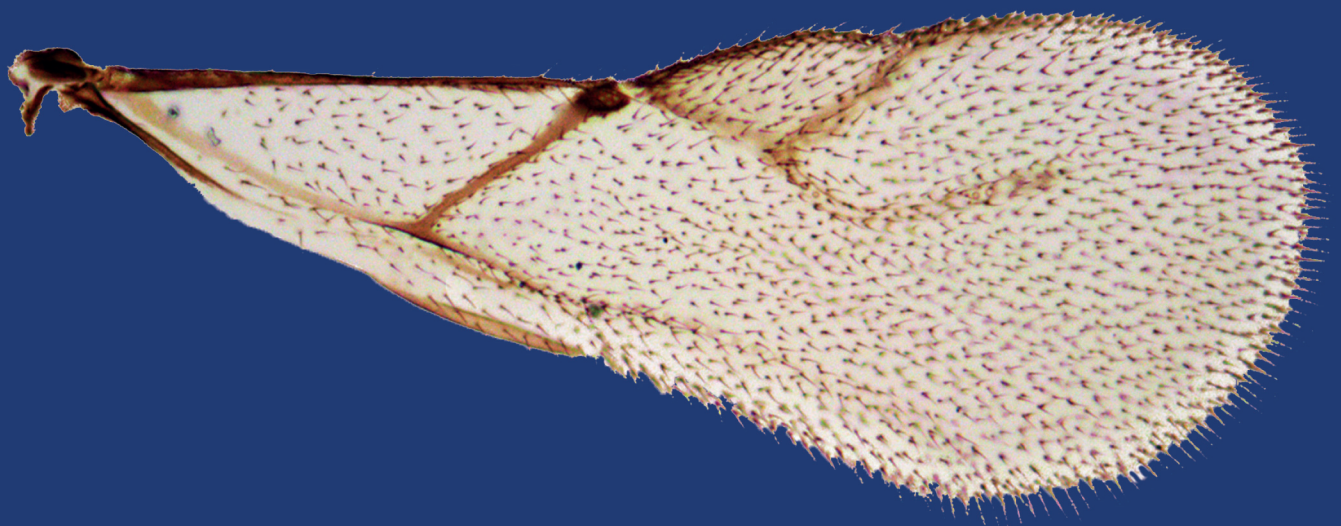


Insights into phylogenetic relationships between
Trixys Haliday, 1833 and *Binodoxys* Mackauer, 1960
(Hymenoptera, Braconidae, Aphidiinae), with
a description of a new species of the genus *Trixys*

Jelisaveta ČKRKIĆ, Andjeljko PETROVIĆ,
Korana KOCIĆ & Željko TOMANOVIĆ



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Trioxyx ulmi Črkić & Tomanović n. sp., male fore wing.

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Insights into phylogenetic relationships between *Trioxys* Haliday, 1833 and *Binodoxys* Mackauer, 1960 (Hymenoptera, Braconidae, Aphidiinae), with a description of a new species of the genus *Trioxys*

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ABSTRACT

Despite extensive research on the taxonomy and phylogeny of the subfamily Aphidiinae Haliday, 1833, certain questions about the relationships between genera remain unresolved. Genera *Trioxys* Haliday, 1833 and *Binodoxys* Mackauer, 1960 are considered closely related, based on morphological and molecular analyses. However, recent studies suggest there is a need for a taxonomic revision of the two genera, since molecular data does not support monophyly of the two groups when a larger number of species is used in the analysis. We examine those relationships using molecular data and including a new species we describe in the present study. *Trioxys ulmi* Črkić & Tomanović, n. sp. is a parasitoid of the Japanese elm aphid (*Tinocallis takachihoensis* Higuchi, 1972) on elm hybrids (*Ulmus x hollandica* Mill.). Despite its probable Asian origin, this species has gone undescribed until its accidental introduction to Europe, highlighting the importance of continued research efforts.

RÉSUMÉ

Aperçu des relations phylogénétiques entre Trioxys Haliday, 1833 et Binodoxys Mackauer, 1960 (Hymenoptera, Braconidae, Aphidiinae), avec la description d'une nouvelle espèce du genre Trioxys.

Malgré des recherches approfondies sur la taxonomie et la phylogénie de la sous-famille des Aphidiinae Haliday, 1833, certaines questions sur les relations entre les genres demeurent non résolues. Les genres *Trioxys* Haliday, 1833 et *Binodoxys* Mackauer, 1960 sont considérés comme étroitement apparentés, d'après des analyses morphologiques et moléculaires. Cependant, des études récentes suggèrent qu'une révision taxonomique des deux genres est nécessaire, puisque les données moléculaires ne supportent pas la monophylie des deux groupes lorsqu'un plus grand nombre d'espèces est utilisé dans l'analyse. Nous examinons ces relations en utilisant des données moléculaires et en incluant une nouvelle espèce que nous décrivons ici. *Trioxys ulmi* Črkić & Tomanović, n. sp. est un parasitoïde du puceron japonais de l'orme (*Tinocallis takachihoensis* Higuchi, 1972) sur les hybrides de l'orme (*Ulmus x hollandica* Mill.). Malgré son origine asiatique probable, cette espèce n'a pas été décrite jusqu'à son introduction accidentelle en Europe, soulignant l'importance des efforts de recherche continus.

