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Research article

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A new *Dicranophragma* (Diptera: Limoniidae) from a hypogeous environment in Catalonia (Iberian Peninsula)

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Abstract. A new species of Limnophilinae (Diptera: Limoniidae), *Dicranophragma (Brachylimnophila) relictum* Mederos sp. nov., is described. This is the third species of this genus recorded from the Iberian Peninsula. This new species was discovered in a cave in the Sant Llorenç del Munt i Serra de l'Obac Natural Park, Barcelona Province (Catalonia) and it is the first record of a species of this genus from a hypogeous environment. The absence of suitable ecological conditions for the survival of this new species, particularly in the vicinity of the cave, suggests that this population of *D. relictum* Mederos sp. nov. is isolated. *Dicranophragma relictum* Mederos sp. nov. is characterized by the following features: a general grey-to-greenish-grey coloration on its thorax and a dark-grey abdomen; wings more than four times longer than wide; pale-brown-to-yellowish tinted wings, with brown veins and a well-marked stigma; in the male genitalia the upper part of the aedeagus is acute when viewed laterally and is longer than the lower part. A key is provided for separating the three species of *Dicranophragma* known to be present in the Iberian Peninsula.

Keywords. Limnophilinae, Tipuloidea, crane flies, cave dweller, Palearctic.

Mederos J., Gago S., Brañas N., Fadrique F., Caballero-López B. & Masó G. 2020. A new *Dicranophragma* (Diptera: Limoniidae) from a hypogeous environment in Catalonia (Iberian Peninsula). *European Journal of Taxonomy* 724: 109–121. <https://doi.org/10.5852/ejt.2020.724.1163>

Introduction

The family Limoniidae Rondani, 1856 contains almost 10 700 described species that are found across the whole globe except Antarctica (Oosterbroek 2020). The genus *Dicranophragma* Osten Sacken, 1860 includes 49 described species and subspecies, grouped into three subgenera: *Brachylimnophila* Alexander, 1966, *Dicranophragma* and *Mixolimnomyia* Savchenko, 1979. Species of this genus have been described from the Nearctic, Palearctic, Afrotropical and Oriental regions (Oosterbroek 2020), the latter region having the greatest number of recorded species. Before the European species were reviewed by Starý & Reusch (2009), *Brachylimnophila* was treated as a subgenus of *Neolimnomyia* Ségué, 1937. From the Iberian Peninsula, two species of *Dicranophragma* (*Brachylimnophila*) have been recorded to date, both of which are widely distributed throughout the Western and Eastern Palearctic: *Dicranophragma* (*Brachylimnophila*) *adjunctum* (Walker, 1848), recorded from Andorra (Canillo) and Spain (Barcelona, Guipúzcoa and Teruel), and *Dicranophragma* (*B.*) *nemorale* (Meigen, 1818), reported from Andorra (Canillo), Portugal (Serra da Estrela) (Eiroa 2002) and more recently from Spain (Jaén) (Hancock 2020; Hancock & Hewitt 2020).

In 2019, a team from the Arthropod Department of the Barcelona Natural Sciences Museum (Museu de Ciències Naturals de Barcelona, MCNB) began a series of visits to Avenc del Daví, a cave in the Sant Llorenç del Munt i l'Obac Natural Park that had been selected as part of a study of two hypogean invertebrates, an isopod crustacean, *Stenasellus virei* Dollfus, 1897, and a leiodid coleopteran, *Troglocharinus kiesewetteri sanllorensi* (Zariquiey, 1924), that are endemic to the area and enjoy a certain degree of protection. During the study, several specimens of a new species of *Dicranophragma* were observed and captured in Avenc del Daví. This cave is well studied since it houses a hibernation colony of the bat *Miniopterus schreibersii* (Kuhl, 1817) and so is regularly monitored (Serra-Cobo *et al.* 1998; Colombi *et al.* 2019). To protect *M. schreibersii*, access to this cave has been controlled since 1994 from November to the end of May.

Material and methods

This work focuses on the results obtained from the explorations of the Avenc del Daví (932 m a.s.l.) (Fig. 1), a deep cavity in NE-facing Eocene montane conglomerates in the Sant Llorenç del Munt i l'Obac Natural Park. This massif is part of an N–S diaclase, deeply degraded by decalcification and with a vertical development of up to -65 meters. The Sant Llorenç and l'Obac massif is predominantly calcareous but with some intercalations of siliceous rocks (DIBA 2020). The characteristic vegetation of the natural park is evergreen oak forest (*Quercus ilex* L.), which at lower altitudes includes typical Mediterranean shrubs such as *Erica* sp. and *Arbutus unedo* L. However, the area where the Avenc del Daví is located, above 800 m a.s.l., enjoys a relatively humid climate that allows forests to be enriched with species from more humid regions such as *Sorbus aria* (L.) Crantz, *Buxus sempervirens* L. and *Quercus pubescens* Willd. The entrance to the cave system itself is surrounded by a vegetation associated with Mediterranean oak forests. Access is through two wide entrances that permit abundant vegetable and animal organic material to fall into its interior. At -40 m, there is a chamber (about 50 × 9 m) where all the fallen material accumulates.

