Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 19 (3): 381–389
https://doi.org/10.15560/19.3.381

PENSOFT

On the identity and distribution of the rare *Rymosia tolleti* Burghele-Balacesco, 1965 (Diptera, Mycetophilidae) encountered in European caves

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Abstract. The identity and distribution of a neglected fungus gnat species, *Rymosia tolleti* Burghele-Balacesco, 1965, in Europe is reviewed based on examination of newly collected specimens as well as available museum materials. *Rymosia tolleti* is widespread but rather rare in Central Europe, with confirmed records from Romania, Slovakia, Germany, and France. All the specimens with known collection details originate from cave environments. Detailed photographs of the male terminalia are provided for the first time, along with two unique DNA barcodes for the species.

Keywords. Central Europe, DNA barcode, faunistics, fungus gnats, taxonomy

Academic editor: Maíra Araújo
Received 1 April 2023, accepted 22 May 2023, published 2 June 2023

Kurina O, Kjærandsen J, Kirik H, Hadbavná D, Dénes A, Oboňa J, Manko P (2023) On the identity and distribution of the rare *Rymosia tolleti* Burghele-Balacesco, 1965 (Diptera, Mycetophilidae) encountered in European caves. Check List 19 (3): 381–389. https://doi.org/10.15560/19.3.381

Introduction

The genus *Rymosia* Tuomikoski, 1966 of the family Mycetophilidae comprises in its strict sense (Tuomikoski 1966) about 80 valid, living species (Søli 2017), and additional nine poorly described extinct species, several of which likely do not belong to the genus (Krzemiński et al. 2019). The majority of the species are recorded from the Holarctic Region (73), whereas just a few species are so far described from the Neotropical (5), Afrotropical (1), and Oriental Regions (1). In Europe, 39 extant species are recorded (Chandler 2005; Chandler et al. 2006), but numerous additional, still undescribed species are known from the Nordic Region (Kjærandsen et al. 2007; Kjærandsen and Søli 2020). As far as known, *Rymosia* species are in their larval stage associated with fruiting bodies of soft fungi. There are rearing records of 12 Palaearctic species (Jakovlev 1994; Ševčík 2010). Moreover, some species of *Rymosia* are, together with some species in other genera of the tribe Exechiini, regularly encountered hibernating in caves in northern Europe (e.g. Kjærandsen 1993; Kurina 1996) and to a higher degree also aestivating further south in Europe (Kjærandsen 1993). According to

Weber et al. (2007), altogether 15 *Rymosia* species have been recorded from caves in the Western Palaearctic Region, and the type species, *Rymosia fasciata* (Meigen 1804), undoubtedly is among the most common dipteran to be found in cave systems throughout Europe (Matile 1970; Kjærandsen 1993).

The scientific history of *R. tolleti* Burghele-Balacesco, 1965 is rather brief. The species was described by Anca Burghele-Bălăcesco (born 1935) in 1965, based on a material collected in cave environment from Romania and France (Hérault department). In the original description, Burghele-Bălăcesco (1965) stated that the species is not rare in Romanian caves. Subsequently it has, however, only been recorded once, from the department of Hautes-Alpes in south-eastern France, without specified collecting data (Matile 1977), but see discussion below.

This communication was prompted by finding a single male specimen of *R. tolleti* during the monitoring of the fauna of Slovakian abandoned mining adits in 2020–2021, which subsequently lead to the study of all available material in museum collections. Additionally, since the type material of *R. tolleti* is regarded as lost, new topotypic material was collected and studied from the same karst area, the Vârghiș (Vargyas) Gorge, Romania. The original description of the species includes a figure of the male terminalia from ventral view only (Burghele-Bălăcesco 1965: fig. 3a). However, close-up photographs, exposing so far undescribed details along with a redescription of the male terminalia and DNA barcodes are provided to secure an unambiguous further interpretation and identification of the species.