KEY WORDS

Aphidiinae,
Trioxina,
Trioxys,
Binodoxys,
DNA barcoding,
new species.

MOTS CLÉS

Aphidiinae,
Trioxina,
Trioxys,
Binodoxys,
DNA barcoding,
espèce nouvelle.

INTRODUCTION

Trioxys Haliday, 1833 is one of the largest genera within the subfamily Aphidiinae Haliday, 1833, with currently over 70 species described worldwide (Yu *et al.* 2016). Classified in the subtribe Trioxina Mackauer, 1961 based on morphology, its main diagnostic characters are accessory prongs on the last abdominal sternite, as well as the absence of secondary tubercles on the petiole (Mackauer 1961). The second character, secondary tubercles on the petiole, is traditionally used to separate *Trioxys* and the closely related genus *Binodoxys* Mackauer, 1960. Recent studies employing morphological and molecular data show there is no clear distinction between the two genera, and that there is a need to revise the current status of *Trioxys* and *Binodoxys*, or at least some of the species within these two genera (Čkrkić *et al.* 2019).

Japanese elm aphid, *Tinocallis takachihoensis* Higuchi, 1972 is an aphid of Asian origin, commonly found on *Ulmus* spp. in its native area, as well as in Europe, North Africa, and North America, where it has been introduced (Blackman & Eastop 1994). Other than *Ulmus* spp., it can be found on *Hemiptelea davidii* (Hance) Planch. in China and eastern Siberia, and it is relatively common on bonsai *Zelkova serrata* (Thunb.) Makino in England (Blackman & Eastop 1994). The introduction of *T. takachihoensis* to Europe has been accidental, and it is probable that it came via bonsai plant trading (Petrović-Obradović *et al.* 2018). While this species is not considered a serious pest, large amount of honeydew deposits can inflict damage on young trees (Petrović-Obradović *et al.* 2018).

There have been reports of parasitoids from the genus *Trioxys* parasitizing *Tinocallis* species (Starý 1978, 1987, 1988; Lumbierres *et al.* 2005). However, only one parasitoid (*Aphidius* sp.) of *T. takachihoensis* has been recorded so far from Algeria (Hemidi *et al.* 2013). Here we report a new species of the genus *Trioxys*, found parasitizing *T. takachihoensis* on *Ulmus x hollandica*, diagnosed by morphological differences and molecular data, using the mitochondrial cytochrome c oxidase subunit I (COI) gene. We also discuss the relationship of the new species with other members of *Trioxys* and with the related genus *Binodoxys*.

MATERIAL AND METHODS

SPECIMEN COLLECTION AND MORPHOLOGICAL ANALYSIS

Specimens were collected in New Belgrade (Belgrade, Serbia; GPS coordinates: 44.48003420, 020.21529524), in June 2017. Plant leaves infested with aphids were collected in plastic boxes covered with mesh to allow for ventilation. Samples were kept under laboratory conditions for 3–4 weeks, or until parasitoid emergence. Adult parasitoids were dissected and slide mounted for detailed examination of external morphology, using a ZEISS® Discovery V8 stereomicroscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). Photographs of the slide mounted specimens were obtained using a Leica DM LS phase contrast microscope (Leica Microsystems GmbH, Wetzlar, Germany), and used to measure relevant

morphological characters with ImageJ software (Schneider *et al.* 2012). Morphological terminology follows Sharkey & Wharton (1997).

MOLECULAR ANALYSIS

DNA was extracted from three individual adult parasitoids using the QIAGEN Dneasy® Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. The barcode region of the COI gene was amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). DNA amplification was performed in a 20 µl volume, containing 1 µl of DNA, 11.8 µl of H₂O, 2 µl of High Yield Reaction Buffer A with 1 × Mg, 1.8 µl of MgCl₂ (2.25 mM), 1.2 µl of dNTP (0.6 mM), 1 µl of each primer (0.5 µM) and 0.2 µl of KAPA Taq DNA polymerase (0.05U/µl) (Kapa Biosystems Inc., Boston, USA). PCR amplification was performed in an Eppendorf Mastercycler® (Hamburg, Germany), using the following thermal profile: initial denaturation at 95° C for 5 min, followed by 35 cycles of 94° C for 60 s, 54° C for 60 s, 72° C for 90 s and a final extension at 72° C for 10 min. Purification of PCR products and DNA sequencing in both direction was performed by Macrogen Inc. (Seoul, Korea).

Sequences were edited with FinchTV ver. 1.4.0 (www.geospiza.com). CLUSTAL W algorithm integrated in MEGA X software (Kumar *et al.* 2018) was used to align sequences. Sequences were trimmed to a length of 638 bp. Newly acquired sequences in this study are deposited in GenBank.

Two additional sequences, identical to those of the new species described here, were acquired from the BOLD database (<http://www.boldsystems.org>; BIN AAU8586). Additional sequences of *Trioxys complanatus* Quilis Perez, 1931, *T. auctus* (Haliday, 1833), *T. sunnysidensis* Fulbright & Pike, 2007, *T. parauctus* Starý, 1960, *T. pallidus* (Haliday, 1833), *Trioxys* sp., *Binodoxys angelicae* (Haliday, 1833), *B. acalephae* (Marshall, 1896), *B. heraclei* (Haliday, 1833), *B. brevicornis* (Haliday, 1833), *B. communis* (Gahan, 1926) and *B. centaureae* (Haliday, 1833) were used to place the newly discovered species within the genus *Trioxys* and its closely related genus *Binodoxys* (Table 1).