Although the description of this new species is based on dry-mounted specimens, part of the type material were preserved in 70% ethanol. Specimens were examined with a Motic SMZ-168 Zoom stereo microscope and a Kyowa Unilux-12 83-483D. Images were taken by multi-stack with iPhone XR and subsequently processed with Helicon Focus 7. Measurements were made with an ocular reticule. When necessary, the male genitalia were macerated in a 4% KOH solution and finally preserved in microvials with 70% alcohol, together with the respective specimens. We have adopted the classification according to Starý (1992), also used by the *Catalogue of the Craneflies of the World (CCW)* (Oosterbroek 2020), that includes *Dicranophragma* within the Limnophilinae Bigot, 1864, family Limoniidae. The

morphological terminology mainly follows that of Gelhaus (2009) and Cumming & Wood (2009), but also Starý & Reusch (2009) for male terminalia.

Depositories

MCNB = Barcelona Natural Sciences Museum, Barcelona, Spain

MNHN = Muséum national d'histoire naturelle, Paris, France

RBINS = Royal Belgian Institute of Natural Sciences, Brussels, Belgium

Full labels are given for each type specimen. Labels are rectangular and white, unless otherwise mentioned. An inventory number starting with the initials MZB (former acronym Museum of

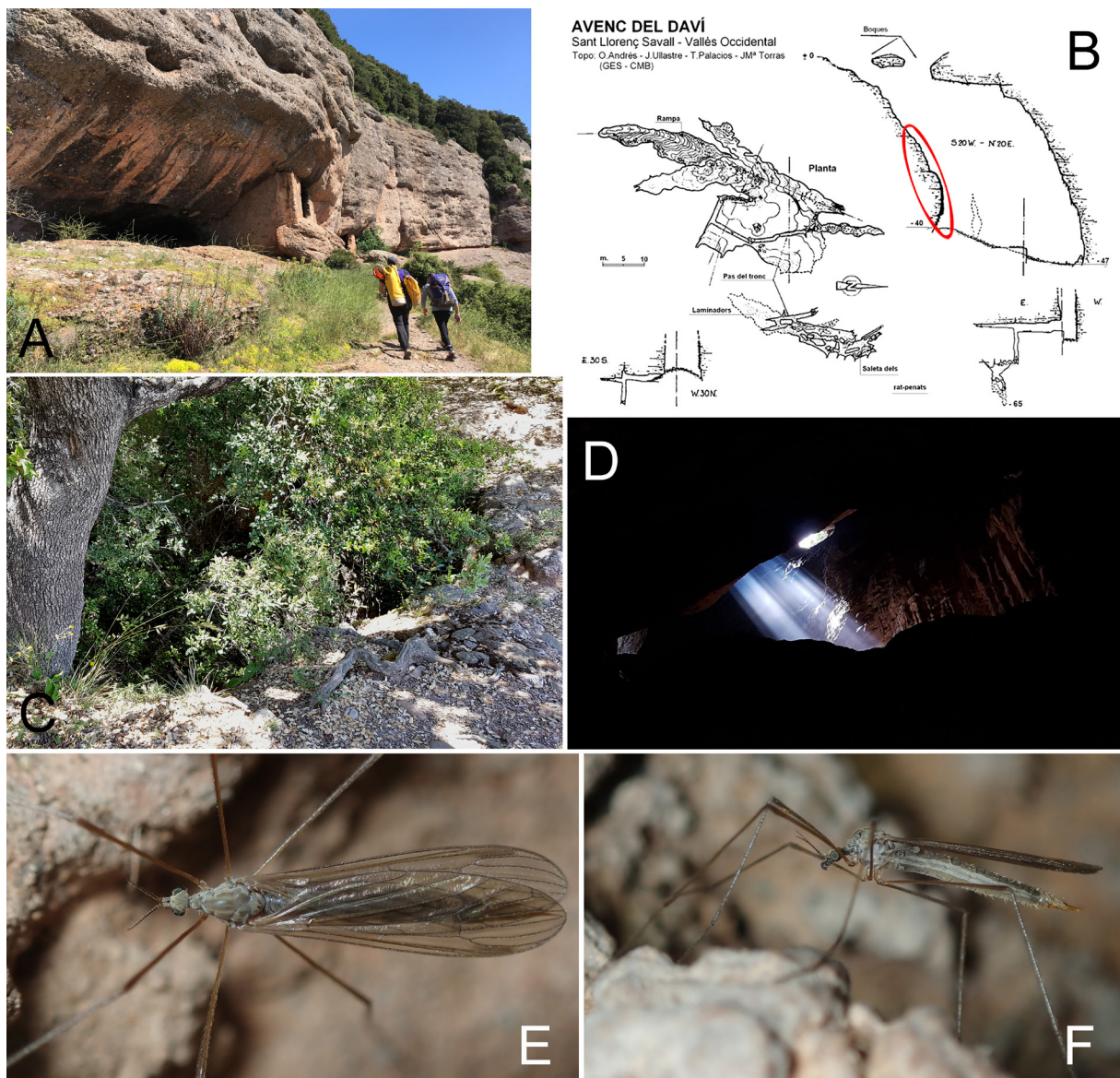


Fig. 1. A. General view of the landscape at the approach to Avenc del Daví. B. Cave topography. Indicated in red where *Dicranophragma (B.) relictum* Mederos sp. nov. was found. C–D. Cave entrance and view from inside. E–F. Female specimen of *D. (B.) relictum* Mederos sp. nov. (MZB 2019-0951, MCNB) photographed in the cave (E, dorsal view; F, lateral view).