Methods

Collecting and morphological studies. The new material from Slovakia was obtained in the framework of a sampling campaign aimed at studying the fauna of abandoned mining adits in the Gelnica-Turzov area. The material was collected by direct collection using forceps or an aspirator. The distance from the entrance, temperature and humidity were recorded at 10 m intervals. Material was collected at monthly intervals for 12 months (years 2021 and 2022). In Romania, collection was targeted for specimens of *Rymosia tolleti*. Using direct collection with entomological forceps and an aspirator, we gathered fungus gnats in a total of eight caves in the Vârghiș (Vargyas) Valley on 02–03.II.2023. Newly collected and studied Slovak and Romanian specimens were initially preserved in about 70% ethyl alcohol. For final preservation the specimen was mounted from alcohol, using a chemical method described by Vockeroth (1966), and double-pinned. For detailed study of the terminalia, they were detached and treated in a solution of KOH, thereafter neutralized in acetic acid, washed in distilled water, and studied in glycerine (for further details see Kurina 2006). Terminalia are stored as glycerine preparations in a small plastic vial attached to the specimen. The re-checked collection material was preserved in ethyl alcohol in the case of material loaned from ZSM and MNHM, or originally pinned in the case of one male loaned from MNHN. The images of the general habitus and terminalia were taken with a Leica K5C camera attached to a Leica 205C stereomicroscope and combined using the software LAS X or Helicon Focus. Subsequently, individual images were processed by the Topaz Sharpen AI software for enhanced sharpness and finally edited and compiled into plates using Adobe Photoshop CS5 (see also Kjærandsen et al. 2022). Morphological terminology follows Søli (2017) and Kjærandsen (2006). The map of sampling localities was generated with QGIS 3.28.3-Hannover and edited with Adobe Photoshop CS5. The studied new material is deposited in the insect collection IZBE – Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences (former Institute of Zoology and Botany), Tartu, Estonia. The re-studied collection material currently on loan is to be deposited in the Museum national d'Histoire naturelle, Paris, France (MNHN) and in the Zoologische Staatssammlung München, Germany (ZSM).

DNA sequencing. One fore leg was detached from both freshly collected specimens and used for DNA sequencing, targeting the widely used mitochondrial cytochrome c oxidase subunit I gene (COI or COX1). DNA extraction was carried out using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) in accordance with manufacturer instructions, after the material was homogenized in the ATL buffer using a handheld Kontes Pellet Pestle (DWK Life Sciences GmbH, Mainz, Germany). From there, "Folmer primers" LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAAT-3′) were used to amplify a 710-bp long partial COI sequences from template DNA (Folmer et al. 1994). The 25 µL PCR mixture consisted of 12.5 µL DreamTaq PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µL of each primer (manufactured by Tag Copenhagen, Frederiksberg, Denmark), 0.5 µL of ddH2O, and 11 µL of template DNA. Further specifics concerning the PCR procedure can be found in a previous article published by Kurina and Kirik (2021). Sanger sequencing was carried out by the Institute of Genomics Core Facility (University of Tartu, Tartu, Estonia). Raw sequences were subjected to quality control in Bioedit v. 7.2.5. (Hall 1999): primer sequences were removed, and forward and reverse strands were combined into consensus sequences. Assembled DNA sequences were run through BLAST (Basic Local Alignment Search Tool, National Institutes of Health, Bethesda, USA) to find related entries. The closest match, a *Rymosia fasciata* sequence (Burdíková et al. 2019), was downloaded from GenBank (National Institutes of Health, Bethesda, USA) and used as comparison material in MEGA v. 11.0.13 (Tamura et al. 2021). Pairwise distances were calculated using the *p*-distance method.

Results

***Rymosia tolleti* Burghele-Balacesco, 1965**

Figures 1–3

Re-examined collection material. FRANCE – **Hautes-Alpes** • Ailefroide; 1500 m a.s.l.; 18.VIII.1966; J.C. Beaucournu leg; 1♂, L. Matile det.; MNHN, pinned, dried from ethanol with detached terminalia in glycerine (SPM-011783). ROMANIA – **Mehedinți county**• Sanat, Env. de-Băile Herculane; 5.X.1968; L. Matile leg.; 1♂, L. Matile det.; MNHN, pinned, terminalia in glycerine (SPM-011655) – **Gorj county** • Cloșani - Peștera no. 6 din Valea Lupsei; 16.X.1968; L. Matile leg.; 1♂, L. Matile det.; MNHN, pinned, dried from ethanol with detached terminalia in glycerine (SPM-011782). GERMANY – Rheinland-Pfalz • Nassau, Grube Holzappel, Neuer Stollen; 25.II.2001; U. Kaiser and D. Kraus leg.; 1♂, #34680, ZSM, in ethyl alcohol; published as *Rymosia cottii* Tollet, 1959 by Zaenker (2008) • Bundenbach, 6110/133 Friedrichsfeld 7; 18.X.2004; D. Weber leg.; 1♂, #38763, ZSM, in ethyl alcohol; published as *R. cottii* Tollet, 1959 by Weber (2010).

New material. SLOVAKIA – **Košice Region** • Turzov, Gelnica, Štôlňa Boží dar; 48.8537N, 20.9131E; 470 m a.s.l.; 20.VIII.2021; D. Hadbavná and P. Manko leg.; hand picking in abandoned mine adit, 30 m from