Average genetic distances were calculated using Kimura's two-parameter method of base substitution (K2P, Kimura 1980) integrated in MEGA X. Phylogenetic relationships between the new species and related species were explored using Bayesian inference of phylogeny. A phylogenetic tree was constructed in MrBayes 3.1.2 software (Ronquist & Huelsenbeck 2003), using the Hasegawa-Kishino-Yano model as the best-fitting model of sequence evolution based on the Akaike Information Criterion, determined by model test integrated in MEGA X. Bayesian inference analysis was conducted by running two Markov Chain Monte Carlo searches, both with one cold and three heated chains. The analysis ran for 1 million generations, with sampling done every 1000 generations and first 25% of trees discarded as a burn-in. Convergence of parameters was confirmed by Tracer 1.7 (Rambaut *et al.* 2018), and the consensus tree with posterior probabilities was viewed in FigTree 1.3.1 (Rambaut 2009).

TABLE 1. — Specimens used in phylogenetic reconstruction (*Trioxys complanatus* Quilis Perez, 1931, *T. auctus* (Haliday, 1833), *T. sunnysidensis* Fulbright & Pike, 2007, *T. parauctus* Stary, 1960, *T. pallidus* (Haliday, 1833), *Trioxys* sp., *Binodoxys angelicae* (Haliday, 1833), *B. aculephae* (Marshall, 1896), *B. heraclei* (Haliday, 1833), *B. brevicornis* (Haliday, 1833), *B. communis* (Gahan, 1926) and *B. centaureae* (Haliday, 1833)). Institution abbreviations: **FBUB**, Faculty of Biology, University of Belgrade, Serbia; **CBG**, Centre for Biodiversity Genomics, Guelph, Ontario, Canada; **ZFMK**, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany; **AO**, Agrocampus Ouest, Rennes, France; **UIBK**, University of Innsbruck, Austria; **IZCAS**, Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

Parasitoid	Country	Date	Plant	Aphid	GenBank acc.num/Deposition of BOLD ID	specimens
<i>Trioxys ulmi</i> Črkić & Tomanović, n. sp.	Serbia	22.VI.2017	<i>Ulmus x hollandica</i> Mill.	<i>T. takachihoensis</i> Higuchi	MT873046	FBUB
<i>T. ulmi</i> Črkić & Tomanović, n. sp.	Serbia	22.VI.2017	<i>Ulmus x hollandica</i>	<i>T. takachihoensis</i>	MT873047	FBUB
<i>T. ulmi</i> Črkić & Tomanović, n. sp.	Serbia	22.VI.2017	<i>Ulmus x hollandica</i>	<i>T. takachihoensis</i>	MT873048	FBUB
<i>T. ulmi</i> Črkić & Tomanović, n. sp.	Canada	23.IX.2009	—	—	ASGLE245-10	CBG
<i>T. ulmi</i> Črkić & Tomanović, n. sp.	Germany	8.VIII.2013	—	—	GMGMC456-14	ZFMK
<i>Trioxys</i> sp.	Canada	20.VI.2014	—	—	CNGSC3555-15	CBG
<i>Trioxys</i> sp.	Canada	20.VI.2014	—	—	CNGSC3595-15	CBG
<i>T. complanatus</i>	Iran	—	—	—	KJ848479	—
<i>T. pallidus</i>	Iran	24.III.2004	<i>Juglans regia</i> L.	<i>Chromaphis juglandicola</i> (Kaltenbach)	KR074105	—
<i>T. auctus</i>	Serbia	31.V.2013	<i>Alisma plantago-aquatica</i> L.	<i>Rhopalosiphum nymphaeae</i> (L.)	MK080163	FBUB
<i>T. sunnysidensis</i>	Germany	—	<i>Triticum aestivum</i> L.	<i>Rhopalosiphum padi</i> (Linnaeus)	KY887944	UIBK
<i>T. sunnysidensis</i>	Germany	—	<i>Triticum aestivum</i> L.	<i>Rhopalosiphum padi</i>	KY887945	UIBK
<i>T. parauctus</i>	Serbia	5.VIII.2011	<i>Galium verum</i> L.	<i>Hydaphis molluginis</i> Börner	MK080164	FBUB
<i>B. brevicornis</i>	Montenegro	27.VII.2012	<i>Sanicula europaea</i> L.	<i>Hyadaphis foeniculi</i> (Passerini)	MF287649	FBUB
<i>B. brevicornis</i>	Montenegro	27.VII.2012	<i>Sanicula europaea</i>	<i>Hyadaphis foeniculi</i>	MK080162	FBUB
<i>B. heraclei</i>	Montenegro	7.VIII.2013	<i>Pastinaca</i> sp.	<i>Cavariella aegopodii</i> (Scopoli)	MF287648	FBUB
<i>B. aculephae</i>	Serbia	28.VI.2014	<i>Robinia pseudoacacia</i> L.	<i>Aphis</i> sp.	MK080160	FBUB
<i>B. aculephae</i>	Finland	22.VII.2016	<i>Vicia cracca</i> L.	<i>Aphis craccae</i> L.	MK080161	FBUB
<i>B. communis</i>	China	15.V.2012	<i>Glycine max</i> (L.) Merr.	<i>Aphis glycines</i> Matsumura	KF597735	IZCAS
<i>B. angelicae</i>	Belgium	15.VI.2015	<i>Tanacetum parthenium</i> (L.) Sch. Bip.	<i>A. fabae</i> Scopoli/ <i>B. cardui</i> (L.)	KY912706	FBUB
<i>B. angelicae</i>	Sweden	2.VII.2014	<i>Malus</i> sp.	<i>Aphis pomi</i> De Geer	MK080159	FBUB
<i>B. centaureae</i>	France	25.VI.2009	—	—	JN620612	AO