Zoology of Barcelona) was attached to each specimen deposited in MCNB and the data were entered into the respective collection database <http://zoologiaenlinia.museuciencies.cat>. Likewise, an inventory number starting with the initials ED (indicating Entomology and Diptera, respectively) was attached to the specimens deposited in MNHN and data were added to the respective collection database <https://science.mnhn.fr/institution/mnhn/collection/ed/item/search>. Finally, an inventory number starting with the initials I.G. (Inventory General) was attached to the specimens deposited in RBINS and data were added to the DARWIN database <https://darwin.naturalsciences.be/> and also on <http://virtualcollections.naturalsciences.be/>.

Abbreviations

Wing venation

A ₁ , A ₂	= anal veins
CuA ₁ , CuA ₂	= branches of anterior branch of cubital vein
dm	= discal medial cell
M ₁ , M ₂ , M ₃	= branches of medial vein
Rs, R ₂₊₃ , R ₂₊₃₊₄ , R ₁₊₂ , R ₃ , R ₄ , R ₅	= posterior branches of radius
r ₃	= radial cell
Sc	= subcostal vein

Female terminalia

iw	= inner wall of tergite 9
spr	= spermathecae
va	= vaginal apodeme

A total of 10 male and 16 female specimens of this new species of *Dicranophragma* were studied and a key is offered for the identification of the three species of this genus present in the Iberian Peninsula.

Key to the species of the genus *Dicranophragma* known from Iberian Peninsula

1. Wing without spots, only stigma marked, sometimes poorly defined; light brown 2
– Stigma dark, well-marked, light brown spots at origin of Rs, median vein and basal end of discal cell *D. (B.) adjunctum* (Walker, 1848)
2. Stigma poorly defined, light-grey pleura, antenna reaching to about mid coxa, body length 6.0–8.5 mm, wing length 6.0–9.5 mm *D. (B.) nemorale* (Meigen, 1818)
– Stigma light-brown, pleura grey to greenish gray, antenna barely reaches fore coxa, body length 7.0–11.5 mm, wing length 7.4–10.5 mm *D. (B.) relictum* Mederos sp. nov.

Results

Phylum Arthropoda von Siebold, 1848
Class Insecta Linnaeus, 1758
Order Diptera Linnaeus, 1758
Family Limoniidae Rondani, 1856
Subfamily Limnophilinae Bigot, 1864

Genus *Dicranophragma* Osten Sacken, 1860

Dicranophragma Osten Sacken, 1860: 240 (as subgenus of *Limnophila*; original description). Type species: *Limnophila fuscovaria* Osten Sacken, 1860 (Nearctic), by monotypy.

Subgenus *Brachylimnophila* Alexander, 1966

Brachylimnophila Alexander, 1966: 119 (as subgenus of *Limnophila*; original description). Type species: *Limnophila brevifurca* Osten Sacken, 1860 (Nearctic), by original designation.

Dicranophragma (Brachylimnophila) relictum Mederos sp. nov.
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 Figs 1E–F, 2–3, 4A–D, G, 5A–B, D

Differential diagnosis

General coloration grey to greenish grey, greenish grey hue on thorax in some specimens; dark grey on abdomen. Wings yellowish-brown, veins brown, a little more than four times as long as wide, stigma light brown, poorly defined in some specimens. Petiole of M_1 and M_2 2.5–3 times as long as these veins. Male terminalia in lateral view with acute distal upper part of aedeagus, longer than the lower part. Female terminalia with three spermathecae. Body length 7.0–11.5 mm, wing length 7.4–10.5 mm. The morphologically closest species is *D. nemorale* (Figs 4E, H, 5E). These two species can be separated by the bi-colored appearance of *D. nemorale* (light-grey thorax and pale-brown abdomen) compared to the more uniform grey to greenish grey coloration of *D. relictum* Mederos sp. nov., by the color of the legs (completely yellow in *D. nemorale* in contrast to the progressively darker brown towards tarsi in *D. relictum* Mederos sp. nov.), and by the shape of the aedeagus in male terminalia (Fig. 4), in both lateral and dorsal views.

Etymology

The new specific name is a qualifying adjective and a term that comes from the Latin word ‘*relictum*’ that refers to the isolated situation of this species.

Material examined**Holotype**

SPAIN • ♂; Catalonia, Barcelona, Sant Llorenç Savall, Parc Natural Sant Llorenç del Munt i l’Obac, Avenc del Daví; 932 m a.s.l.; 25 Jul. 2019; J. Mederos, S. Gago, F. Fadrique and X. Oller leg.; specimen collected at -40 m depth; dry specimen, pinned with minute pin on foam; MZB 2019-0985.

Paratypes

SPAIN • 1 ♂, 2 ♀♀; same collection data as for holotype; dry specimens; MZB 2019-0986 to 2019-0988 • 1 ♂, 2 ♀♀; same collection data as for holotype; J. Mederos leg.; specimens collected at -20 m depth; dry specimens; MZB 2019-0989 to 2019-0991 • 3 ♂♂, 1 ♀; same locality as for holotype; 19 Jun. 2019; J. Mederos, S. Gago and F. Fadrique leg.; specimens collected at -40 m depth; dry specimens; MZB 2019-0944 to 2019-0947 • 1 ♂, 2 ♀♀; same locality as for holotype; 19 Jun. 2019; J. Mederos, S. Gago and F. Fadrique leg., specimens collected at -40 m depth; in ethanol 70%; MZB 2019-0949 to 2019-0951 • 1 ♂, 1 ♀; same locality as for holotype; 19 Jun. 2019; J. Mederos leg.; specimens collected between -7 to -27 m depth; in ethanol 70%; RBINS I.G. 34107 • 1 ♂, 1 ♀; same locality as for holotype; 19 Jun. 2019; J. Mederos leg.; specimens collected between -7 to -27 m depth; in ethanol 70%; MNHN ED10669, ED10670 • 5 ♀♀; same locality as for holotype; 19 Jun. 2019; J. Mederos leg.; specimens collected between -7 to -27 m depth; in ethanol 70%; MZB 2019-0954, MZB 2019-0955, MZB 2019-0980.