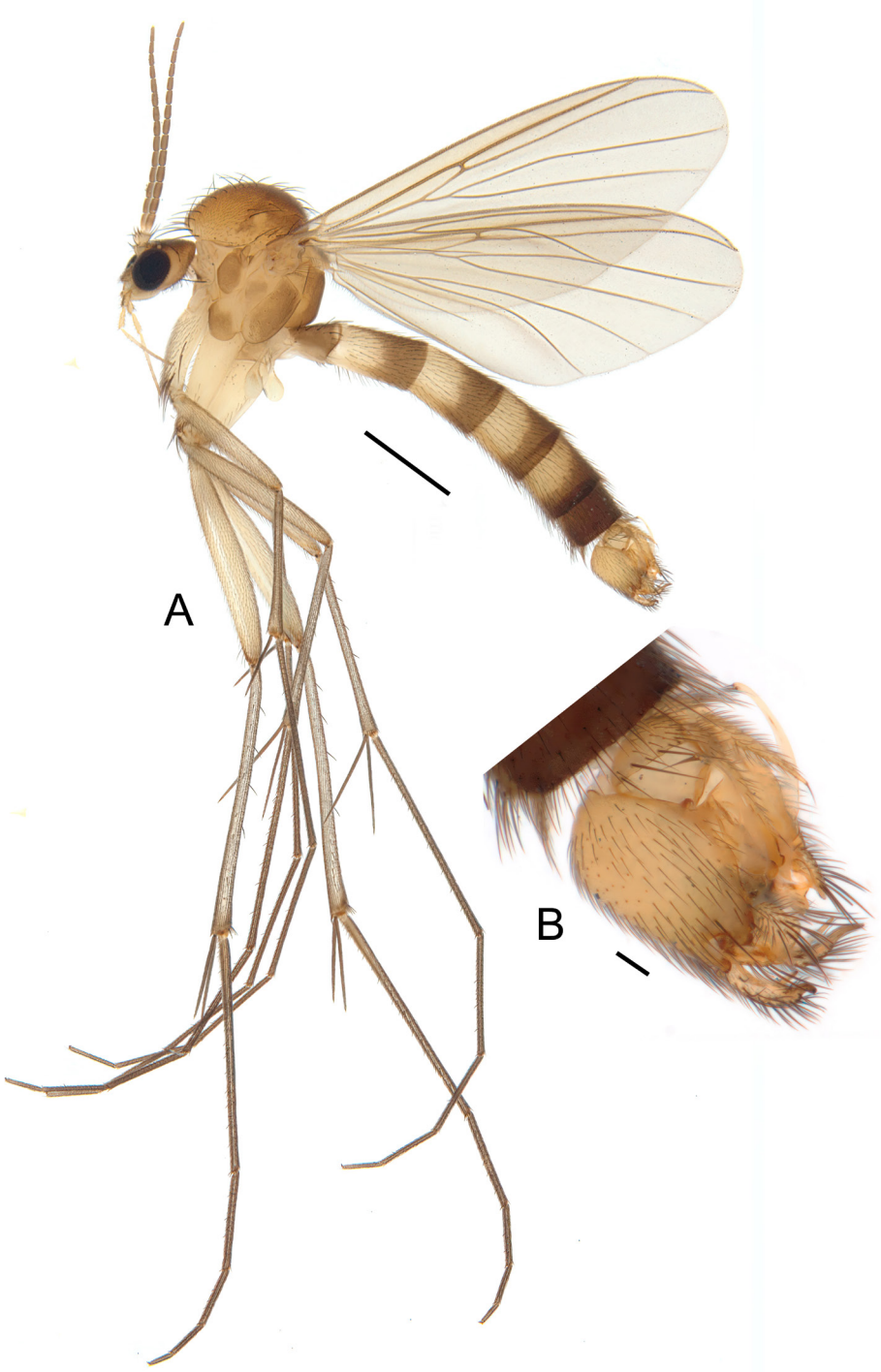

Figure 1. *Rymosia tolleti*, male (Slovakian specimen: IZBE0228868). **A.** General habitus. **B.** Close up view of terminalia. Scale bars: 1 mm (A) and 0.1 mm (B).

