

RE-DISCOVERY OF *LEPTOCHILUS QUINTUS* GUSENLEITNER FROM MOSOR MOUNTAIN, CROATIA (HYMENOPTERA; VESPIDAE), AFTER 136 YEARS OF PRESUMED EXTINCTION

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Six males of *Leptochilus quintus* Gusenleitner were collected in May 2021 on Mosor Mountain in Croatia. This species was assumed to have become extinct since no specimens were collected during the past 136 years. In contrast to the only previously known (holotype) specimen with an entirely black clypeus, the specimens examined have variable amounts of yellow markings on the clypeus, and only one specimen has an entirely black clypeus. Two specimens were sequenced for the mitochondrial COI gene, revealing that their sequences differed in two base pairs and suggesting a certain level of retained genetic diversity in the population. This finding suggests the need for a more detailed faunistic analysis of Croatia and the entire Balkans as an intersection of Central, Eastern and Southern European faunistic elements.

Keywords: near-extinction, Eumeninae, sequencing

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Šest mužjaka vrste *Leptochilus quintus* Gusenleitner prikupljeno je u svibnju 2021. na planini Mosoru u Hrvatskoj. Za tu vrstu pretpostavljalo se da je izumrla, jer u posljednjih 136 godina nije prikupljen nijedan primjerak. Za razliku od prethodno poznatog primjerka (holotipa) s potpuno crnim klipeusom, prikupljeni primjerci na klipeusu imaju različite količine žutih oznaka, a tek jedan primjerak ima potpuno crni klipeus. DNA dva primjerka sekvencionirana je za mitohondrijalni gen COI te je otkriveno da se njihove sekvence razlikuju u dva para baza, što ukazuje na određen stupanj genetičke raznolikosti koja se zadržala u populaciji. Ovaj nalaz ukazuje na potrebu detaljnije faunističke analize Hrvatske i čitavog Balkana kao sjecišta srednje-, istočno- i južnoeuropskih faunističkih elemenata.

Cljučne riječi: izumiranje, Eumeninae, sekvencioniranje

INTRODUCTION

The genus *Leptochilus* Saussure 1853 is represented by five subgenera in Europe, comprising 29 species (GUSENLEITNER, 1993, 2003; SANZA, 2003). The greatest number of species is reported from the Iberian Peninsula; diversity declines further east, with only eight species reported from Greece (ARENS, 2012). Eight species were reported from Croatia, namely *L. regulus* (Saussure, 1855), *L. limbiferus* (Morawitz, 1867), *L. duplicatus* (Klug, 1835), *L. quintus* Gusenleitner, 1991, *L. alpestris* (Saussure, 1855), *L. tarsatus* (Saussure, 1855), *L. crassipunctatus* (Maidl, 1922), *L. josephi* Giordani Soika, 1947,

while the status of *L. membranaceus* (Morawitz, 1867) remains unclear (VOGRIN, 1955; GUSENLEITNER, 1991, 1993; JOZAN, 2009, 2012). *L. mimulus* Gusenleitner, 1970 was additionally reported from some neighbouring countries, raising the possibility that this species might also be present in Croatia (GUSENLEITNER, 1993). However, the most interesting species from Croatia is *Leptochilus (Lionotulus) quintus* Gusenleitner, described in 1991. The only known male specimen (holotype) was collected in 1885 in Podvežica, a part of the city of Rijeka in the Kvarner region of Croatia. The characteristics used for species determination include a black clypeus, tegula marked in yellow, and ventral side of antennal scape with yellow markings (GUSENLEITNER, 1993). Since only one specimen of this species was ever recovered, the species was presumed to likely be extinct in both the original description and in a subsequent revision of European species (GUSENLEITNER, 1991, 1993).

MATERIAL AND METHODS

The examined specimens were collected by a hand net during a field trip to Mosor Mountain in southern Croatia. The entire region (encompassing about 1.5 km², from the parking lot to the Umberto Girometta mountain house) was systematically investigated over five years, yielding 184 specimens belonging to 41 Eumeninae species. The micro-location where the examined specimens were collected has scattered *Quercus pubescens* Willd and *Juniperus communis* L. bushes and predominantly semi-arid Mediterranean vegetation (Fig. 1). Notably, the forest wildfire severely affected the region on 17 July 2017, which caused substantial devastation and deforestation of almost 4,500 acres of the *Pinus nigra* forests (Figs. 2a, b).



Fig. 1. The Mosor mountain path, May 2021.

The specimens were photographed with a Leica S9i camera, and image stacking was performed using Helicon Focus 6.8.0 software (Helicon Soft, Kharkiv, Ukraine; <https://www.heliconsoft.com>). The taxonomic analysis included 37 new specimens of *Leptochilus* from the study location and 21 specimens from other locations in Croatia (all in



Fig. 2. July 2017 forest fire in the region, a) the view from the city of Split, demonstrating the extent of the fire, b) the situation in May 2021, near the village of Žrnovnica.

the Polašek Collection). In addition, specimens from the Hungarian Museum of Natural History (HMNH) and the British Museum of Natural History (BMNH) were examined, including the type specimens of *L. torretassoi* (Giordani Soika) and *L. hermoni*, Gusenleitner, alongside the *L. crassipunctata* (Maidl) holotype photograph, from the Naturhistorisches Museum in Vienna (NHM).