Other material examined

SPAIN • 1 ♂, 2 ♀♀; same collection data as for holotype; in absolute ethanol; MZB 2019-0992 to 2019-0994.

Description

Male

MEASUREMENTS. Body length (without antennae) 7.0–7.2 mm. Antenna length 1.3–1.5 mm. Wing length 7.4–8.0 mm.

HEAD (Fig. 2). Grey pruinosity, some specimens with small dark grey dots; small concavity (depression) on vertex, which seems darker in contrast to the surrounding area due to reflection of light. Antenna

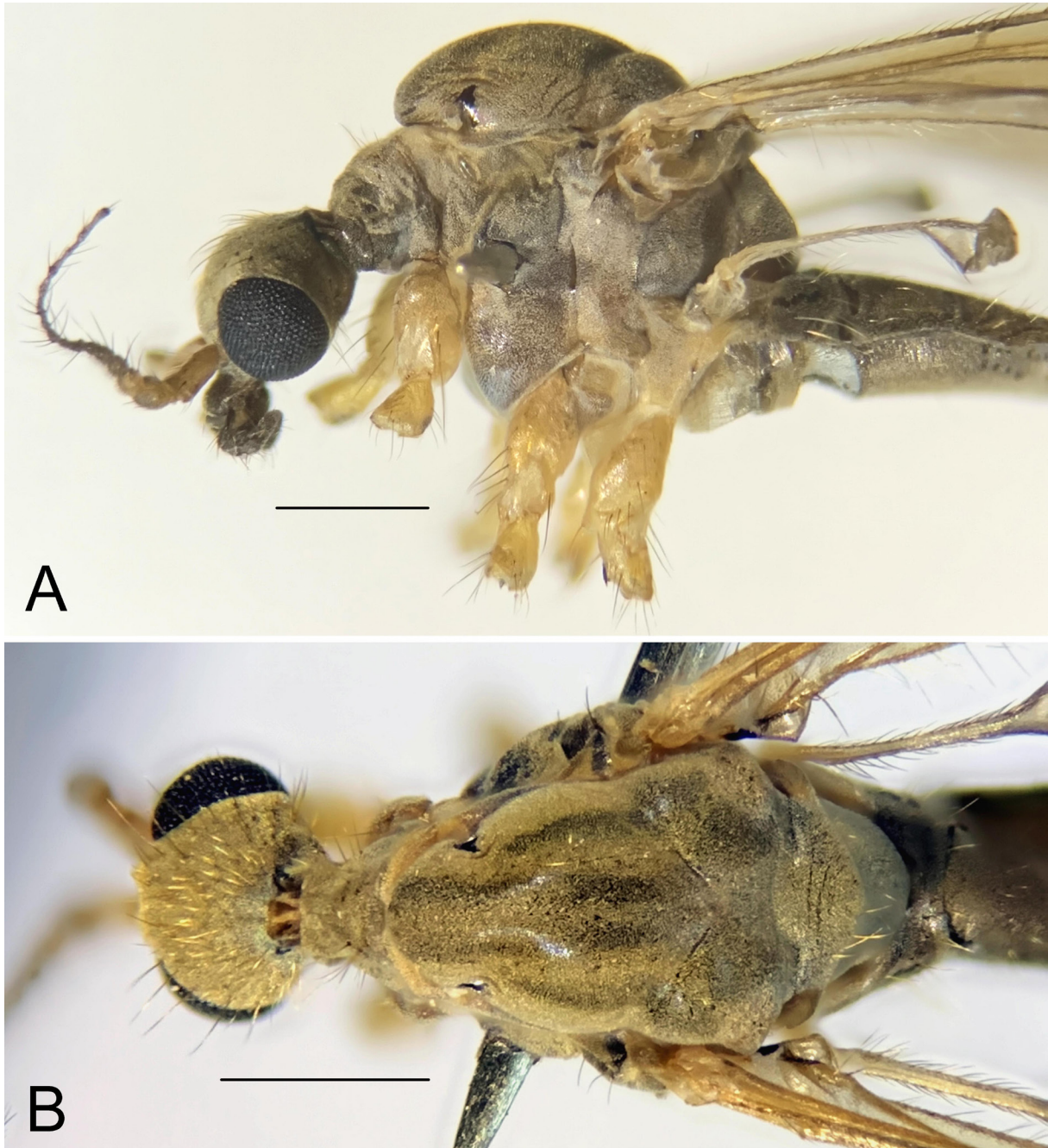


Fig. 2. *Dicranophragma (B.) relictum* Mederos sp. nov., holotype, ♂ (MZB 2019-0985, MNCB). Head and thorax. **A.** Lateral view. **B.** Dorsal view. Scale bars = 0.5 mm.

barely reach fore coxa; scape and pedicel yellow to yellowish brown, pedicel brown in distal half; first flagellomere yellow to yellowish brown, other flagellomeres brown to dark brown towards tip; terminal flagellomere longer than penultimate one; flagellomeres ovoid, narrower and shorter towards tip; verticils about 1.5–2 times as long as their respective flagellomeres.