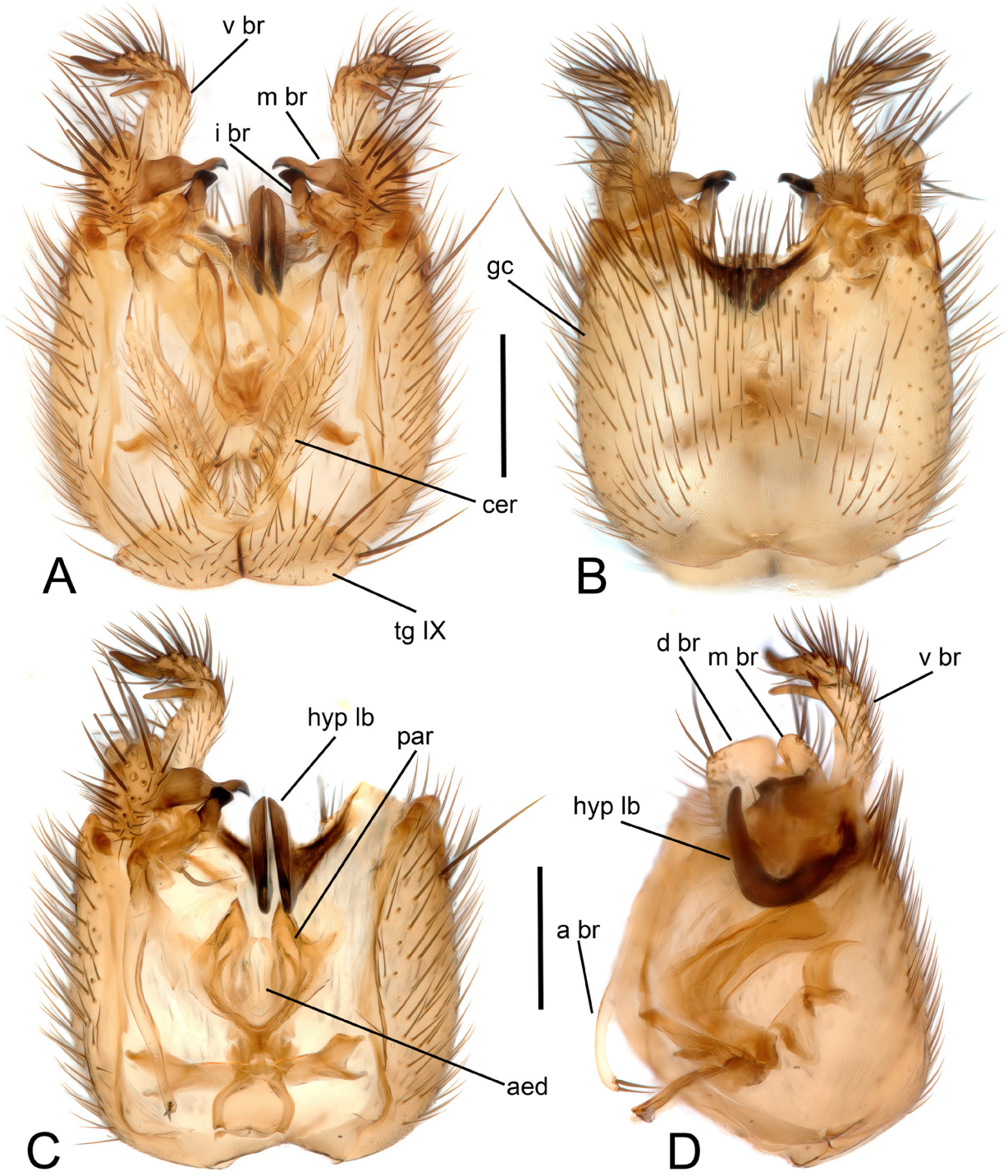

Figure 2. *Rymosia tolleti*, male terminalia (Slovakian specimen: IZBE0228868). **A.** Dorsal view. **B.** Ventral view. **C.** Dorsal view, tergite IX and cerci removed. **D.** Lateral view. Scale bars: 0.2 mm. Abbreviations: a br = anterior branch of gonostylus, aed = aedeagus, d br = dorsal branch of gonostylus, gc = gonocoxite, cer = cercus, hyp lb = hypandrial lobe, i br = internal branch of gonostylus, m br = medial branch of gonostylus, par = paramere, tg IX = tergite IX, v br = ventral branch of gonostylus.

entrance; 1♂, IZBE0228868, mounted from alcohol, terminalia in glycerine; GenBank No OQ628077. ROMANIA – **Harghita county** • Vârghiș (Vargyas), Cheile Vârghișului (Vargyas-szoros), Șura Cailor (Lócsűr) cave; 46.2194°N, 025.5447°E; 02.II.2023; P. Manko, J. Oboňa, A. Dénes and M. Pál leg.; cave wall, hand picking; 1♂, IZBE0228869, mounted from alcohol, terminalia in glycerine; GenBank no. OQ628078.

Identification. Both in wing characters, where the furcation point of posterior fork is clearly before that of medial fork, the branches of the medial and the posterior forks are without setulae above, and the subcostal vein ends in first radial vein (cf. keys by Søli et al. 2000; Chandler 2022), as well as the characteristic habitus with pale abdominal markings situated at the base of the abdominal segments, and male terminalia with

