

First record of *Gymnetron tibiellum* Desbrochers des Loges, 1900 (Coleoptera, Curculionidae, Mecinini) from Poland

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Abstract: From several dozen *V. anagallis-aquatica* seed bags collected from a small astatic pond in Zalesie-Podlipie (Eastern Poland), one adult *Gymnetron tibiellum* was grown in laboratory conditions. The weevil was identified based on its morphology and mtCOI DNA sequence. This is the first record of *G. tibiellum* in Poland. The result of the revision of *G. veronicae* specimens published by Smreczyński & Cmoluch (1961) is also given.

Keywords: *Gymnetron tibiellum*, *Veronica anagallis-aquatica* L., first record, E Poland, DNA mtCOI

Introduction

The curculionid genus *Gymnetron* Schoenherr, 1825 consists of 70 Afrotropical species (Caldara 2003) and approximately 35 Palaearctic species (Caldara 2008, Alonso-Zarazaga *et al.* 2023); seven of the latter have been reported from Poland (Wanat & Mokrzycki 2018, Wanat & Ruta 2018). The Palaearctic *Gymnetron* species live on many plants from the genus *Veronica* L. (speedwells) (Plantaginaceae) in various types of habitats, both dry (roadsides, ruderal communities, edges of arable fields) and wet (banks of rivers, ponds, wetlands) (Burakowski *et al.* 1997, Caldara 2008, Benedikt *et al.* 2022).

G. tibiellum Desbrochers des Loges, 1900 (Fig. 1) is distributed from the Middle East, Anatolia and the Caucasus, through Crimea and south-eastern Europe (incl. the Balkans), to the Italian Peninsula. The northernmost localities of *G. tibiellum* hitherto reported are in Austria,

Moravia (Czechia) and Slovakia (Caldara 2008, Benedikt *et al.* 2022, Alonso-Zarazaga *et al.* 2023) (Fig. 2).

Adults of *G. tibiellum* are morphologically very similar to *G. veronicae* (Germar, 1821), so it is very difficult to identify this species solely on the basis of the external morphology. The most conspicuous external character differentiating the two species is the shape of the rostrum: on *G. tibiellum* it is almost straight, though visibly shorter in the male than in the female, whereas in *G. veronicae* it is slightly curved, elongate, and almost the same length in both sexes. But the crucial diagnostic differences between *G. tibiellum* and *G. veronicae* are to be found 1) in the shape of the penis – the apex pointed; the aedeagal and tegminal apodemes elongated in *G. tibiellum* vs the apex rounded; the aedeagal and tegminal apodemes short in *G. veronicae* (Fig. 3A–D); and 2) in the shape of the

spermatheca – with a short, slightly curved cornu and a short, narrow ramus in *G. tibiellum*

vs an elongate, strongly curved cornu and massive ramus in *G. veronicae* (Caldara 2008).



Fig. 1. *Gymnetron tibiellum* Desbr., habitus, dorsal view (photo I. Toševski).



Fig. 2. Distribution of *Gymnetron tibiellum* Desbr.

