB0003687, male holotype), OR960969 (*D. porczynski*, RB0003782, male paratype), OR960970 (*D. porczynski*, RB0003799c, male paratype), OR960967 (*D. porczynski*, RB0003799, female paratype), PP538723 (*D. leptoclada*, RB0003786, female) and PP538724 (*D. leptoclada*, RB0003930, male).

The new name has been registered in Zoobank: urn:lsid:zoobank.org:act:D84D7897-4D5D-4422-B3C4-83F50573CA31.

Systematics

Family Ceratopogonidae Newman, 1834

Subfamily Forcipomyiinae Lenz, 1934

Tribe Dasyheleini Lenz, 1934

Genus *Dasyhelea* Kieffer, 1911

Subgenus *Pseudoculicoides* Malloch, 1915

Dasyhelea porczynskii Szadziewski, Blatrix et Dominiak, **n. sp.**

Material examined

Holotype male: RB0003687, Site Naturel Protégé des Salines de Villeneuve, collected 22.04.2021, altitude 1 m, latitude 43.51757, longitude 3.82667, within a flower of *Aristolochia clematitis*, leg. Rumsais Blatrix.

Paratypes: male RB0003782, 03.05.2021, Réserve Naturelle Nationale de l'Estagnol, altitude 6 m, latitude 43.53905, longitude 3.83624, within a flower of *Aristolochia clematitis*, leg. Rumsais Blatrix; male RB0003799c, all data as above; female RB0003799, all data as above.

Diagnosis

Male of the new species is unique in having gonostyli with short ventral finger-like projection at base, long cylindrical apicolateral processes of tergite 9 and stout F-like right paramere with blunt apex. Female is in general similar to many other species of the subgenus, except notum with transverse lumen and small anterior lobe, and a pair of oval and well-visible sclerites under sternite 8.

Description

Male (n=2). Body, including scutellum and halteres, black, tarsi pale (Fig. 1D). Eyes with long macrotrichia between ommatidia. Antennal flagellum (Figs 1A-B) with 13 flagellomeres, distal 4 flagellomeres elongated, terminal flagellomere with slightly pointed apex; length 0.67-0.70 mm, antennal ratio AR 0.97. Palpus 5 segmented; palpomere 3 relatively long (Fig. 1C), length 68-75 μ m, palpal ratio PR 4.2-4.3. Scutellum black with some marginal setae. Wing with numerous

macrotrichia (Fig. 1E), first radial cell absent; wing length 0.96-1.07 mm, costal ratio CR 0.44-0.45. Tarsi slender, unmodified, tarsal ratio of hind leg TR(3) 2.0-2.2. Genitalia (Figs 2A-H). Sternite 9 (Fig. 2A) extended, tapering towards apex. Tergite 9 (Figs 2A, C) with long, cylindrical and slender apicolateral processes bearing single apical seta. Gonocoxite without modifications. Gonostylus (Figs 2A-B) slender, slightly curved, armed at basal third with short

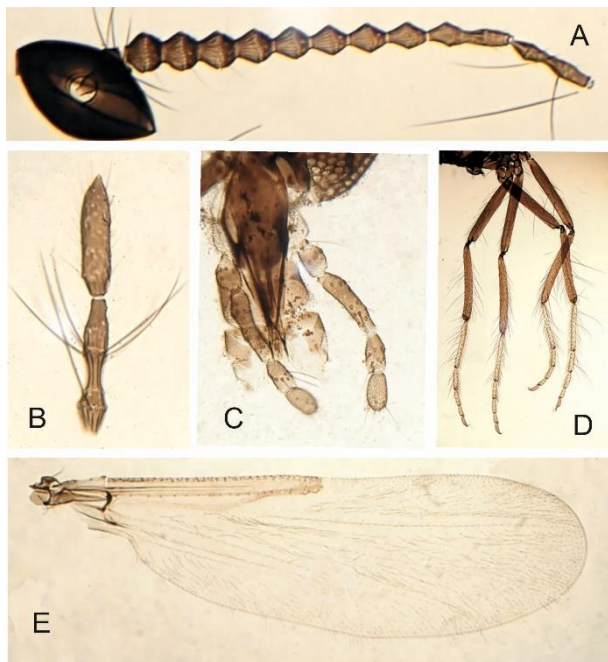


Fig. 1. *Dasyhelea porczynskii* Szadziewski, Blatrix et Dominiak, n. sp., male: A – flagellomeres 1-11; B – flagellomeres 12-13; C – palpi; D – legs; E – wing.

