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Short communication

REVEALING THE PRESENCE OF THE EAST ASIAN LEAFHOPPER *TAUTONEURA POLYMITUSA* (HEMIPTERA: AUCHENORRHYNCHA: CICADELLIDAE: TYPHLOCYBINAЕ) IN SERBIA THROUGH DNA BARCODING

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Current data on diversity of the subfamily Typhlocybinae of the Cicadellidae (Hemiptera: Auchenorrhyncha) in Serbia comes from the comprehensive research of Auchenorrhyncha fauna in the former Yugoslavia documenting at least 30 typhlocybina genera with more than 100 species (Janković, 1975; Janković, 1978; Janković & Papović, 1985). A decades-long gap in faunistic research has most likely allowed the unnoticed intrusion of at least some of the alien Auchenorrhyncha species reported in Europe (D'Urso *et al.*, 2019).

During 2017 and 2018, several Typhlocybinae specimens with a white-ochraceous-reddish mosaic pattern were observed by Ivan Pančić in the city of Zrenjanin (Fig. 1A): park Šumice, 45°23'31.14" N 20°24'35.09" E, 25.10.2017; same town but in the suburb Bagljaš, 45°22'46.54" N 20°22'21.06" E, 12.11.2017; same suburb at 45°22'45.79" N 20°22'12.83" E, 23.11.2017; same suburb but at 45°22'54.26" N 20°22'5.59" E, 07.08.2018 and 13.08.2018; same suburb but at 45°22'47.46" N 20°22'6.19" E, 25.09.2018; and in the village Melenci by Marko Šćiban: Banja Rusanda, 45°31'39.87" N 20°18'20.33" E, 21.09.2018, swept from *Fraxinus excelsior*. In 2019, four female individuals with the same habitus were collected by sweeping or combining light traps and yellow sticky traps: 3 ♀♀ in Belgrade and 1 ♀ in the Special Nature Reserve (SNR) Deliblatska peščara (Fig. 1B). Sampled individuals were stored in 2 ml plastic tubes (Sarstedt, Germany) filled with 96% ethanol and kept in a laboratory refrigerator at 4°C until further analysis. Observing

the outer morphological characters under a Leica MZ7.5 stereomicroscope suggested its affiliation to the genus *Tautoneura* Anufriev 1969. First records of this genus in Europe come from the recent findings of the species *Tautoneura polymitusa* Oh & Jung 2016 in Hungary and Slovenia (Tóth *et al.*, 2017; Seljak, 2018).

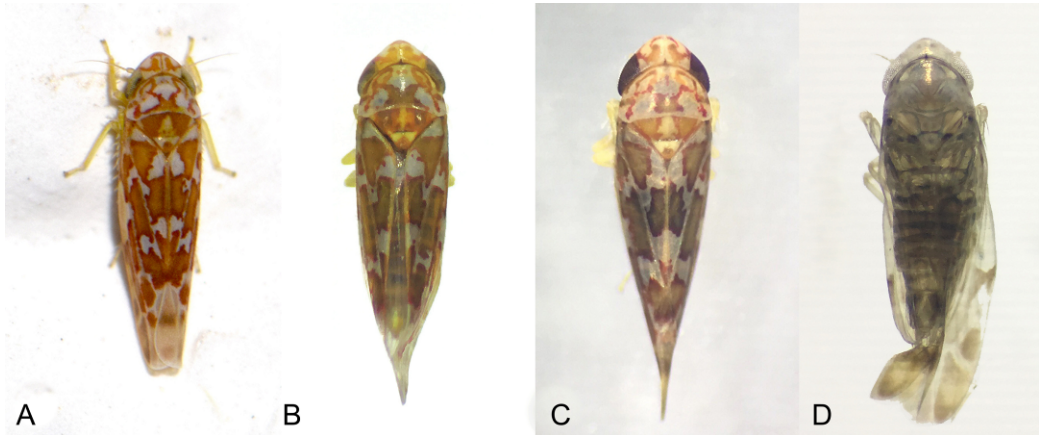


Figure 1. *Tautoneura polymitusa* individuals: A - *T. polymitusa* in obs. (Zrenjanin, 2018); B - female *T. polymitusa*, swept from *U. minor* (Belgrade, 2019); C - male *T. polymitusa*, collected on hyperactinic light tube (Budapest, 2019); D - *T. polymitusa* specimen after DNA extraction.

Since male-based taxonomic keys could not be used in determining the species of collected individuals, the chosen methodology was molecular identification based on the barcoding region of the mitochondrial COI gene, a successful tool in biodiversity research that supplements traditional taxonomy (Sheth & Thaker, 2017). Genotyping solely Serbian specimens would not be informative, since barcoding sequences of *T. polymitusa* are not available in on-line genetic databases (NCBI, BOLD). In order to provide a reliable referent molecular dataset, 13 specimens of *T. polymitusa* (11 ♂♂ and 2 ♀♀) were collected in Budapest (HU) between September 11 and 20, 2019, on an 8W hyperactinic light tube, set on the balcony of an 8th-floor flat located in Káposztásmegyér, the type site of the species first occurrence in Europe (Fig. 1C).