THORAX (Fig. 2). Grey to greenish grey, occasionally with some brown hues; some specimens have small, dark grey to black dots scattered overall; prescutum with four, dark grey to brownish grey longitudinal stripes, two long central stripes and two other short lateral stripes; in some specimens, the two longitudinal central stripes merge into one broad stripe. Pleura light grey to greenish grey. Coxae and trochanters yellow to yellowish brown. Legs yellowish brown, progressively browner towards tarsi.

WING (Fig. 3). Tinged pale brown to yellowish brown, just over four times as long as wide, veins brown to dark brown, stigma light brown and in some specimens poorly defined. Venation: R_{2+3} of variable length, from just longer than R_{2+3+4} to more than twice as long; petiole of M_1 and M_2 between 2.5–3 times as long as these veins but in some specimens up to six times this length due to reduction of cell m1 (Fig. 3H); cell dm hexagonal, with some variability in shape and length.

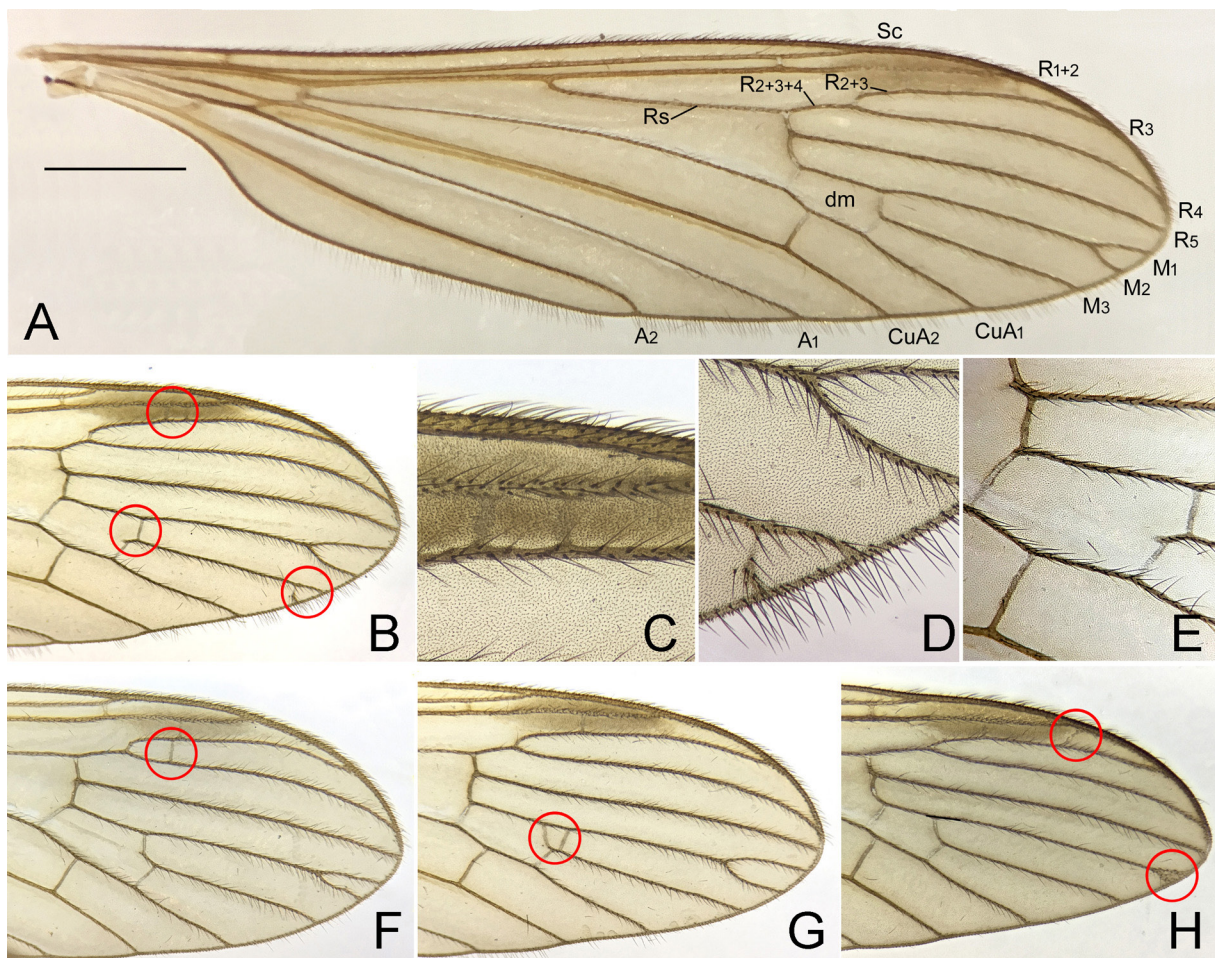


Fig. 3. *Dicranophragma (B.) relictum* Mederos sp. nov. **A.** Wing venation. **B–H.** Variations detected in some specimens such as extra cross veins (C, F–H) and the split at the end of M_3 vein creating a cell (D), as well as variations in the length and shape of cell dm and cell m1 (B, F–H). Scale bar = 1.0 mm.

ABDOMEN. Grey to greyish brown, covered with short fine silver hairs; apical edge of tergite 7 and tergite 8 dark grey to brown, together with corresponding sternites; tergite 9 yellowish brown, terminalia yellow.