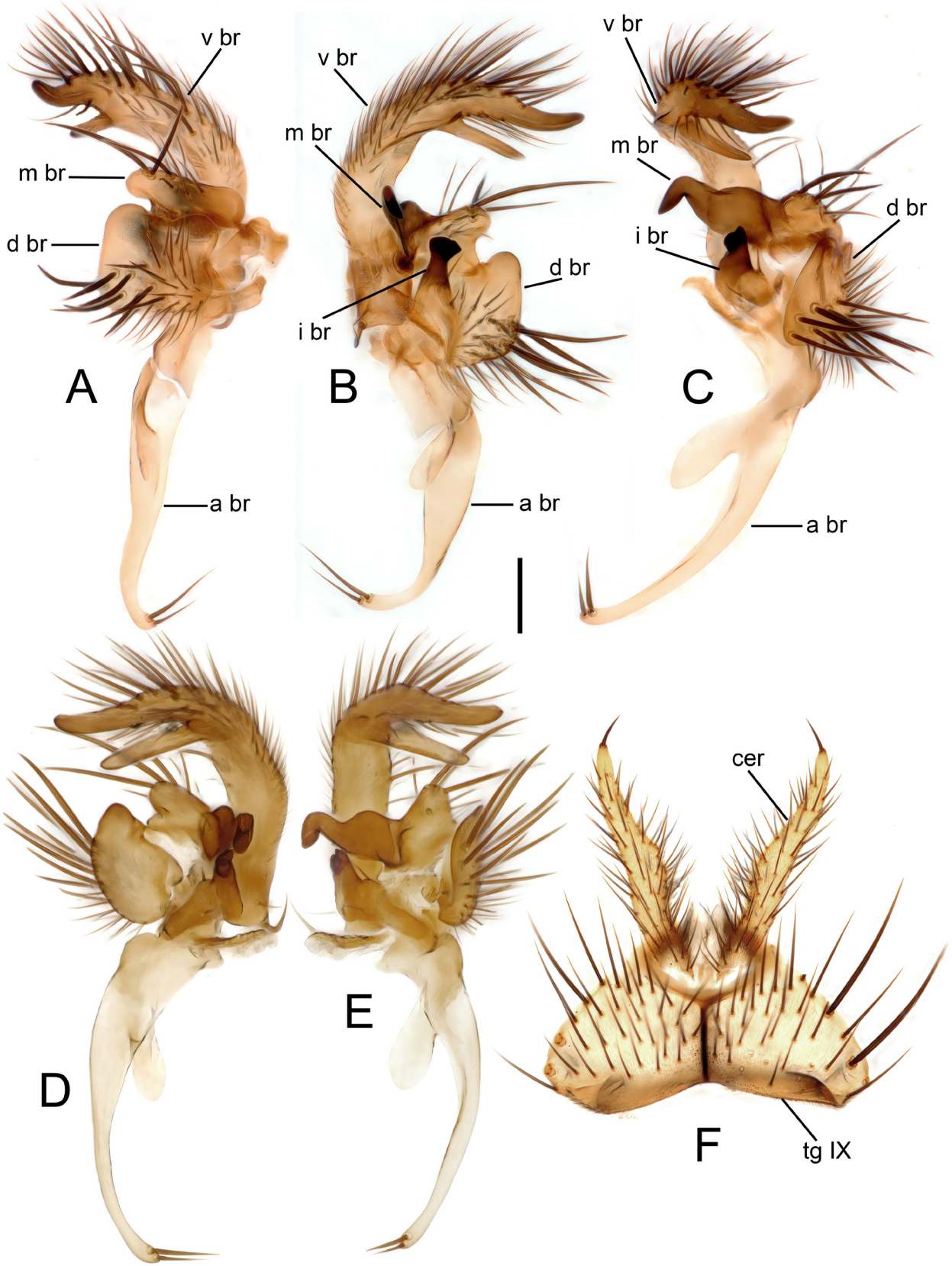

Figure 3. *Rymosia tolleti*, male terminalia. **A, D.** Gonostylus, laterodorsal view. **B.** Gonostylus, internal view. **C, E.** Gonostylus, posterodorsal view. **F.** Tergite IX and cerci, dorsal view. **A–C, F.** Slovakian specimen (IZBE0228868). **D, E.** French specimen (SPM-011783). Scale bar: 0.1 mm. Abbreviations: see Fig. 2.

a ventrally closed synsclerite leaves no doubt about the placement of *R. tolleti* in *Rymosia* sensu stricto. Further species identification of *Rymosia* relies largely on the characters of the male terminalia.

Redescription of male terminalia. Males of *R. tolleti* have terminalia (Figs 1B, 2, 3) yellow, with hypandrial

lobes and apical part of the medial branch of the gonostylus dark brown to blackish. Posteroventral margin of the ventrally closed synsclerite (gonocoxites) slightly emarginated medially. Hypandrial lobe well developed, consisting of pair of inwardly recurved prongs; in lateral view, hypandrial lobe is apically tapered and hooked medially at right angle. Tergite IX (Fig. 3F) obcordate. Cercus narrow and apically pointed, somewhat broadened medially, setose, except subapically, with single distinct apical seta. Gonostylus divided into five branches (Fig. 3A–E, cf. Kjærandsen 2006). Ventral branch elongated, tapering, curved dorsad and laterad; setose, except for apical part; split into additional non-setose lobe subapically, well exposed in dorsal view; anteromedial corner of ventral branch with strong medially directed seta. Medial branch L-shaped, extending transversally, lateral part swollen, setose, medial part sclerotized, smoothly bending into beak-shaped apex, well exposed in dorsal view. Dorsal branch subquadrate, flat, lateral surface setose. Internal branch short, subapically constricted, apically blunt and sclerotized. Anterior branch elongated, about as long as the ventral branch, with subbasal thumb-like lobe, non-setose, except for two apical setae. Aedeagal apparatus (Fig. 2 C) with parameres apically spathulate, extending over aedeagus posteriorly.