Female. Similar to male. Antennal flagellum 0.48 mm long, antennal ratio AR 0.88. Flagellomeres slightly and gradually increasing in length from 2 to 13 (Figs 3A-B). Clypeus with 11 setae in two rows, at base with distinct lateral lobes. Third palpal segment relatively long (Fig. 3B); length 65 μm , palpal ratio PR 3.7. Wing membrane with numerous macrotrichia, first radial cell reduced (Fig. 3C), wing length

0.94 mm, costal ratio CR 0.45. Legs (Fig. 3D) with hind leg tarsal ratio TR(3) 2.0. Genitalia (Fig. 3E). Two ventral, ovoid and well-visible sclerites present under abdominal sternite 8. Notum of subgenital plate with indistinct, transverse, narrow lumen and small tongue-like anterior lobe. Seminal capsule single, ovoid and small, 50 x 40 μm , with short neck. Cerci short.

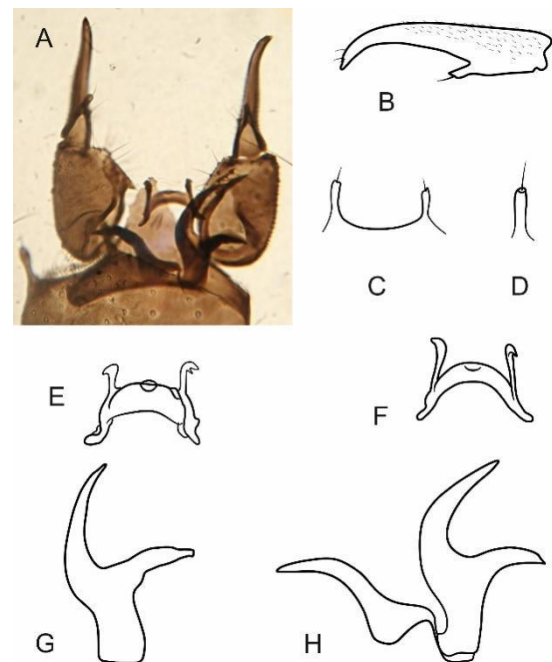


Fig. 2. *Dasyhelea porczynskii* Szadziewski, Blatrix et Dominiak, n. sp., male genitalia: A – ventral view; B – gonostylus; C, D – apicolateral processes of tergite 9; E, F – aedeagus; G – right paramere; H – left and right parameres.

0.94 mm, costal ratio CR 0.45. Legs (Fig. 3D) with hind leg tarsal ratio TR(3) 2.0. Genitalia (Fig. 3E). Two ventral, ovoid and well-visible sclerites present under abdominal sternite 8. Notum of subgenital plate with indistinct, transverse, narrow lumen and small tongue-like anterior lobe. Seminal capsule single, ovoid and small, 50 x 40 μm , with short neck. Cerci short.

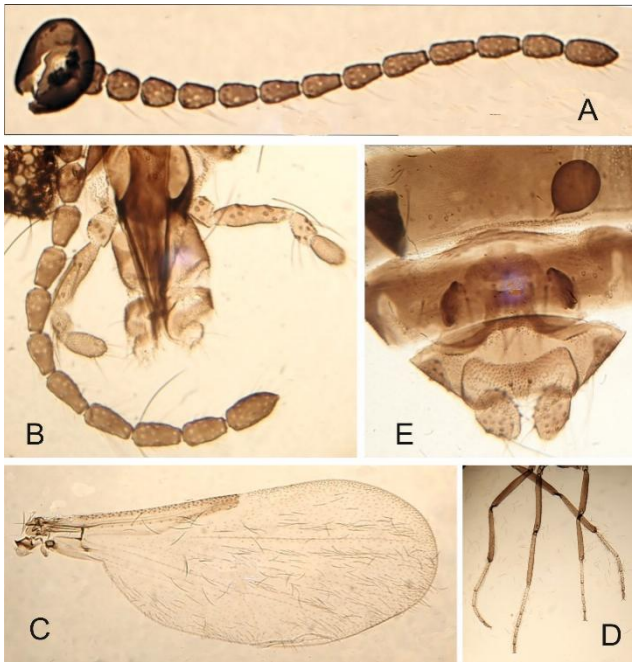


Fig. 3. *Dasyhelea porczynskii* Szadziwski, Blatrix et Dominiak, n. sp., female: A – flagellum; B – palpi; C – wing; E – genitalia; D – legs.

Etymology

The new species is dedicated to Józef Porczyński (1848-1916), a prominent dipterist of Polish origin who worked mostly on taxonomy of dipterans in Russian empire when Poland was occupied by imperialistic neighboring states: Austria, Germany and Russia from 1795 to 1918.

Distribution

So far, the newly described *Dasyhelea* is known only from Occitania region in southern France. The sampling localities are characterized by presence of saline habitats, and we suspect that *D. porczynskii* may be a halophilic species. The males and female of the new species were collected from flowers of European birthwort together with *D. leptoclada*. The latter species is halobiontic (Szadziwski 1983, Dominiak 2012).