MALE TERMINALIA (Fig. 4A–D, G). Gonocoxite conical, less than twice as long as outer gonostylus; outer gonostylus stouter, moderately arched on apical half, paler in two proximal thirds and darker in distal third, with bi-toothed apex. Inner gonostylus fleshy, conical, shorter than outer gonostylus, slightly

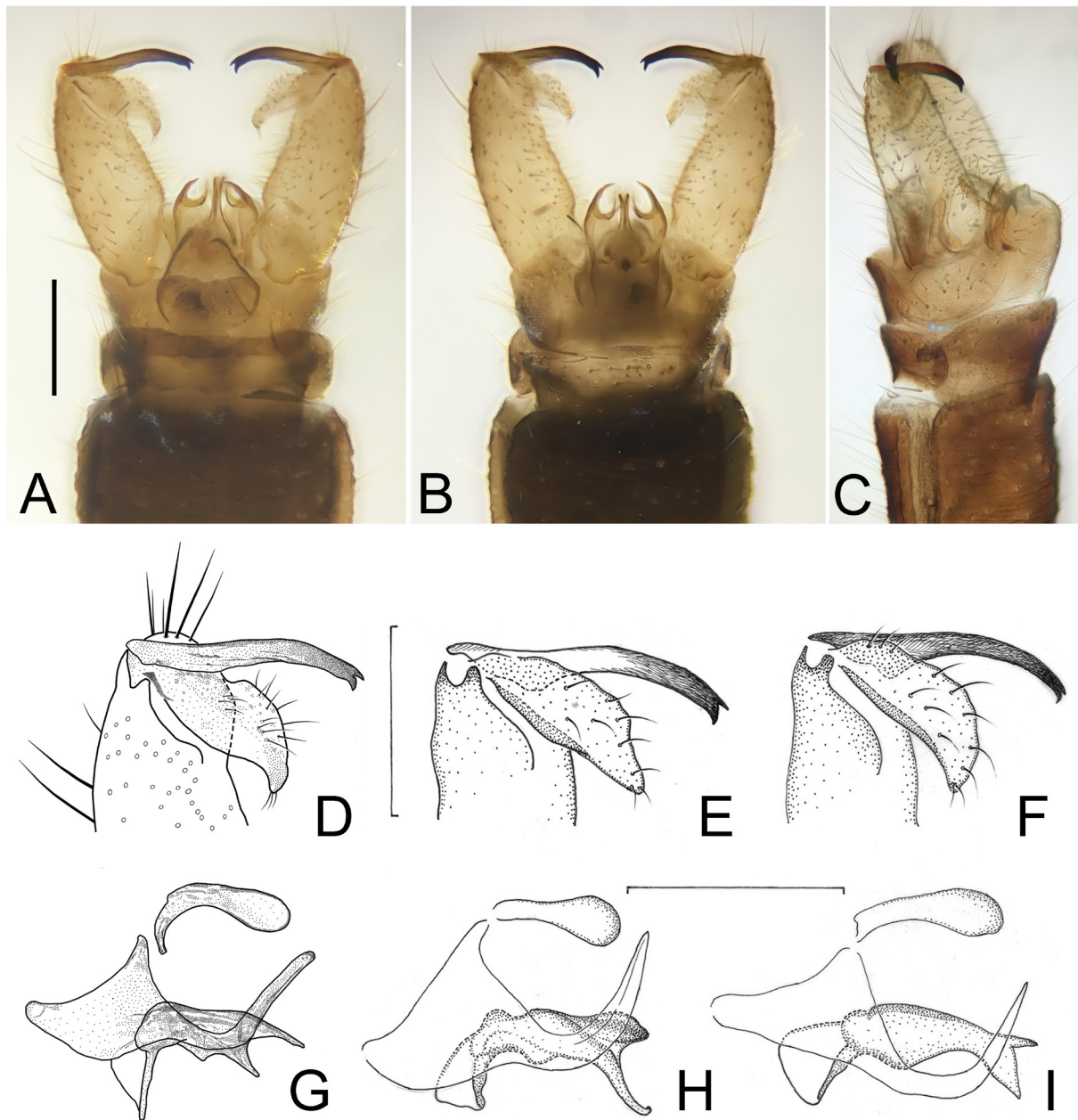


Fig. 4. A–C. Male terminalia of *Dicranophragma (B.) relictum* Mederos sp. nov. A. Dorsal view. B. Ventral view. C. Lateral view. D–I. Detail of inner and outer gonostylus (dorsal view) and aedeagal complex (lateral view). D, G. *D. (B.) relictum* Mederos sp. nov. E, H. *D. (B.) nemorale* (Meigen, 1818). F, I. *D. (B.) adjunctum* (Walker, 1848). Scale bars = 0.25 mm (images E–F, H–I after Starý & Reusch 2009).

curved at apex and with several hairs at tip; interbase spoon-like; paramere moderately stout, long and obtuse at apex; aedeagus bipartite distally in lateral view, with upper part acute and longer than lower part.

Female

MEASUREMENTS. Body length 10.5–11.5 mm. Antennae 1.6–1.8 mm. Wing length 9.7–10.5 mm.

As male in general aspect and color, but larger in body size and wingspan. Verticils shorter, about twice as long as their respective flagellomeres. Female terminalia (Fig. 5A–B, D) with internal structures as shown in Fig. 5D. Vaginal apodeme (genital fork) short and rather broad. Another prominent feature to consider, in addition to the shape of the vaginal apodeme, is the significant reduction of the inner walls of tergite 9 in the internal structure of the terminalia compared to those of the other species present in the Iberian Peninsula. Spermathecae dark brown, ovoid, the length of the sclerotized parts of their ducts about 2 times as long as the spermatheca (Fig. 5D).