ABBREVIATIONS

FBUB Institute of Zoology, Faculty of Biology, University of Belgrade;
 MNHN Muséum national d'Histoire naturelle, Paris.

SYSTEMATICS

Subfamily APHIDIINAE Haliday, 1833
 Subtribe TRIOXINA Mackauer, 1961
 Genus *Trioxys* Haliday, 1833

RESULTS :

MOLECULAR ANALYSIS

Phylogenetic tree constructed based on COI sequences of *Trioxys* and *Binodoxys* in MrBayes is shown in Figure 1. *Trioxys ulmi* Črkić & Tomanović, n. sp. forms one clade in the tree, with *T. complanatus* and *T. pallidus* positioning as sister species. The other clade is comprised of the remaining species used in the analysis, from both analysed genera, with *T. parauctus* clustering with *B. brevicornis*.

Interspecific genetic distances calculated based on COI range from 6% between *T. complanatus* and *T. pallidus*, to 14.8% between *T. complanatus* and *B. brevicornis* (Table 2). Divergence rates between *T. ulmi* Črkić & Tomanović, n. sp. and other species used in the analysis range from 10.2% with *T. sunnysidensis* to 14.1% with *B. heraclei*.

Trioxys ulmi Črkić & Tomanović, n. sp.
 (Figs 2; 3)

urn:lsid:zoobank.org:act:A6C5F36F-5CC4-4188-A5A9-1EC300CC562B

TYPE LOCALITY. — Serbia, Belgrade, New Belgrade (44°28'47"N, 20°12'55"E).

TYPE MATERIAL. — **Holotype**. Serbia • 1 ♀; Belgrade, New Belgrade 44°28'47"N, 20°12'55"E; 22.VI.2017; Korana Kocić leg.; reared from *T. takachihoensis* on *Ulmus x hollandica*, slide mounted; FBUB. **Paratypes**. Serbia • 21 ♀, 14 ♂; same data as holotype; preserved in alcohol and slide mounted (2 ♀, 2 ♂); FBUB (17 ♀, 9 ♂); MNHN (5 ♀, 5 ♂).

DISTRIBUTION. — The current known distribution of the new species is Serbia, although we suspect a much broader distribution of this

TABLE 2. — Genetic distances between *Trioxys* Haliday, 1833 and *Binodoxys* Mackauer, 1960 species based on COI sequences (*Trioxys complanatus* Quilis Perez, 1931, *T. auctus* (Haliday, 1833), *T. sunnysidensis* Fulbright & Pike, 2007, *T. parauctus* Starý, 1960, *T. pallidus* (Haliday, 1833), *Trioxys* sp., *Binodoxys angelicae* (Haliday, 1833), *B. aculephae* (Marshall, 1896), *B. heraclei* (Haliday, 1833), *B. brevicornis* (Haliday, 1833), *B. communis* (Gahan, 1926) and *B. centaureae* (Haliday, 1833)).

	<i>T. ulmi</i> Čkrkić & Tomanović, n. sp.	<i>Trioxys</i> sp.	<i>T. complanatus</i>	<i>T. auctus</i>	<i>T. sunnysidensis</i>	<i>T. parauctus</i>	<i>T. pallidus</i>	<i>B. angelicae</i>	<i>B. aculephae</i>	<i>B. heraclei</i>	<i>B. brevicornis</i>	<i>B. communis</i>
<i>T. ulmi</i> Čkrkić & Tomanović, n. sp.	—	—	—	—	—	—	—	—	—	—	—	—
<i>Trioxys</i> sp.	0.114	—	—	—	—	—	—	—	—	—	—	—
<i>T. complanatus</i>	0.124	0.114	—	—	—	—	—	—	—	—	—	—
<i>T. auctus</i>	0.119	0.102	0.132	—	—	—	—	—	—	—	—	—
<i>T. sunnysidensis</i>	0.102	0.084	0.128	0.083	—	—	—	—	—	—	—	—
<i>T. parauctus</i>	0.131	0.115	0.141	0.113	0.117	—	—	—	—	—	—	—
<i>T. pallidus</i>	0.113	0.115	0.060	0.138	0.138	0.124	—	—	—	—	—	—
<i>B. angelicae</i>	0.135	0.107	0.142	0.118	0.084	0.117	0.142	—	—	—	—	—
<i>B. aculephae</i>	0.128	0.096	0.123	0.098	0.092	0.106	0.126	0.101	—	—	—	—
<i>B. heraclei</i>	0.141	0.109	0.134	0.123	0.101	0.103	0.137	0.114	0.098	—	—	—
<i>B. brevicornis</i>	0.131	0.109	0.148	0.128	0.119	0.081	0.142	0.111	0.103	0.097	—	—
<i>B. communis</i>	0.124	0.088	0.121	0.094	0.088	0.094	0.125	0.101	0.032	0.096	0.105	—
<i>B. centaureae</i>	0.127	0.099	0.136	0.123	0.108	0.111	0.132	0.087	0.117	0.118	0.123	0.115