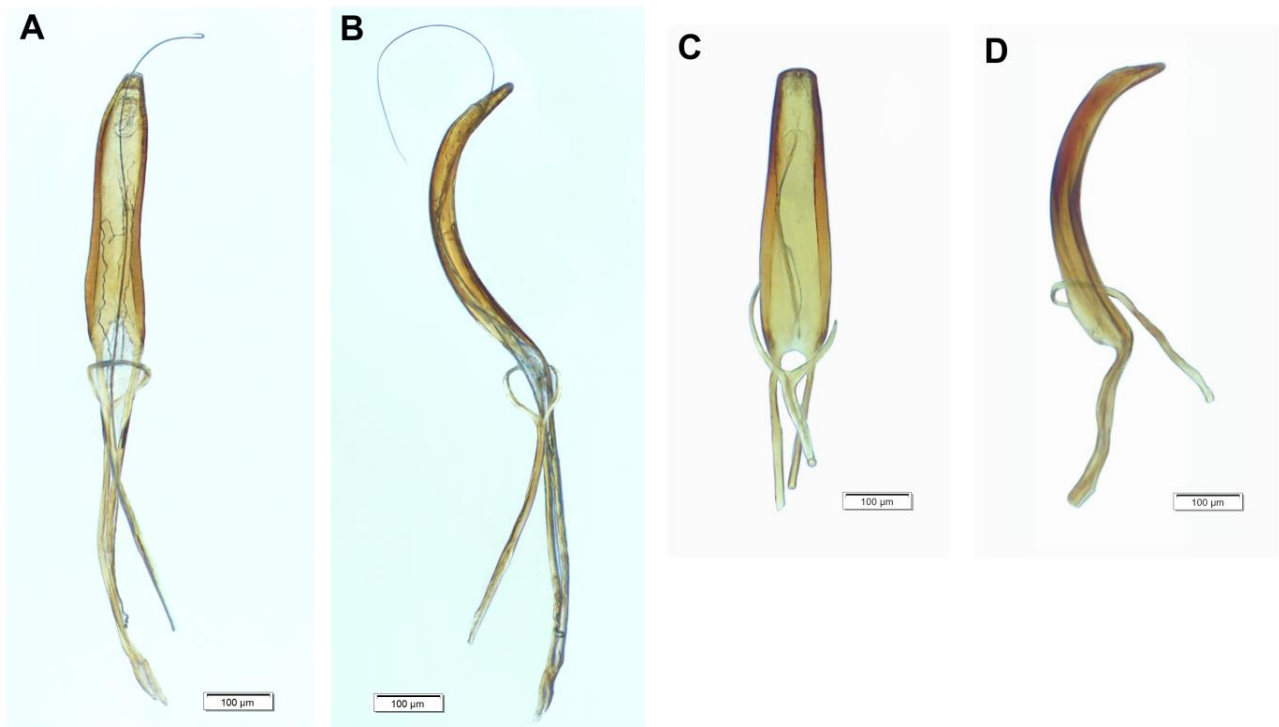


Fig. 3. Comparison of aedeagus shapes in *G. tibiellum* (A, B) and *G. veronicae* (C, D) (photo R. Gosik).

The biology and morphology of immatures of the two species are well known. Adults are active from mid-April, and oviposition takes place from early June to mid-August. The larvae develop in the seed capsules of water speedwell (*Veronica anagallis-aquatica* L.). Seed capsules attacked by larvae are readily detected by the dark colour of the frass deposited within them (Fig. 4A). Pupation also takes place in the seed capsules. The eclosed adults leave the capsules directly after pupation. Despite the similarities in their biology and habitat requirements, no competition has to date been found to occur between *G. tibiellum* and *G. veronicae* (Skuhrovec *et al.* 2022).

Material and Methods

On 2 September 2023, several dozen seed capsules of *V. anagallis-aquatica* were

collected from a small astatic pond surrounded by arable fields in Zalesie–Podlipie (51.241986 22.849490) (near Lublin, eastern Poland). The material was treated in accordance with the method proposed by Toševski (in Skuhrovec *et al.* 2022), i.e. it was placed on wet tissue paper in Petri dishes (Fig. 4B) kept indoors. The level of moisture in the Petri dishes was checked daily and water was added if necessary. On 5 September 2023, a single freshly eclosed adult of *Gymnetron* sp. was collected and preserved in 99% alcohol. It was identified by Ivo Toševski based on its morphology, and the DNA mtCOI sequence provided confirmation. The specimen is deposited in the collection of the Institute for Plant Protection and Environment, Zemun, Serbia.

