

Zombrus bicolor (Enderlein) (Hymenoptera: Braconidae: Doryctinae), a new allochthonous species for the fauna of Serbia

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Abstract. The paper presents all the findings of the braconid species *Zombrus bicolor* in Serbia so far and gives a brief history of the arrival of this allochthonous wasp species in Europe. The first report of *Z. bicolor* on the continent was in 2011 in the European part of Russia. After that, it was found in four more European countries: Italy in 2012, Greece in 2021, and Romania in 2022. Considering Serbia, we report on five localities where this species was found from 2020 to 2022.

Keywords: wasp, alien species, ectoparasitoid, Holcobraconini, Cerambycidae.

Zombrus bicolor (Enderlein, 1912) is a large braconid wasp (Fig. 1) belonging to the subfamily Doryctinae, tribe Holcobraconini, subtribe Odontobraconina. The Holcobraconini contains seven genera widely distributed in tropical and subtropical areas, primarily in Africa (Belokobylskij & Samartsev 2011). About 50 species are known (Yu 2019); only four of them have been recorded in the Palaearctic (all from the genus *Zombrus* Marshall, 1897). The species inhabiting Palaearctic are *Z. anisopus* Marshall, 1897, recorded in Egypt and Saudi Arabia (Fahringer 1930), also Morocco (Belokobylskij & Samartsev 2011); *Z. flavipennis* (Brullé, 1848) with distribution in Iran (Fallahzadeh & Saghaei 2010) and Pakistan (Shenefelt & Marsh 1976); *Z. sikkimensis* Enderlein, 1920 from Afghanistan (Fahringer 1930) and India (Enderlein 1920), and *Z. bicolor* (Enderlein, 1912), which is already known from Europe (Belokobylskij & Samartsev 2011). *Zombrus bicolor* is a species native to Far East Asia whose autochthonous distribution range includes China, Mongolia, Japan, and the Korean Peninsula. It has also been recorded in Central Asia (Kazakhstan and Kyrgyzstan) (Belokobylskij & Samartsev 2011, Yu 2019).

In Europe, *Z. bicolor* was observed for the first time in the European part of Russia in the Astrakhan province (Belokobylskij & Samartsev 2011) and Dagestan (Castañeda-Osorio et al. 2019). The following country where it was found was Italy, in the province of Siena (Loni et al. 2012). In Serbia, the very first information on the potential presence of this species is given in the Alciphron database of insects of Serbia (Radeka 2020). An unpublished record from 2021 indicates the occurrence of *Z. bicolor* in Greece; more precisely in Kozani regional unit, on the Facebook group "European Ichneumonoidea" (2023), unpublished. The last unofficial publication and information on this species from Europe comes from the same internet source, the record from Romania, Black Sea coast, 2022 ("European Ichneumonoidea" 2023, unpublished).

It is a solitary ectoparasitoid of several wood-boring coleopteran species larvae in its native distribution. The hosts are primarily cerambycids from the genera *Xylotrechus* Chevrolat, 1860, i.e., *X. pyrrhoderus* Bates, 1873; *Turanoclytus* Sama, 1994, i.e., *T. namanganensis* (Heydel, 1885), and *Chlorophorus* Chevrolat, 1863, i.e., *C. annularis* (Fabricius,

1787), and *C. japonicus* (Chevrolat, 1863) (Cao et al. 2015, Yu et al. 2019). Also, the following cerambycid species are recorded as hosts: *Allotraeus sphaerioninus* Bates, 1877 (Cao et al. 2015); *Dere thoracica* White, 1855 (Chen et al. 2009); *Trichoferus campestris* (Faldermann, 1835), and *Massicus raddei* Blessig, 1872, (Belokobylskij & Maeto 2009, Cao et al. 2015, Yu 2019).