FIG. 1. — Phylogenetic tree constructed based on sequences of COI. Bayesian posterior probabilities are shown above branches. *Monoctonus crepidis* (Haliday, 1834) (MT080072), *Ephedrus* sp. (KR927633) and *Venturia* sp. (KP072612) were used as outgroups.

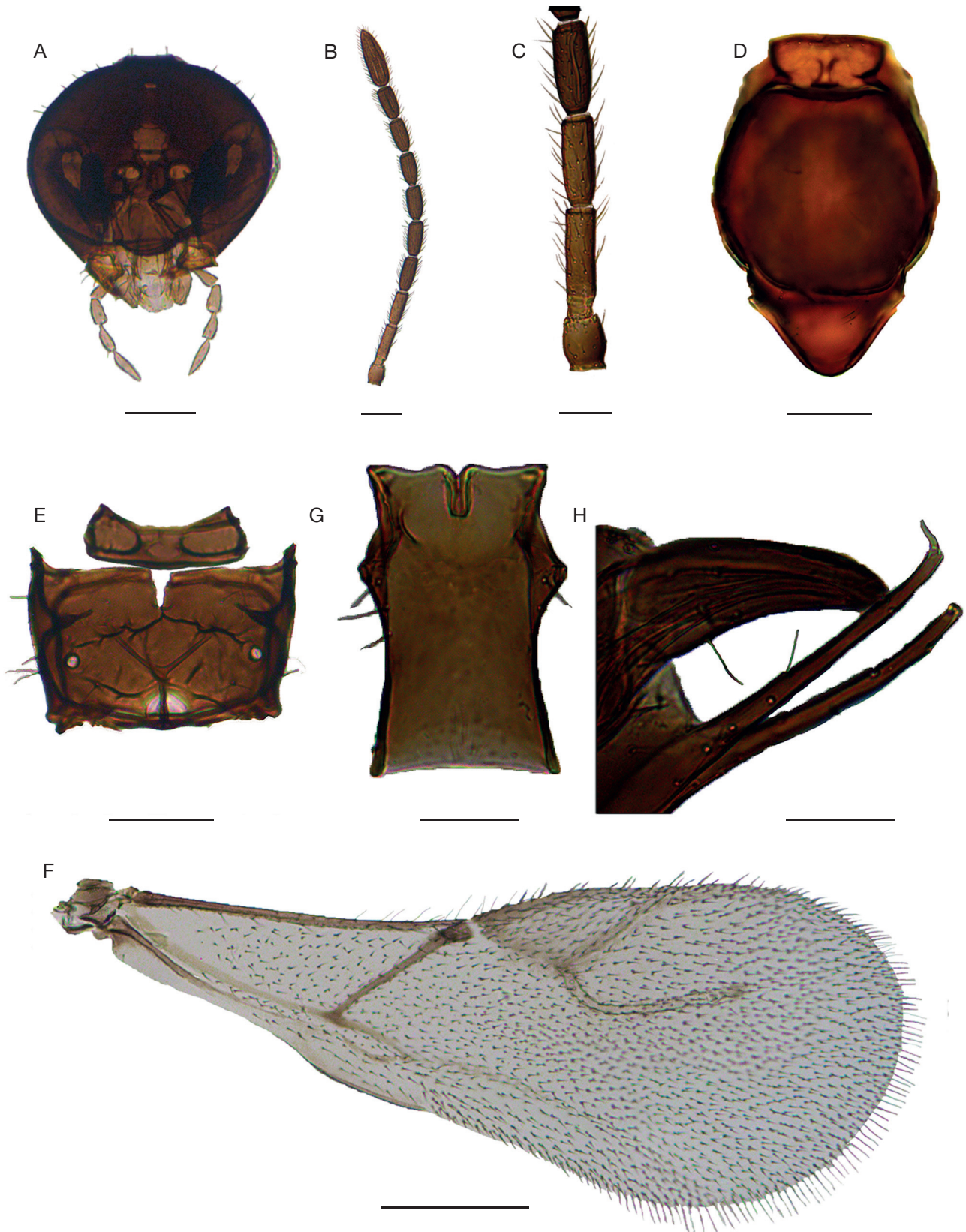


FIG. 2. — *Trioxys ulmi* Čkrkić & Tomanović, n. sp., female: **A**, head; **B**, antenna; **C**, flagellomeres 1-3; **D**, mesoscutum; **E**, propodeum; **F**, fore wing; **G**, petiole; **H**, ovipositor sheath and prongs. Scale bars: A, B, D, E, 100; C, G, 50; F, 200 μm .