Biology

The specimens of *D. relictum* Mederos sp. nov. reacted little to human contact, either when we were taking photographs just a few centimeters away or when captured. No flight reactions were observed in any specimen of either sex, they only walked on the walls of the chamber or substrate when moving away from their initial resting sites. Only once was a slight wing fluttering observed but without taking flight. An analogous behavior was observed in specimens of the crane fly *Tricyphona* (*Tricyphona*) *contraria* Bergroth, 1888 in another cave with a similar speleogenesis, also in conglomerates and of similar age, in the Montserrat massif, approximately 20 km from Avenc del Daví (Mederos *et al.* 2018). The temperature and relative humidity during the study period oscillated between 7.2–9.3°C and 89.3–91.8% RH. Venation in crane flies is prone to individual anomalies, including the addition of cross veins and the opening of cells due to vein atrophy (Edwards 1938; Gelhaus 2009), and we found anomalies in several specimens of this population of *D. relictum* Mederos sp. nov.

Larvae of *Dicranophragma* have been reported to be semi-aquatic, being found in springs and beds of fine-grained sediments in large water courses, in marginal situations along flowing and standing waters, in the more or less saturated organic mud of lake margins and marshes, and in rich organic mud near reservoirs (Reusch & Schrankel 2006; Kramer & Withers 2007; Podenas & Podeniene 2008; Cranston & Drake 2010). Adults have been observed and collected from a variety of habitats including wet mineral and peaty soils, especially wet meadows, small swampy wooded areas, wet scrapes in a field, marshland, a forest above riverbanks, emerging from a forest floor in a beech (*Fagus* sp.) forest; with a preference for mildly calcareous soils within wet wooded habitats, and in a semi-wet *Fraxinus* forest (Crossley 2003; Boardman 2007, 2016; Nielsen & Nielsen 2009; Starý & Reusch 2009; Drake 2011; Chandler 2015). The absence of the aforementioned ecological conditions in the massif, particularly in the vicinity of the cave, suggests that *D. relictum* Mederos sp. nov. is isolated in Avenc del Daví and a remnant of a time when the species was found elsewhere in the region when it was characterized by different conditions.

The population of *D. relictum* Mederos sp. nov. in Avenc del Daví is located approximately from a depth of -7 m to the chamber located at approximately -40 m. Several other species of arthropods were observed and sampled in this area, including *Limonia nubeculosa* Meigen, 1804 (Diptera: Limoniidae), the only other crane fly observed in this cave and very abundant during the prospecting dates (June–July 2019). The specimens of *L. nubeculosa* were distributed uniformly over the walls of the cave, while *D. relictum* Mederos sp. nov. was found only in specific spaces on the west wall (descent ramp) and on the floor of the chamber located at -40 m.