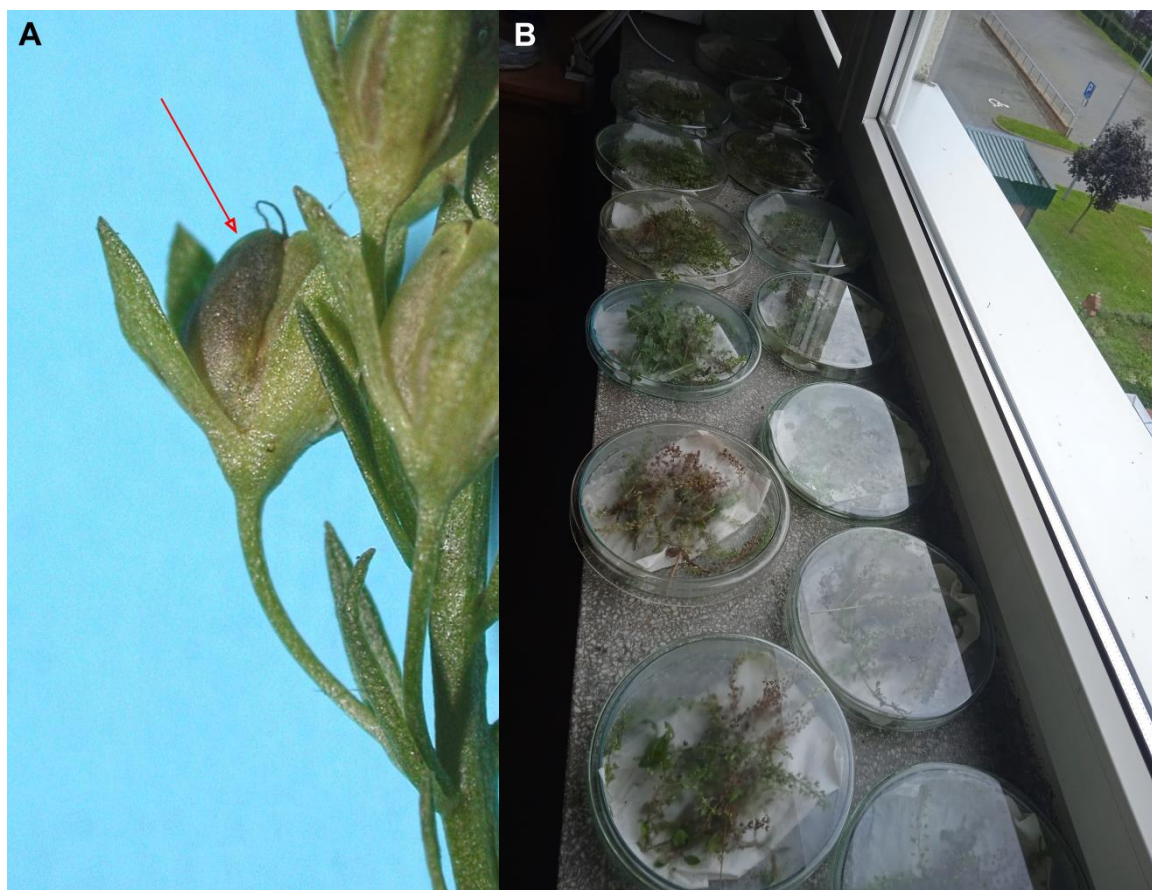


Fig. 4. **A.** A seed capsule of water speedwell attacked by a larva cf. *G. tibiellum* Desbr. (photo I. Toševski). **B.** Rearing *Gymnetron* species from water speedwell seed capsules (photo R. Gosik).

Molecular analysis

For molecular analysis, DNA was extracted from adult emerged from seed capsules of *Veronica anagalis-aquatica*. The barcoding region of the mitochondrial cytochrome c oxidase subunit I gene (mtCOI) was used to confirm the identity of the emerged adult. Genomic DNA was extracted using the DNeasy® Blood & Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. The barcoding region was amplified in a were performed in a Mastercycler ep Gradient S (Eppendorf, Hamburg, Germany) using the primer pair LCO1490 (Folmer *et al.* 1994) and HCOd (Chetverikov *et al.* 2015). PCR reaction was carried out in a volume of 20 µl [1 µl of DNA, 11.8 µl of H₂O, 2 µl of High Yield Reaction Buffer A (1 × 1.5 mM MgCl₂), 1.8 µl of MgCl₂

(2.25 mM), 1.2 µl of dNTP (0.6 mM), 1 µl of each primer of the pair LCO1490/HCOd (0.5 µM) and 0.2 µl of FastGene Taq DNA polymerase (0.0375U/µl) (NIPPON Genetics Europe, Dueren, Germany). The PCR protocol consisted of an initial denaturation at 95°C for 5 min, 35 cycles, each consisting of three steps, i.e., 1 min at 94°C, 1 min at 48°C and 1.5 min at 72°C, with a final extension step at 72°C for 7 min at the end of amplification protocol. After PCR amplification, the products were run on 1% agarose gel, stained with ethidium bromide and visualized under a UV transilluminator. The amplified product was sequenced using the Eurofins sequencing service (Eurofins Genomics-MWG, Germany). The sequence data were deposited in the NCBI GenBank database (www.ncbi.nlm.nih.gov) under accession number MK891708.