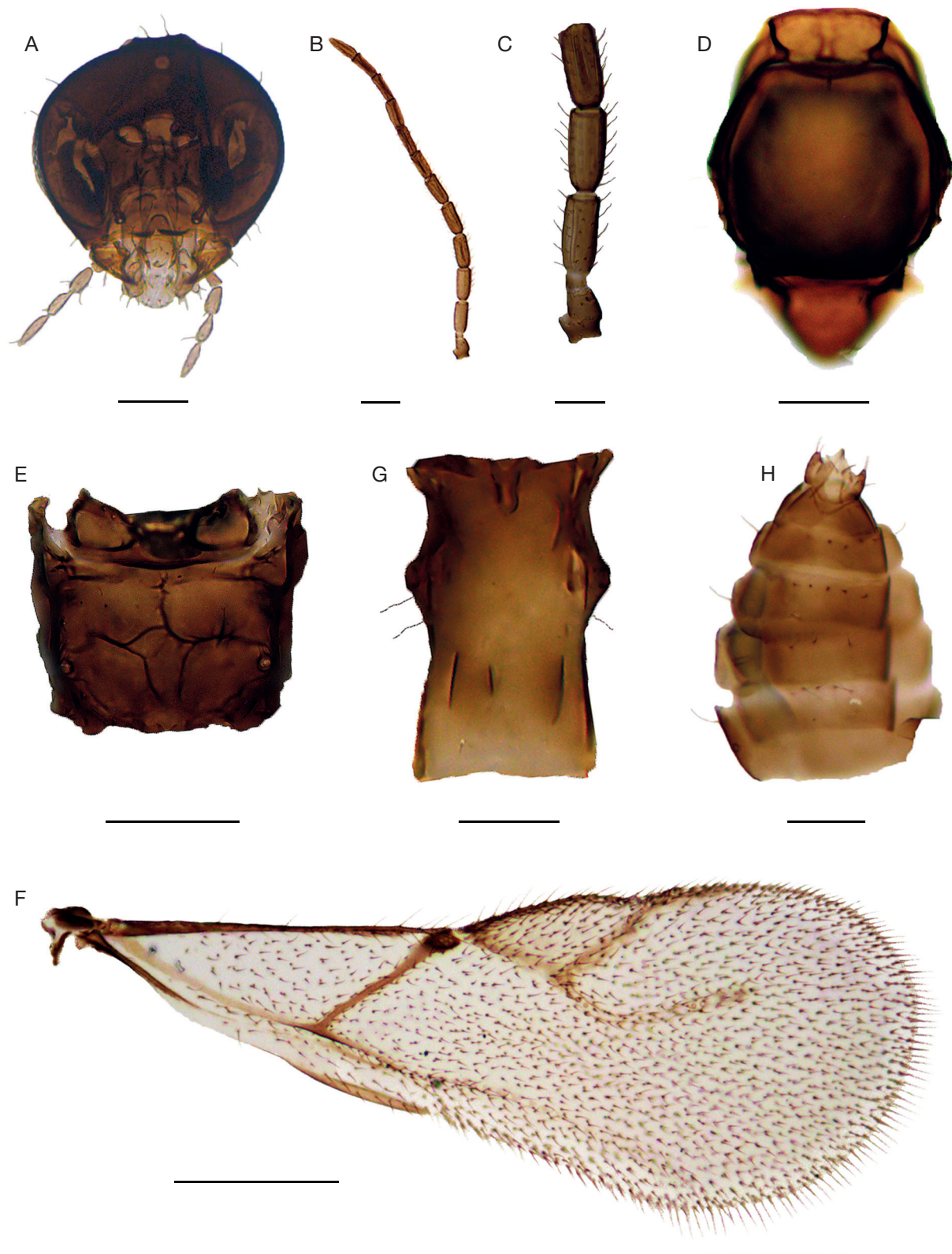


FIG. 3. — *Trioxys ulmi* Čkrkić & Tomanović, n. sp., male: **A**, head; **B**, antenna; **C**, flagellomeres 1-3; **D**, mesoscutum; **E**, propodeum; **F**, fore wing; **G**, petiole; **H**, tip of metasoma and aedeagus. Scale bars: A, B, D, E, H, 100; C, G, 50; F, 200 µm.

species in association with *T. takachihoensis*/ *Ulmus* spp. Sequences of the COI gene identical to those from Serbian specimens are also registered in Canada and Germany.

ETYMOLOGY. — The name of the new species is derived from the most common host of *T. takachihoensis*, *Ulmus* spp.

DIAGNOSIS. — Morphologically most similar to *T. complanatus* (Tomanović & Kavallieratos 2002). Transverse carinae present on dorsal surface of propodeum (Fig. 2E), irregular postmedian carinae and the beginning of a closed central areola present in some specimens (in *T. complanatus* transverse carinae sometimes present, but discontinuous; no signs of a central pentagonal areola). Petiole with a slight constriction behind spiracular tubercles (almost parallel-sided in *T. complanatus*).

HOST. — *Tinocallis takachihoensis*.

REMARK

Since the main diagnostic character, the sculpturing of the dorsal surface of the propodeum, varies to some extent in *T. ulmi* Čkrkić & Tomanović, n. sp., it is advisable to take aphid hosts (*T. ulmi* Čkrkić & Tomanović, n. sp. is a parasitoid of *Tinocallis takachihoensis* while *T. complanatus* mainly parasitizes aphids from the genus *Therioaphis* Walker, 1870 on legumes) and DNA data into account when identifying this new species.