Discussion

According to the original description, the type material of *Rymosia tolleti* is stated to be deposited at the "Emil Racovită" Institute of Speleology, Bucharest, Romania (Burghele-Bălăcesco 1965). Unfortunately, this material appears not to have been preserved, as was also concluded for crane flies (Diptera: Limoniidae) described by Burghele-Bălăcesco (Krzemiński 1982; Oosterbroek and Reusch 2008). A study of the historical material from Romania and France (on loan to JK from MNHN) and newly collected topotypic material is therefore highly valuable. The record from the department of Hautes-Alpes in France (Matile 1977) relies probably on the same specimen studied by us, whereas data of the two specimens collected by Loïc Matile (1938–2000) from Romania in 1968 have not previously been published.

The original description of *R. tolleti* includes a figure of the male terminalia from ventral view (Burghele-Bălăcesco 1965: fig. 3a) that allows us to determine the conspecificity with our studied material with very high confidence. The slight variability in the structure of the male terminalia seen in the studied specimens (cf. gonostylus of Slovakian and French specimens in Fig. 3) is considered to be intraspecific variation and/or caused by a slightly different angle of view. Moreover, analyses of the DNA barcodes of the newer material confirms that materials from Slovakia and the topotypic material from Romania is conspecific and will make identifying *R. tolleti* easier in the future. The partial COI sequences of the two specimens collected from Slovakia and Romania are 0.6% different from each other—only four nucleotide substitutions can be found out of the total 658 positions (Fig. 4). This variation appears to be restricted to the third-codon position, which is known to be less functionally constrained than the first- and second-codon positions, and therefore likely to degrade the fastest (Bofkin and Goldman 2007). Meanwhile, the closest related entry currently available in GenBank and on BOLD is a *R. fasciata* (Meigen, 1804) sequence (Burdíková et al. 2019), which shows a 9.1% difference from both *R. tolleti* samples. This distinct barcode gap further strengthens our conclusion that the specimens collected from Slovakia in 2021 and Romania in 2023 likely belong to the same species.

Within the Western Palaearctic Region *Rymosia tolleti* forms a group with *R. beaucornui* Matile, 1963, *R. cottii* Tollet, 1959, *R. fasciata*, *R. lauricola* Chandler & Ribeiro, 1995, and *R. tenuivittata* Santos Abreu, 1920. All six species have the male terminalia with a strongly

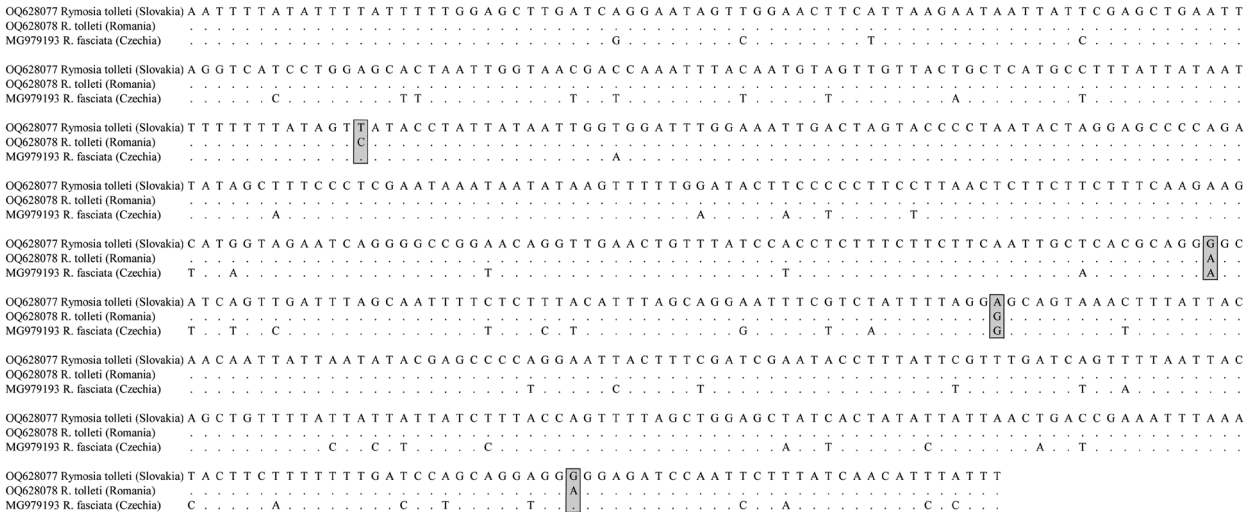

Figure 4. 658 bp partial COI sequences from the two new *Rymosia tolleti* specimens compared to *R. fasciata* (Burdíková et al. 2019), the closest related currently available sample in GenBank. Sites containing nucleotide variation between *R. tolleti* sequences from Slovakia (OQ628077) and Romania (OQ628078) have been highlighted in gray. Codon start: second nucleotide.