Genomic DNA was isolated from 3 ♀♀ *T. polymitusa* in obs. specimens (SRB) and 7 ♂♂ *T. polymitusa* specimens (HU) by using a partly modified sodium dodecyl sulphate (SDS) extraction method (Kosovac *et al.*, 2018). All 10 specimens whose DNA content was extracted were more or less damaged during the isolation process (Fig. 1D) and are deposited in Marko Šćiban's private entomological collection along with the rest of the intact individuals (2 ♀♀ 4 ♂♂ HU). The desired COI barcoding gene region was amplified for all 10 samples in a PCR protocol using primer pair LCO1490/HCOd (Folmer *et al.*, 1994; Chetverikov *et al.*, 2015) and following the reaction conditions according to Stepanović *et al.* (2016). PCR products of seven samples (2 ♀♀ SRB and 5 ♂♂ HU) were sequenced by MacroGen Inc. (Seoul, South Korea). Obtained sequences were edited in FinchTV v.1.4.0 (<http://www.geospiza.com>) and aligned in Mega v.5.2 using an incorporated ClustalW algorithm (Thompson *et al.*, 1994; Tamura *et al.*, 2011). The final 596 bp-long alignment has revealed a 100% genetic match of Serbian and Hungarian specimens, i.e. the presence of a single haplotype, named Tp1, in all seven samples (GenBank acc. nos. MT024247-8). The lack of nucleotide differences on the barcoding COI gene region between Hungarian male specimens and Serbian female

individuals unequivocally confirms the presence of the Typhlocybae species *T. polymitusa* in Serbia, surmounting the scarce number of sampled individuals and male deficiency.

Examined material:

Serbia: Belgrade: Zemun, 44°49'38.5" N 20°23'11.8" E, 06.08.2019, 1 ♀, swept from *Ulmus minor* along the A1 motorway (part of the European route E-75), leg. A. Kosovac and M. Šćiban, DNA isolate AK1961, COI haplotype Tp1, GeneBank acc.no. MT024247.

Serbia: Belgrade: Zemun (Gornji Grad), 44°51'19.3" N 20°22'39.1" E, 27.08.2019, 1 ♀, caught on yellow sticky trap attached to outdoor LED light, leg. A. Kosovac and M. Šćiban, DNA isolate AK2037, COI haplotype Tp1; same locality, methodology and leg., but 03.09.2019, 1 ♀, DNA isolate AK2038, not genotyped.

Serbia: SNR Deliblatska peščara: Labudovo okno, 44°50'50.8" N 21°16'44.9" E, 26.08.2019, 1 ♀, at light lamp; leg. A. Kosovac and M. Šćiban. Note: physically damaged individual, by outer morphological features and coloring pattern most probably *T. polymitusa*.

Hungary: Budapest: Káposztásmegyer, 47°35'09.6" N 19°06'46.8" E, 11-20.09.2019, 1 ♂, at hyperactinic light tube, leg. L. Ronkay & M. Tóth, DNA isolate AK2061, COI haplotype Tp1, GeneBank acc.no. MT024248; same locality, collecting methodology and leg., but 1 ♂, DNA isolate AK2062, haplotype Tp1; same but 1 ♂, DNA isolate AK2063, haplotype Tp1; same but 1 ♂, DNA isolate AK2064, haplotype Tp1; same but 1 ♂, DNA isolate AK2065, haplotype Tp1; same but 1 ♂, DNA isolate AK2066, not genotyped; same but 1 ♂, DNA isolate AK2067, not genotyped; same but 4 ♂♂ and 2 ♀♀, intact specimens.

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ОТКРИЋЕ ПРИСУСТВА ИСТОЧНОАЗИЈСКЕ ЦИКАДЕ
TAUTONEURA POLYMITUSA (HEMIPTERA: AUCHENORRHYNCHA:
CICADELLIDAE: TYRHLOCYBINAЕ) У СРБИЈИ
ПУТЕМ ДНК БАРКОДИНГА

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Извод

Након првих запажања јединки цикада подфамилије Tyrhlocybinae (Hemiptera: Auchenorrhyncha: Cicadellidae), специфичне бело-наранџасто-црвене обојености у Зрењанину и Меленцима 2017. и 2018. године, четири женке истог хабитуса су сакупљене у августу 2019. у Београду и Специјалном резервату природе Делиблатска пешчара. Анализе спољашње морфологије су указале да су најсличније источноазијској инвазивној врсти присутној у Европи, *Tautoneura polymitusa* Oh & Jung 2016. Услед недостатка узоркованих мужјака, поуздана детерминација врсте је захтевала употребу молекуларних метода. С обзиром да у банкама гена нису доступне нуклеотидне секвенце баркодинг региона COI гена врсте *T. polymitusa*, генетичкој идентификацији су прикључени примерци мужјака сакупљени у Будимпешти на локалитету првог налаза врсте у Европи. Након успешног умножавања и секвенцирања COI баркодинг региона утврђено је 100% генетичко подударање секвенци анализираних женки из Србије и мужјака из Мађарске. Добијени резултати потврђују присуство алохтоне цикаде *T. polymitusa* у Србији и указују на значај баркодинг методе која у контексту истраживања биодиверзитета успешно превазилази проблеме узорковања као што су мали број примерака или одсуство мужјака.

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