DESCRIPTION

Female

Head (Fig. 2A). Eyes oval, medium sized, sparsely setose. Malar space equal to 0.14–0.15 of longitudinal eye diameter, tentorial index 0.25. Clypeus oval with 4–5 setae. Maxillary palps with 4 palpomeres, labial palps with 2 palpomeres. Antenna with 11 antennomeres, filiform, setae on flagellomeres semierect, subequal to flagellomere diameter (Fig. 2B). Flagellomere 1 (F1) 3.6 times as long as wide, with 0–1 longitudinal placodes. F2 2.6 times as long as wide, with 1–2 longitudinal placodes (Fig. 2C). F1 1.2 times longer than F2. F3, F4 and F5 with 1–2, 0–3 and 0–4 longitudinal placodes, respectively.

Mesosoma. Mesoscutum without notaulices, dorsal surface smooth (Fig. 2D). Head width/mesoscutum width ratio 1.2. Propodeum without closed central pentagonal areola (Fig. 2E). Antemedian carina very short; transverse carinae present, sometimes irregular. Postmedian carinae present and irregular in some paratypes.

Fore wing (Fig. 2F). Wing length 1 mm, width 0.4 mm. Stigma triangular, 2.6 times as long as wide and 1.7 times as long as distal abscissa of R1. Wing venation reduced, fused r and RS (r&RS) visible, reaching distally as far as R1 or shorter.

Metasoma. Petiole 1.45 times as long as wide at spiracles. Dorsal disc smooth, with 2–3 long setae on each side (Fig. 2G). Ovipositor sheath slightly curved downwards, length/width ratio 2.6. Prongs straight, length 0.16 mm, with 2 dorsal hairs and one claw-like apical bristle (Fig. 2H).

Colour. Head brown, eyes black, mouthparts yellow. Scapus, pedicel, F1 and F2 light brown, remainder of antenna brown.

Mesonotum brown, propodeum and legs light brown. Wings hyaline with brown venation. Petiole light brown, rest of metasoma, including ovipositor sheaths, brown.

Body length. 1.6 mm.

Male (Paratype)

Head (Fig. 3A). Eyes oval, medium sized, sparsely setose. Malar space equal to 0.2 of longitudinal eye diameter, tentorial index 0.3. Clypeus oval with 2–3 setae. Maxillary palps with 4 palpomeres, labial palps with 2 palpomeres. Antenna with 13 antennomeres, filiform, setae on flagellomeres semierect, subequal to half of flagellomere diameter (Fig. 3B). Flagellomere 1 (F1) 2.3 times as long as wide, with 2–4 longitudinal placodes (Fig. 3C). F2 2.2 times as long as wide, with 1–4 longitudinal placodes. F1 subequal to F2.

Mesosoma. Mesoscutum without notaulices, dorsal surface smooth (Fig. 3D). Head width/mesoscutum width ratio 1.2. Propodeum in most specimens with closed central pentagonal areola (Fig. 3E), but some rare specimens lack the areola and have irregular transverse carinae.

Fore wing (Fig. 3F). Wing length 1 mm, width 0.4 mm. Stigma triangular, 2.6 times as long as wide and 2.1 times as long as distal abscissa of R1. Wing venation reduced, fused r and RS (r&RS) visible, reaching distally as far as R1 or shorter.

Metasoma. Petiole with prominent spiracles, 1.5 times as long as wide at spiracles. Dorsal disc smooth, with 2 long setae on each side (Fig. 3G).

Colour. Head brown, eyes black, mouthparts yellow. Scapus, pedicel, F1 and F2 light brown, remainder of antenna brown. Mesonotum and propodeum brown, legs light brown. Wings hyaline with brown venation. Petiole light brown, rest of metasoma brown (Fig. 3H).

Body length. 1.5 mm.

DISCUSSION

Genera *Trioxys* and *Binodoxys* are considered closely related, and were initially considered to be one genus, *Trioxys* (Mackauer 1959). Mackauer (1960) separated *Binodoxys* as a subgenus of *Trioxys* based on the presence of secondary tubercles on the petiole. Since then, *Binodoxys* has been considered as a separate genus (Mackauer 1961) or as a subgenus of *Trioxys* (Mackauer 1960). Morphological data seem to support the separation of two groups (Lazarević *et al.* 2017). Molecular analyses of the phylogeny of Aphidiinae recovered *Trioxys* and *Binodoxys* as closely related, but usually with a small number of species used in the analysis (Belshaw & Quicke 1997; Smith *et al.* 1999; Kambhampati *et al.* 2000; Sanchis *et al.* 2000; Shi & Chen 2005; Derocles *et al.* 2012). Recent studies that focused solely on the two genera, rather than the whole sub-