developed and bi-forked hypandrial lobe with a pair of long prongs recurved inwards/upwards in combination with a narrow, tube-shaped ventral branch of the gonostylus that is often dilated apically. Four of them are known to occur in caves, making it likely that the species group developed this habit early in their evolution. Among these species, *R. tolleti* is most similar to *R. cottii* Tollet, 1959, another species described from a material collected from cave environment in Switzerland (Fornet cave in Tremona, Ticino canton). *Rymosia cottii* was described from a single discoloured specimen that was stained with eosin and mounted on a microscopic preparation (Tollet 1959). The terminalia were figured in dorsal and ventral views and discussed as unique (Tollet 1959: figs 4, 5). Unfortunately, the holotype of *R. cottii* is also lost (P. Limbourg pers. comm. to OK), but figures of the terminalia allow distinguishing it from *R. tolleti*. *Rymosia cottii* has the ventral branch of the gonostylus with apical part setose, well tapering, and bearing an strong apical seta deviating from other vestiture of the gonostylus (apical part bare, smoothly tapering, and without an apical seta in *R. tolleti*). Subsequently, Zaenker (2008) and Weber (2010) recorded *R. cottii* from two different caves in Rheinland-Pfalz, Germany. However, as a result of our study, this material—in both cases one male specimen (loan to OK from ZSM)—turned out to be *R. tolleti* and is listed above. In addition, Weber (2010: 1421) quoted *R. cottii* also from Sweden based probably on a male specimen in ZSM (SWEDEN – **Lule Lappmark** • Abisko; 11–18. VIII.1975; lichtfang, K. Müller leg; 1♂, in ethyl alcohol, #13107 ZSM; E. Plassmann det.). However, details of this record have not been published in a synopsis of light-trapping results of fungus gnats from Abisko (Plassmann 1980) nor in any other publications by E. Plassmann (1938–2014; German dipterologist: specialist of fungus gnats). Our re-examination revealed that this specimen is conspecific with *Rymosia connexa* Winnertz, 1864, a species listed from northern Sweden by Kjærandsen et al. (2007). Consequently, because the only known specimen of *R. cottii* is lost, the identity of the species relies only on the figures by Tollet (1959: figs 4, 5).

Considering the likely destroyed type material of *R. tolleti* (19♂, 12♀; Burghele-Bălăcesco 1965: 176), the studied material from France (1♂), Germany (2♂), Slovakia (1♂), and Romania (3♂) represents all known subsequent records of this species (Fig. 5). The species is probably widespread in Central Europe but seems relatively rare. The record of a single individual from Slovakia comes from collections carried out in three abandoned mining adits at monthly intervals for 12 months, and the record of a single individual from Romania also comes from collections from eight mostly smaller caves. On the other hand, based on historical records, this species was abundant in Romanian caves.