Examined material

The material is chronologically listed and presented in Figure 2: 1f, Vršac, 08.09.2020; 1f, 21.06.2021; 1f, 15.06.2022, leg. B. Radeka; 1f, Paraćin, 05.06.2021, leg. A. Petrović (Fig. 1); 2f, Bogatić, 06.06.2021, leg. S. Gajić; 1m, Koviljski Rit, 23.07.2021, leg. G. Farkaš; 1m, 21.07.2022. Stara Oreškovića (Donji Milanovac), leg. N. Vesović; 1f Bački Brestovac, 09.11.2022, leg. B. Rapajić.



Figure 1. Female of *Z. bicolor* found in Serbia locality of Paraćin. Photo A. Petrović.

Barcoding and phylogenetic tree construction

A barcoding method is applied for species confirmation on the molecular level. The entire genomic DNA was extracted using Dneasy® Blood & Tissue Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions.



Figure 2. Map of occurrences of *Z. bicolor* in Europe. Countries where the species has been recorded are green, and the year of the first finding is also indicated (right). The enlarged map of Serbia shows the sampling localities within the country (left). Localities (yellow circles) in Serbia: 1. Vršac, 2. Paraćin, 3. Koviljski Rit, 4. Stara Oreškovica (Donji Milanovac), 5. Bački Brestovac, 6. Bogatić.

DNA extraction was successfully performed from a single female (1f, Paraćin, 05.06.2021, leg. A. Petrović). The barcoding region of the mitochondrial cytochrome oxidase subunit I (COI) was used to compare the examined specimens with available corresponding parasitoid sequences from the public database NCBI GenBank (1988). Amplification of the barcoding COI fragments was done using standard primer pairs LCO1490 and HCO2198 (Folmer et al. 1994).

The PCR reactions took place in an Eppendorf Mastercycler® according to the following protocol: initial denaturation at 95°C for 5 min; 35 cycles consisting of 1 min at 94°C; 1 min at 54°C and 1.5 min at 72°C; and a final extension at 72°C for 7 min. Thus, amplified products were run on 1% agarose gel, stained with Midori Green (Nippon Genetics Europe) safe DNA/RNA stain, and visualized under a UV transilluminator.

Barcoding COI fragments were sequenced using automated equipment (Macrogen Europe, Amsterdam, the Netherlands).

The read sequence was edited using FinchTV (Geospiza, Inc., Seattle, WA) and prepared for alignment using Clustal W incorporated in the MEGA 11 software package (Tamura et al. 2021). Three available sequences of the genus *Zombrus* were used from the NCBI GenBank (*Z. bicolor* from Russia, *Zombrus* sp. from Benin, and *Zombrus* aff. *croceipes* (Brues, 1826) from Kenya), and one sequence of the genus *Monarea* Szépligeti, 1904, which is a member of the same tribe, with the only represented species *M. fridae* Belokobylskij,

Zaldívar-Riverón & Coronado-Blanco, 2014 in the GenBank database. The COI sequence of *Z. bicolor* that was successfully read (marked with the code Zb1 COI) is a female collected at locality 1 – Vršac. Together with the sequences taken from the BOLD gene bank (Table 1), a phylogenetic tree was constructed (Fig. 3). We used COI sequences of two braconid species, *Dendrosoter protuberans* (Nees, 1834) (Doryctinae) and *Cotesia tibialis* (Curtis, 1830) (Microgastrinae) as outgroup taxa (Table 1, Figure 3).

Table 1. The list of COI sequences/taxa used for phylogenetic tree construction.

