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 mate-
rials. *Rymosia tolleti* is widespread but rather rare in Central Europe, with confirmed records from Romania, Slo-
vakia, 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

Introduction

The genus *Rymosia* Tuomikoski, 1966 of the family Mycetophilidae comprises in its strict sense (Tuomi-
koski 1966) about 80 valid, living species (Soli 2017), and additional nine poorly described extinct spe-
cies, 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 Neotropi-
cal (5), Afrotopical (1), and Oriental Regions (1). In Europe, 39 extant species are recorded (Chandler et al. 2006), but numerous additional, still undescribed species are known from the Nordic Region (Kjærandsen et al. 2007; Kjærandsen and Soli 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 Palaeartic 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; Kuri-
na 1996) and to a higher degree also aestivating fur-
ther 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 diperan to be found in cave systems throughout Europe (Matile 1970; Kjerandsen 1993).

The scientific history of R. tolleti Burgehe-Balacesco, 1965 is rather brief. The species was described by Anca Burgehe-Bălăcesco (born 1935) in 1965, based on a material collected in cave environment from Romania and France (Hérault department). In the original description, Burgehe-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 led 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 (Burgehe-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 vals for 12 months (years 2021 and 2022). In Romania, temperature and humidity were recorded at entrance. The material was collected directly in glycerine (for further details see Kurina 2006). The new material was collected in cave environment from Romania and France (Hérault department). In the original description, Burgehe-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.

**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 COXI). 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′-GGTCAACAATTCTACATAAGATATTTGG-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, Frederiksborg, 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 (Burdikova 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

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, Štolňa 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...
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...
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 gonostyli dark brown to blackish. Posteroventral margin of the ventrally closed synsclerite (gonoxites) slightly emarginated medially. Hypantral lobe well developed, consisting of pair of inwardly recurved prongs; in lateral view, hypantral 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 lateral; 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, medially part sclerotized, smoothly bending into beak-shaped apex, well exposed in dorsal view. Dorsal branch subquadrate, flattened, 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.

Discussion

According to the original description, the type material of Rymosia tolleti is stated to be deposited at the "Emil Racoviță" Institute of Speleology, Bucharest, Romania (Burghele-Bălăcescu 1965). Unfortunately, this material appears not to have been preserved, as was also concluded for crane flies (Diptera: Limoniidae) described by Burghele-Bălăcescu (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 (Burghel-Bălăcescu 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.

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**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*.

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