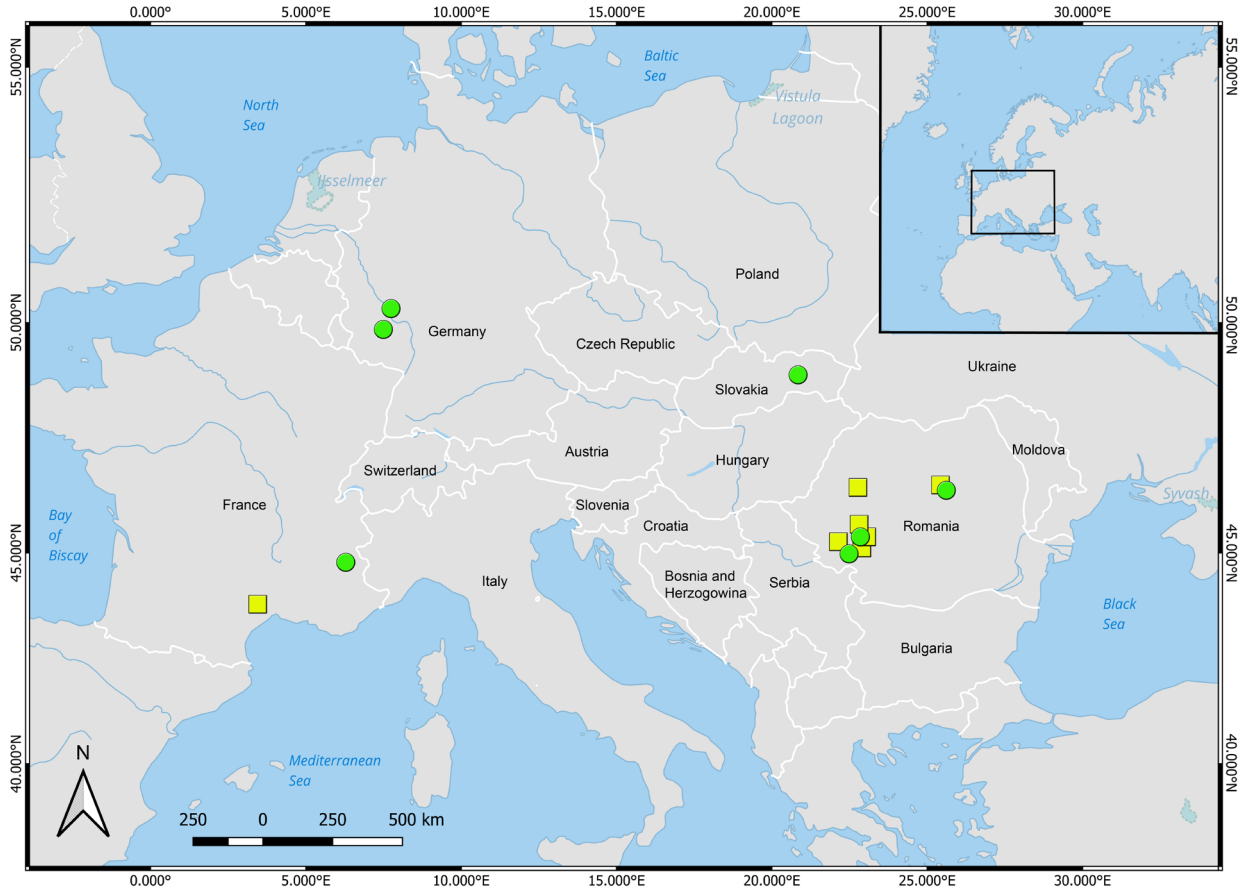

Figure 5. Sampling localities of *Rymosia tolleti* in Europe. Green circles denote material checked during the current study. Yellow rectangles denote material included in the original description by Burghele-Bălăcesco (1965); in Romania, the rectangles may represent several nearby localities.

All specimens of *R. tolleti* with known collecting details are from caves or artificial subterranean environments. According to Weber et al. (2007), the species has been classified as an eutrogloxene, i.e. taxa that accidentally get into caves. Adults of *R. tolleti* use caves probably as shelter during unfavourable weather conditions (dry, warm summers) and for hibernation. There are no data on the larval feeding habitat of the species, but two species of this species-group—*R. beaucornui* and *R. fasciata*—are found to be fungivorous (Jakovlev 1994; Chandler 2010).

The specimen of *R. tolleti* from Slovakia represents the first country-record. In the list of mycetophilid species of Germany, *R. cottii* must be replaced by *R. tolleti*.

Acknowledgements

We are much obliged to Dieter Weber (Hassloch, Germany), Stefan Zaenker (Fulda, Germany), Levente-Péter Kolcsár (Matsuyama, Japan), Lujza Keresztes (Cluj-Napoca, Romania), and Peter Chandler (Melksham, UK) for invaluable comments and discussion during the preparation of the manuscript. Marion Kotrba and Dieter Doczkal (both Munich, Germany) are thanked for arranging the loan of *Rymosia* material from the Zoologische Staatssammlung München (ZSM). We are grateful to Pol Limbourg (Brussels, Belgium) for information on *R. cottii* type material at the Royal Belgian Institute of Natural Sciences (RBINS) and to Demeter László (Agenția Națională Pentru Arii Naturale Protejate Serviciul Teritorial Harghita, Romania) for the willingness and permission to collect in the Cheile Vârghișului Protected Area. OK was supported by institutional research funding from the Ministry of Education and Research of Estonia and funding from the Estonian Research Council (TT14). JK was supported and funded by UiT—The Arctic University of Norway. JO and PM were supported by a project of the Slovak Research and Development Agency under the contract no. APVV-20-0140.

Author Contributions

Conceptualization: OK, PM, JO. Data curation: OK, JK, AD, PM. Funding acquisition: OK, JK, PM. Investigation: OK, JK, HK, DH, AD, JO, PM. Methodology: OK, JK, HK, JO, PM. Resources: OK, JK, HK, JO, PM. Supervision: OK, JK, JO, PM. Visualization: OK, JK, HK. Project administration: OK, PM. Writing – original draft: OK, JK, HK. Writing – review and editing: OK, JK, HK, DH, AD, JO, PM.

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