Species	Country	Accession number (code)
<i>Zombrus bicolor</i>	Serbia	OR113697 (Zb1 COI)
<i>Zombrus bicolor</i>	Russia	MK728579.1
<i>Zombrus</i> sp.	Benin	MK728578.1
<i>Zombrus</i> aff. <i>croceipes</i>	Kenya	MK728577.1
<i>Monarea fridae</i>	Mexico	MK728567.1
<i>Dendrosoter protuberans</i> (outgroup)	Russia	KP268028.1
<i>Cotesia tibialis</i> (outgroup)	Austria	JF963164.1

Mounting specimens and imaging

The examined specimens of *Z. bicolor* previously preserved in 96% alcohol were air dried, then mounted on cards, and labeled. The whole insect was photographed outdoors using a SONY Snapshot G10 camera. The body parts were photographed using a Leica M165C stereomicroscope with Leica Flexacam C3 (Leica Microsystems, Wetzlar, Germany) at the Faculty of Sciences and Mathematics, University of Niš, Serbia. One male (1m, 21.07.2022. Stara Oreškovica (Donji Milanovac), leg. N. Vesović) and one female (1f, Paraćin, 05.06.2021, leg. A. Petrović) were deposited in the entomological collection of the mentioned institution. The remaining specimens of *Z. bicolor* in Serbia are registered solely as photo data, which are deposited on the Alciphron database and the “Insekti Srbije (Insects of Serbia)” Facebook group (2023).

Phylogenetic status

The phylogenetic position of the analyzed taxa using the neighbor-joining method is presented in Figure 3. The topology of the presented phylogenetic tree shows that all *Zombrus* species are clustered, leaving *M. fridae* branch out. The sequence of *Z. bicolor* (MK728579.1) from the GeneBank database, which was used in the phylogenetic analysis of the tribe Holcobraconini (Castañeda-Osorio et al. 2019), and the analyzed specimen of *Z. bicolor* (Zb1 COI) are very closely related, i.e., they differ only in one nucleotide base. Being clustered together, with such a small distance, clearly indicates that the two sequences belong to the same species, *Z. bicolor*.

Diagnosis

Zombrus bicolor clearly differs morphologically from the congeneric species by completely dark wings, a body covered with long dense setae, and occipital carina present dorsally and partly laterally on the occiput.

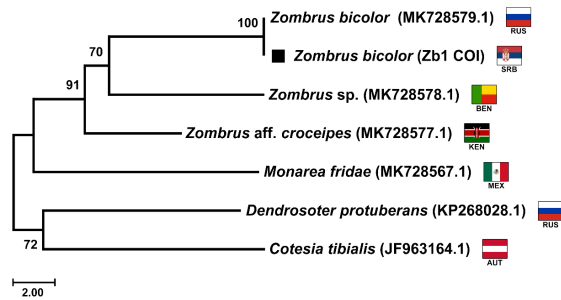


Figure 3. Phylogenetic tree inferred using the neighbor-joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved seven nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). A total of 570 positions were in the final dataset. The sequence marked with a black square represents the *Z. bicolor* from Serbia.

Female (Fig. 4). Body reddish-brown, 12.5 mm in length. Head: smooth, 1.5 times wider than its medial length. Eyes glabrous, small, 1.2 times longer than the temple. Ocelli arranged in an equilateral triangle. Maxillary palps with 6 palpomeres reddish-brown, labial palps with 4 palpomeres, reddish to brown. Antennae black, thick, 48-segmented bearing long setae. The scape 1.5 times longer than wide on its half-length; the pedicel is short, and the first flagellar segment is 3.3 times longer than wide. Mesosoma: twice as long as its height. Pronotum with deep longitudinal medial depression. Mesoscutum with deep notauli, sparsely crenulate. Mesonotum and mesopleuron smooth, sternauli deep. The propodeum is sculptured and reticulate with short lateral tubercles. Both pairs of wings are completely black, including pterostigma—length of forewing 8.5 mm. Legs are entirely black. Tarsal segments of the mid legs are shorter than those in the fore and hind legs. Hind coxa with long curved spine dorsally. Hind basitarsus half as long as fore basitarsus. Metasoma: First tergite (T1) trapezoid, broadening apically, 1.4 times longer than wide. The second tergite (T2) has deep anterolateral oblique grooves. The first three tergites striated longitudinally. Ovipositor sheath is 0.6 times as long as metasoma.