Discussion

The new species is very similar to *D. leptoclada* which is widely distributed in Europe (Dominiak 2012). Males of the two *Dasyhelea* species differ in many details of genitalia, especially the shape of the apical projection of their right paramere. In *D. porczynskii* it is stout and rather short, while in *D. leptoclada* the apical projection is slender and distinctly curved (Figs 5A-B). Females of both species look very much alike, and their identification was based on DNA match with the males. The redescription of *D. leptoclada* female given by García Sánchez & Sahuquillo Herráiz (1987) has only formal information as it is totally devoid of diagnostic specific characters. Here, the female of the latter species is for the first time illustrated (Figs 4A-E) and briefly diagnosed based on the specimen collected in France (RB3786). *Dasyhelea leptoclada* differs from the female of *D. porczynskii* in having more cylindrical antennal flagellomeres, yellow scutellum, pale halteres, and subgenital plate with more circular lumen and lacking the characteristic small anterior lobe present in the newly described species. The K2p genetic distance between pairs of specimens of *D. porczynskii* and *D. leptoclada* varied between 13 % and 13.6 %, confirming that the two taxa are distinct species, but are genetically related. The new *Dasyhelea* species resembles closely *D. franzella* Goetghebuer, 1950 from Austria (Fig. 5C), especially in the shape of right paramere and gonostylus bearing ventral projection. However, it differs in having blunt, not bilobed apex of right paramere and slender apicolateral processes of tergite 9. Gonostylus with ventral, finger-like projection is present also in male of *D. borbonica* Clastrier, 1959 from Reunion (Fig. 5D), but in contrary to *D. porczynskii*, apex of the right paramere in this species is bilobed.

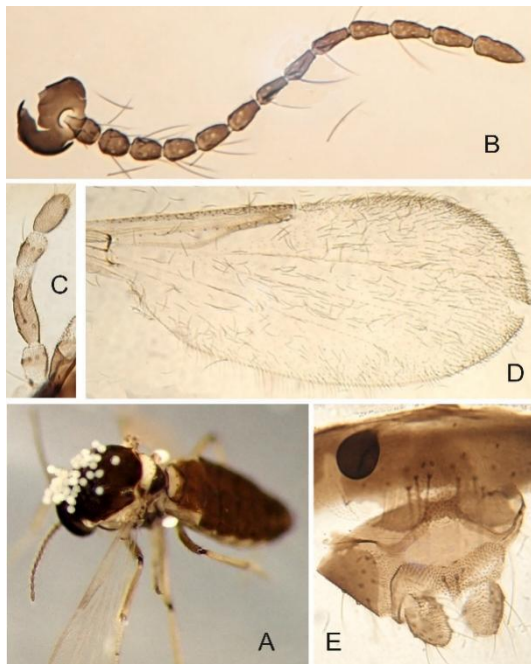


Fig. 4. *Dasyhelea leptoclada* Remm, 1967, female (RB3786): A – total habitus; B – flagellum; C – palpus; D – wing; E – genitalia.

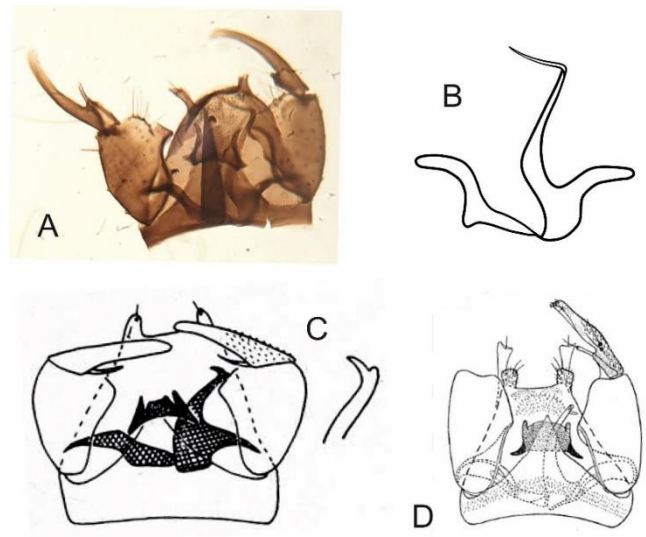


Fig. 5. Male genitalia of *Dasyhelea*: A – *D. leptoclada* Remm, 1967, ventral aspect (RB3930); B – *D. leptoclada* Remm, 1967, parameres; C – *D. franzella* Goetghebuer, 1950 (from Szadziewski 1986); D – *D. borbonica* Clastrier, 1959 (from Clastrier 1959).

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