KEY TO SPECIES OF APHIDIINAE PARASITIZING *TINOCALLIS* MATSUMURA, 1919 APHIDS IN EUROPE, BASED ON FEMALES

1. Forewing m-cu vein present; ovipositor sheath without prongs; pupation in a cocoon under mummified aphids *Praon flavinode* (Haliday, 1833)
- Forewing m-cu vein absent; ovipositor sheath with prongs; pupation in a cocoon inside the mummified aphid 2
2. Abdominal prongs bent knee-like behind the middle, apex of prongs brush-like with 5 bristles on apex *Betuloxys hortorum* (Starý, 1960)
- Abdominal prongs not bent, apex of prongs with 1 simple or claw-shaped bristle 3
3. Abdominal prongs with 1 simple bristle at apex *Trioxyx tenuicaudus* Starý, 1978
- Abdominal prongs with 1 claw-shaped bristle at apex 4
4. Prongs curved upwards, with 2 long hairs on dorsal surface *Trioxyx curvicaudus* Mackauer, 1967
- Prongs straight, with 2 or 4 setae on dorsal surface 5
5. Prongs with 4 long setae on dorsal surface *Trioxyx pallidus* (Haliday, 1833)
- Prongs with 2 long setae on dorsal surface *Trioxyx ulmi* Čkrkić & Tomanović, n. sp.

family, showed that molecular data is not in concordance with morphological analyses, implying that *Binodoxys* and *Trioxyx* are not monophyletic groups (Čkrkić *et al.* 2019; Rakhshani *et al.* 2020). The results of this study confirm the ambiguities shown by molecular results – while all *Binodoxys* species cluster together, the positioning of *Trioxyx* species used in the analysis does not support monophyly of this genus. Genetic distances between species of the two genera based on COI are relatively high for Aphidiinae (Table 2), a situation that is becoming increasingly common with new studies of various Aphidiinae genera (Čkrkić *et al.* 2019, 2020; Kocić *et al.* 2019). In addition, the divergence rates between congeners are not significantly different from those between species designated to different genera, as previously shown (Čkrkić *et al.* 2019). The grouping of some *Trioxyx* species does not reflect ecological similarities either. *Trioxyx ulmi* Čkrkić & Tomanović, n. sp. and *T. pallidus*, parasitoids of arboricolous aphids, cluster with *T. complanatus*, a parasitoid of the spotted alfalfa aphid, *Therioaphis trifolii* on *Medicago* and *Trifolium* (Tomanović & Kavallieratos 2002). These results suggest that a comprehensive revision is needed, where all available species of both genera will be included and subject to an integrative approach. Combining morphology, several molecular markers and biology and ecology of analysed species with thorough sampling for greater resolution will allow for a clearer picture of the phylogenetic relationships between *Trioxyx* and *Binodoxys* species.

Taxonomic studies of Aphidiinae wasps throughout Europe have been ongoing for decades (Starý 1966a, b; Kavallieratos *et al.* 2004, 2016; Kos *et al.* 2012; Žikić *et al.* 2012). Despite continuous research efforts, new species for science or for European fauna are reported very often (e.g. Tomanović *et al.* 2009; Petrović *et al.* 2013, 2017; Žikić *et al.* 2015), including some *Trioxyx* species (Rakhshani *et al.* 2017, 2020; Čkrkić *et al.* 2019). Aphidiinae are often accidentally introduced into new areas due to their small size and intercontinental traffic and trade. Based on the origin of its aphid host, it is

probable that *T. ulmi* Čkrkić & Tomanović, n. sp. also has an Asian origin, and that it was accidentally introduced with *T. takachihoensis* and bonsai plant material. A European origin is unlikely, given the numerous taxonomic studies conducted in Europe lately, where this species would probably have been recorded by now (Kavallieratos *et al.* 2004; Žikić *et al.* 2012; Rakhshani *et al.* 2017, 2020). Its currently known host, *T. takachihoensis* is not considered a serious pest, and *T. ulmi* Čkrkić & Tomanović, n. sp. most likely has little significance in terms of biological control of aphids at this moment. However, there are some concerns that *T. takachihoensis* may become a pest of ornamental plants in non-native areas, especially in the light of global warming, and that it may adapt to more severe climatic conditions (Kanturski *et al.* 2018). On the other hand, as a suspected alien species, *T. ulmi* Čkrkić & Tomanović, n. sp. should be carefully monitored, because there is always a possibility of adaptation to new hosts in non-native areas (Roy *et al.* 2011; Petrović *et al.* 2013). Although Hemidi *et al.* (2013) listed *Aphidius* sp. as a parasitoid of *T. takachihoensis*, this finding should be considered with caution, since all known parasitoids of *Tinocallis* aphids belong to the genera *Betuloxys* Mackauer, 1960 and *Trioxyx* (Starý 1978, 1987, 1988; Lumbierres *et al.* 2005), with the exception of *Praon flavinode* (Haliday, 1833) (Starý 1987). This study represents the first record of a *Trioxyx* species parasitizing *T. takachihoensis*. Other *Tinocallis* species in Europe are parasitized by three *Trioxyx* species – *T. curvicaudus* Mackauer, 1967, *T. pallidus* and *T. tenuicaudus* Starý, 1978 (Starý 1988).

These findings highlight the importance of ongoing taxonomic research efforts, since most studies continue to yield new data about species distributions and tritrophic associations with aphids and host plants. Given the recent studies showing alarming extinction rates in insects (Hallmann *et al.* 2017; Sánchez-Bayo & Wyckhuys 2019), studies that contribute to our knowledge of insect biodiversity are of paramount importance.

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