Male (Fig. 5). Body length 11.2 mm. The coloration of all body parts as in female. Head: as in female, including the number of maxillary and labial palpomeres. Antennae are 50-segmented, thick, equal to the length of the body. Mesosoma: as in female. Forewing length 7 mm. Metasoma: slender, dorsoventrally flattened, 1.2 times longer than in females. Aedeagus is slightly visible from the outside.

Although the examined specimens of *Z. bicolor* were collected without knowing their hosts, this parasitoid may have adapted to autochthonous xylophagous beetle fauna. Potential host candidates, for example, could be from the genus *Chlorophorus* since it has been registered from *C. annularis* and *C. japonicus* in its original habitats (Belokobylskij & Samartsev 2011, Yu 2019). Moreover, in the host spectrum of *Z. bicolor*, there are important pest cerambycid species such as the tiger longhorn beetle, *Xylotrechus chinensis* (Chevrolat, 1852), and *Anoplophora*

chinensis (Forster, 1771) which have been already registered in several European countries and listed in EPPO global database. The tiger longhorn beetle causes serious damage to mulberries, but its harmful effect on apples, pears, and grapevines has also been noted primarily in Greece and Spain (EFSA Panel on Plant Health et al. 2021). It is known that *Z. bicolor* shows certain variability in color; for example, there are specimens with rather dark-colored metasoma (Belokobylskij & Samartsev 2011), in Serbia were only registered specimens with reddish-brown colored metasoma. A more detailed description of the variability is given in Belokobylskij & Samartsev (2011).

Since *Z. bicolor* is a relatively recently recorded species in Europe and there is no data on its ecological impact, it could still be beneficial to keep the population of the aforementioned cerambycid pests under control.

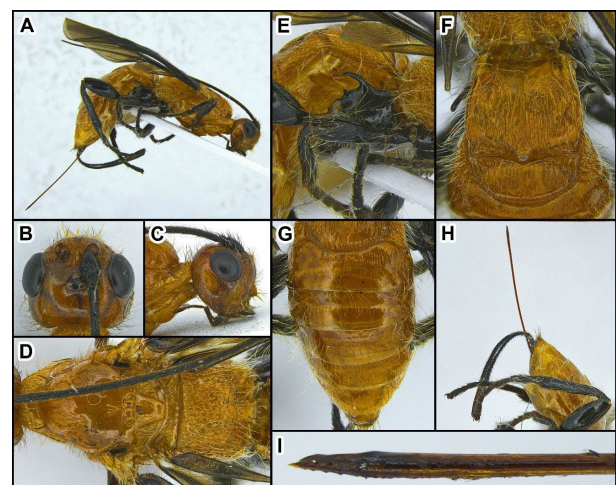


Figure 4. *Zombrus bicolor* (female). A. general habitus (lateral view); B. head (dorsal view); C. head (lateral view); D. mesosoma (dorsal view); E. hind coxa (outer view); F. petiole (dorsal view); G. metasoma (dorsal view); H. metasoma (lateral view); I. ovipositor (lateral view).

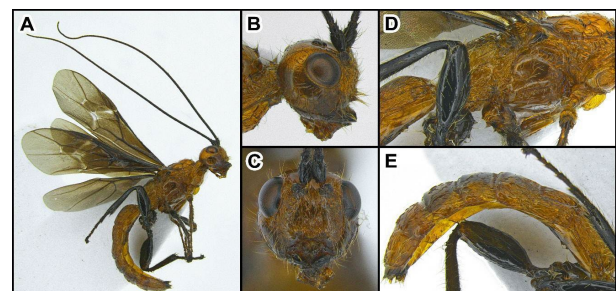


Figure 5. *Zombrus bicolor* (male). A. general habitus (lateral view); B. head (lateral view); C. head (frontal view); D. mesosoma (lateral view); E. metasoma (lateral view).

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