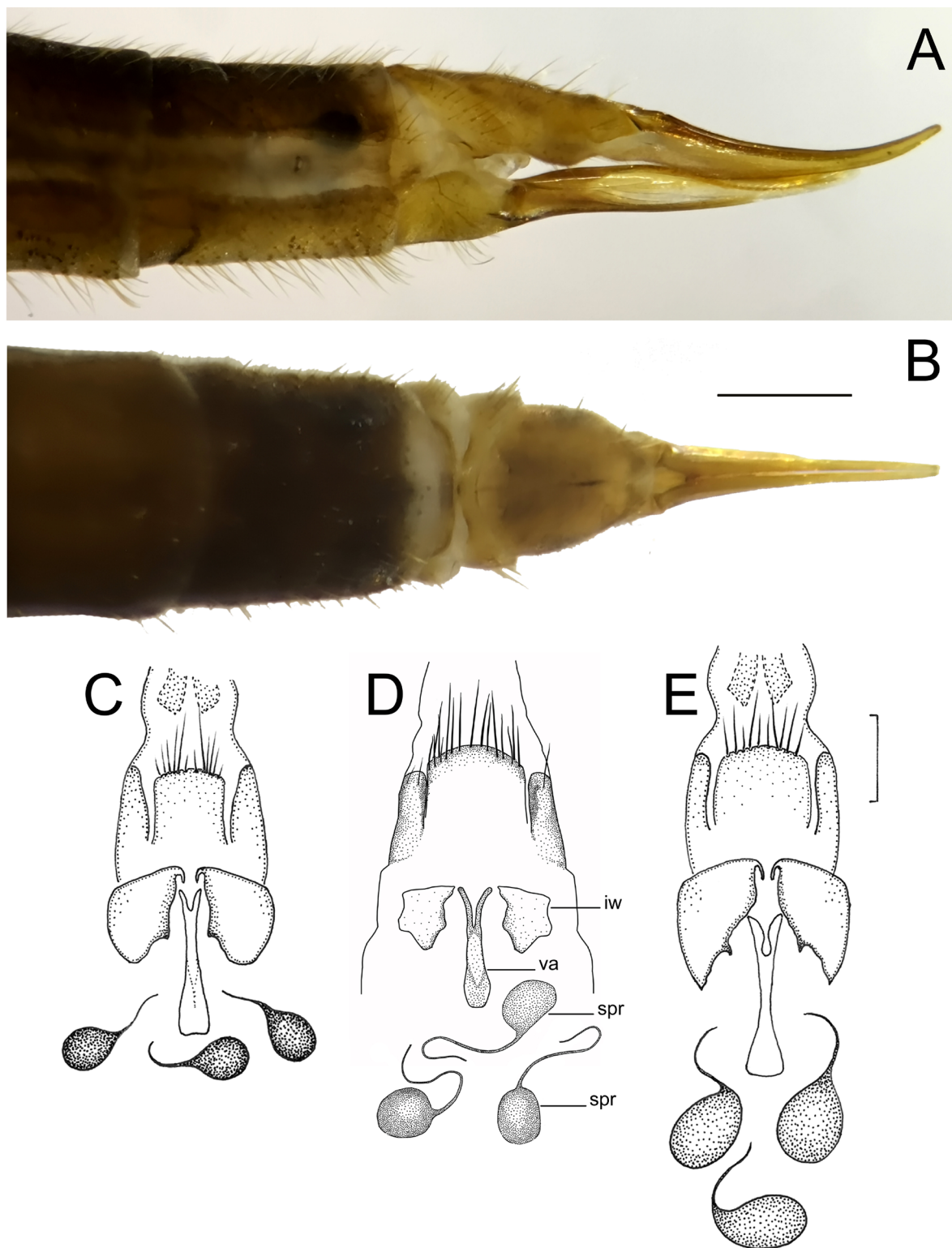


Fig. 5. A–B. Female terminalia of *Dicranophragma (B.) relictum* Mederos sp. nov. A. Lateral view. B. Dorsal view. C–E. Comparison of the internal structures of female terminalia of three *Dicranophragma* species (ventral view). C. *D. (B.) adjunctum* (Walker, 1848). D. *D. (B.) relictum* Mederos sp. nov. E. *D. (B.) nemorale* (Meigen, 1818). Abbreviations: see Material and methods. Scale bars = 0.25 mm (images C and E after Starý & Reusch 2009).

Distribution

Species known only from the type locality, Avenc del Daví cave in Catalonia.

Remarks

The examination of the specimens captured in Avenc del Daví reveals differences in structure, both in male terminalia as well as the spermathecae and shape of the inner wall of tergite 9 in female terminalia, that are constant and sufficient to regard this as a distinct from other European species of *Dicranophragma*. *Dicranophragma relictum* Mederos sp. nov. can also be distinguished by external characters, such as the body coloration, wing venation and wing pattern. The closest species is *D. nemorale*, as shown in the differential diagnosis section, widely distributed in the Palearctic and cited in the Iberian Peninsula from Andorra, Portugal and more recently from Spain (Eiroa 2002; Hancock 2020; Hancock & Hewitt 2020; Oosterbroek 2020).

Discussion

During the study, *Dicranophragma relictum* Mederos sp. nov. was not observed in any of the other caves in the Sant Llorenç del Munt i Serra de l'Obac Natural Park. Despite the presence of caves with a similar speleogenesis, altitude, development and age, no other cave visited during the study offered similar conditions of humidity, temperature and orientation.

In Europe, there are no records of species of this genus from caves (Oosterbroek 2020) and so we believe that *D. relictum* Mederos sp. nov. could be a eutroglophile species lacking a population outside the cave. According to Sket (2008), a eutroglophile is an aboveground species that is also able to maintain a permanent underground population. It is possible that life conditions changed drastically for *D. relictum* Mederos sp. nov. when the ecological parameters that allowed the survival of the population outside the cave were modified by some specific historical event.

Dicranophragma relictum Mederos sp. nov. is a very interesting species that joins the few European members of this genus. Presumably, the population in Avenc del Daví is a remnant of an era when conditions were more appropriate for its survival in the territory, different from those of today. This population seems to be isolated in the Avenc del Daví, which offers a suitable substrate and more or less stable humidity and temperatures that are more favorable than the conditions outside the cave. A more exhaustive exploration of caves with appropriate conditions for *D. relictum* Mederos sp. nov., as well as in nearby outdoor environments, is still pending. This new discovery obliges the administration of this natural park to review the current access protocol to this cave, given the likelihood that *D. relictum* Mederos sp. nov. is a vulnerable and endangered species, with a single known population and dependent on an ecosystem as fragile and threatened as the hypogean environment.

Acknowledgments

Jaroslav Starý (Silesian Museum), Eulalia Eiroa (University of Santiago de Compostela), and Boris Sket (University of Ljubljana) are thanked for their valuable comments and support. Thanks to Xavier Oller (Espeleo Club Sabadell) for assistance in the field. We are grateful to Jordi Serra-Cobo (IRBio, University of Barcelona), Wouter Dekoninck (RBINS), Christophe Daugeron and Emmanuel Delfosse (MNHN) for their help and facilities provided during the preparation of the manuscript. We also thank Mike Lockwood for the English revision. We feel grateful to Daniel Pons Julià (DIBA, Barcelona) and the park staff who provided support during the study. We acknowledge financial support through the agreement signed between the Barcelona Provincial Council (DIBA), the Barcelona Natural Science Museum (MCNB), the Catalan Biospeleological Association (BIOSP), and the Catalan Speleological Federation (FCE), to promote collaboration in research projects in karst environments and in the heritage of collections belonging to the network of Natural Parks run by DIBA. This paper is the result of the

study *Artròpodes del medi hipogeu del Parc Natural de Sant Llorenç del Munt i l'Obac 2019*. The authors also thank the observations offered by two anonymous referees, which have improved the final quality of the manuscript.

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Manuscript received: 18 June 2020

Manuscript accepted: 21 September 2020

Published on: 25 November 2020

Topic editor: Nesrine Akkari

Desk editor: Pepe Fernández

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