DNA-sequence of the mtCOI gene:

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1  ggagcttgat  caggatagtg  aggaacatct
ataagaatta tcattcgcac agaattagga
61  aaccgggaa  aattcattgg  taacgaccaa
attacaatt caattgtaac agctcatgct
121  tttatcataa  tttttttat  agttatacct
attataattg ggggttttgg gaattgatta
181  atcccttaa  tattaggggc  accgatata
gctttccctc gattaaataa tataagattc
241  tgacttctc  ccccatctt  aactttatta
ttaataagaa gagttatcga taaaggagta
301  ggaacaggat  gaactgtcta  tccaccttta
tcaactaata ttgctcatgc aggagcttca
361  gttgacttag  caatttttag  tttacatata
gctggagttt catctatctt aggagcaata
421  aattttatct  caacaatttt  aatataaaa
atagatgaaa taaaacttga tcgtatacct
481  ctttttattt  gatcagtaaa  aatcacagca
atcttattac tttatctttt gccggtttta
541  gcaggagcta  ttactatgct  attaacagat
cgtaatatta atacatcttt tttgaccca
601  gcaggagggg  gggaccaat  tttataccaa
catttattt

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Results and Discussion

The successful breeding of a specimen of *G. tibiellum* from a seed capsule clearly demonstrates that this was not an accidental introduction of a single individual, but that reproduction and development actually occurred in Poland. According to Ivo Tošovský, many more *Gymnetron* adults are usually obtained from seeds in the laboratory than are scooped up from the seed collection site. This highlights the very secretive lifestyle of these weevils and may also indicate that their numbers and actual occurrence have been underestimated.

During previous research (in June and July 2023), several hundred specimens of *G. villosulum* Gyll. but only 3 specimens of *G. veronicae* were obtained from seeds collected in Zalesie-Podlipie. This may indicate that both *G. tibiellum* and *G. veronicae* are in fact rare here. Moreover, because *G. tibiellum* closely resembles the more common *G. veronicae*, it is

highly probable that some specimens were mislabelled and that the distribution of *G. tibiellum* was erroneously identified. For example, all the specimens from Bulgaria identified as *G. veronicae* by Smreczyński & Cmoluch (1961) turned out to be *G. tibiellum*, so these records should be treated as the first of this species from Bulgaria. Furthermore, data on the distribution of this species are based mainly on the reviews of archival materials rather than on new records. For example, Yunakov *et al.* (2018) reports the occurrence of *G. tibiellum* in Ukraine based on the work of Caldara (2008), which is again based on specimens found in Crimea by Strejček in 1983. With the exception of Italy, Serbia, the Czech Republic and Slovakia, all records of *G. tibiellum* localities are probably from the 20th century. This makes it difficult to precisely determine the direction and pace of migration of *G. tibiellum*.

Up to now, the locality of *G. tibiellum* closest to Zalesie-Podlipie was Nový Zámok in Slovakia (Benedikt *et al.* 2022), almost 600 km away in a straight line. Therefore, it seems that, unlike *G. rotundicollis* (Wanat & Ruta 2018), *G. tibiellum* probably did not migrate into east part Poland via the Moravian Gate. However, it cannot be ruled out that due to the high similarity to *G. veronicae*, some specimens of *G. tibiellum* have been incorrectly identified and its distribution in Poland and in Ukraine is underestimated and that it is expanding its range from the south-east. Therefore, a revision of all *G. veronicae* specimens in collections will be welcomed. On the other hand, *G. tibiellum* is capable of active flight, which makes it easier for it to spread freely. It will be possible to precisely determine the direction of migration of *G. tibiellum* once new locations are found. Because *Veronica anagallis-aquatica* is native and widely distributed in the Palaearctic (also common in Poland), the expansion of *G. tibiellum* is probably not associated with changes in the host plant's distribution. Moreover, this speedwell is neither an ornamental nor an

agricultural plant, so it is not intentionally spread or transmitted by humans; the accidental introduction of the insect along with its host plant is therefore unlikely. Wanat & Mokrzycki (2005, 2018) observed that the vast majority of the over 30 species of weevils found in Poland for the first time in the 21st century, including *G. tibiellum*, turned up in anthropogenic biotopes, though rarely as a result of direct human participation. Besides *G. rotundicolle* Gyllenhal, 1838 (Wanat & Ruta 2018), *G. tibiellum* is another southern species that is expanding its range northwards. Hence, the reasons for this presence, as for that of *G. rotundicolle*, should probably be sought in climate changes, especially the declining frequency of regularly recurring long-lasting cold winters and the synanthropization of plant species (Tokarska–Guzik *et al.* 2012).

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