The genetic analysis was based on COI gene sequencing, using a specifically developed primer, in line with the previous study (POLAŠEK *et al.*, in press). Two types of analyses were performed. The first was performed in BOLD (<http://www.boldsystems.org>), where the tested sequence was compared to all other deposited sequences. This analysis measured the inter-specific distance with the closest taxonomic group. The second analysis was performed to understand the phylogenetic relationship of multiple species from the genus *Leptochilus*. For this purpose, we used all the available sequences of the Palearctic *Leptochilus* from BOLD, supplemented with the 11 sequences of newly genotyped specimens, namely nine specimens of *Leptochilus* species and two Eumeninae species collected at the same location, which were used as outgroups. The phylogenetic analysis was based on the MAFFT alignment protocol, BMGE alignment and FastME tree build, performed on the NGPhylogeny.fr website (<http://www.ngphylogeny.fr>; DEREPPER *et al.*, 2008; LEMOINE *et al.*, 2019). The two sequences of *L. quintus* males were deposited in the BOLD database (records id 15219040 and 15219041).

RESULTS

Material examined. Croatia, Gornje Sitno, Mosor [43.516439, 16.618167; 740 m above sea level], 27.05.2021, 1♂, leg. O. Polašek; 3♂♂ from the same location, 29.05.2021; 1♂ from the same location, 01.06.2021; Croatia, Gornje Sitno, Mosor [43.519548, 16.606366; 617 m above sea level], 01.06.2021., 1♂, leg. O. Polašek. Two more ♂♂ of the same species were collected in the first location on 01.06.2021, but they were only photographed and released.

The location where the specimens were collected is a part of the nearly 1.2 km long mountain path. All but one specimens originated from the top of this narrow path, while one was collected at the lower altitude of the same path. Two more males with yellow markings on clypeus were observed in the first location and released on 01.06.2021. No females were collected in either location, therefore, the female of this

species remains undescribed. Finally, three more *Leptochilus* species were collected on the same mountain path, namely *L. limbiferus* (Morawitz, 1867), *L. tarsatus* (de Saussure, 1855), and *L. josephi* Giordani Soika, 1947, while *L. alpestris* (de Saussure, 1855) was collected on the neighbouring mountain of Kozjak. Interestingly, there were no specimens of *L. crassipunctatus* (Maidl, 1922) in this area, despite the expectation of this being the most common species in the region (ARENS, 2012).

All specimens were collected very near to the ground, no higher than 10 cm from the surface. In all but one instance, males were collected on the surface of small stones at the edge of the mountain path, where they exhibited territorial behaviour and a lek-related pattern of exposing themselves on the edge of the stones.

Taxonomic analysis

The six examined specimens were first compared to the holotype. Their general appearance did not suggest substantial differences; notably, the colour pattern of the fresh specimens was overall darker (darker wings and legs; Fig. 3a), but this could reflect the colour loss due to the holotype age (Fig. 4a). In addition, only one examined specimen has a completely black clypeus, in line with the holotype (Fig. 4b); the remaining specimens have variable amount of yellow colour on the clypeus, bordered by black (Fig. 3b). A very interesting resemblance was seen in the pubescence pattern on the clypeus and lower part of the vertex, characterised by dense silvery pubescence in the apical fourth of the clypeus, which extends to the interantennal area and along the inner eye margin (Fig. 4b).

The specimen with the black clypeus also has an entirely black ventral side of the antennal scape. These characteristics would have resulted in difficulties determining if the key by GUSENLEITNER (1993) had been used, since the black scape would indicate *L. torretassoi* ssp. *venerensis* Blüthgen. That (sub)species is known only by the holotype specimen from Mt Taormina in Sicily. The tegula colour would be critical in that account, with predominantly yellow tegula in *L. quintus*, Gusenleitner (Fig. 3a). In addition, the examination of the *Leptochilus torretassoi* (Giordani Soika) holotype stored in BMNH (BM.TYPE HM18.1360) suggests that this species has much finer second abdominal tergite punctuation and numerous colour differences, most notably the dark colour of the legs.



Fig. 3. *Leptochilus quintus* Gusenleitner, 1991, male; a) habitus, b) head

One of the *L. quintus* specimens would be misclassified as *L. aegineticus* Gusenleitner; this specimen has longer pubescence on the vertex (about 1.4 longer than the mesonotal pubescence), while the remaining specimens have equally long pubescence of the vertex and mesonotum. The defining feature distinguishing this species is the well-developed angle of the pronotal carina at humeri, which is not present in *L. aegineticus* Gusenleitner (GUSENLEITNER, 1993; ARENS, 2012), while it is well developed in all examined *L. quintus* specimens (Fig. 3a).

The largest taxonomic problem was separating these specimens from two similar species, *L. crassipunctatus* (Maidl) and *L. hermon* Gusenleitner. The comparison of the available specimens revealed that the most pronounced difference was in the T2 lamina structure. The T2 lamina in *L. quintus* is mostly flattened and impunctate (Figure 5a), while the remaining two species have more strongly developed interdigitations in the T2 lamella (extensions of the punctuation structure of the T2 surface), which commonly extend to at least half of the lamella length (Fig. 5b, c). Notably, the overall punctuation of T2 is much stronger in *L. crassipunctatus* (Fig. 5b), while the examined males of *L. hermon*, including one paratype, always have dark brown or blackish markings on the tibia. Lastly, the pronotum is black in all examined *L. quintus* males, with variably sized yellow markings in *L. crassipunctatus* and large markings in *L. hermon*.



Fig. 4. *Leptochilus quintus* Gusenleitner, 1991, holotype; a) habitus, b) head, c) labels; the photos were kindly made by Zoltan Vas from HNHM

Genetic analysis

The genetic analyses were based on the European specimens of *Leptochilus* that were available in the BOLD database, supplemented by the nine newly sequenced specimens of *Leptochilus* and two specimens treated as outgroups (Fig. 5).

The initial analysis and the identification in the BOLD database suggested no species matches for these sequences, with the highest value of similarity found for *L. tarsatus* (89.4%), followed by *L. moustirsensis* (88.6%) and *L. alpestris* (88.3%). The inclusion of newly sequenced specimens from Croatia revealed a distinct sequence not shared with previously sequenced and available species (notably, this analysis also included a pre-



Fig. 5. Second metasomal tergum, dorsally, a) *L. quintus* Gusenleitner, b) *L. crassipunctatus* (Maidl), c) *L. hermon* Gusenleitner

viously unreported sequence of *L. josephi* Giordani Soika; Fig. 6). Unfortunately, there were no fresh specimens of *L. crassipunctatus* (Maidl) or *L. hermon* Gusenleitner available for sequencing, preventing a more detailed genetic analysis. Notably, the two examined specimens of *L. quintus* did not have the same genetic sequence but differed in two loci (positions 31 and 232).

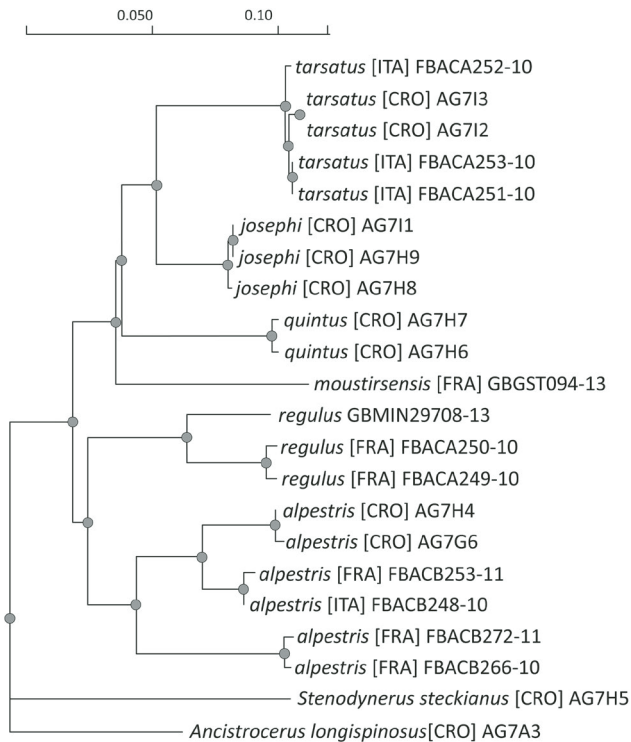


Fig. 6. FastMe tree of the Palearctic *Leptochilus* sequences available in BOLD, supplemented by the 11 newly genotyped specimens from the study location.

DISCUSSION

The finding of *Leptochilus quintus* Gusenleitner, 1991 males on Mosor Mountain in Croatia means that this species is no longer considered extinct, but rare and exhibiting a geographically patchy occurrence. The genetic analysis of the two specimens collected in the same location revealed that they were dissimilar, meaning that the population retained a certain level of genetic diversity. This suggests an even larger population pool, and that the apparent lack of specimens in the examined collections might simply reflect a very patchy occurrence. Interestingly, the 2017 forest fire did not cause the disappearance of this species from the region. However, it remains impossible to define the extent of that event on the population size, as there were no pre-fire records of this species.

The taxonomy of this entire genus in Europe requires revision, especially in light of these genetic analyses. Several European *Leptochilus* species were described on a single examined specimen (GUSENLEITNER, 1993), and previous papers note difficulties in species separation (ARENS, 2012). This is further aggravated by the high levels of intra-specific variability, resulting in the description of numerous subspecies of these species in the past (GUSENLEITNER, 1993); the most extreme example of this in Europe is *L. alpestris*, with several European subspecies and multiple genetic lineages (Figure 6). Finally, large regions of Croatia and the entire Balkans were not adequately assessed in terms of Eumeninae wasps, and notably, these are the most interesting as they are at an intersection of the Central, Eastern and Southern European fauna. Therefore, one of the main priorities for the region is expanding collection efforts and increasing the number of sequenced specimens, allowing for better comparison and clarification of some of the previously raised taxonomic issues.

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