



Research article

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The taxonomic status of recently described *Isophya* taxa from Serbia (Tettigoniidae, Phaneropterinae)

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Abstract. During recent decades, increasing research of the taxonomy of the genus *Isophya* resulted in taxonomic descriptions of several new species. The delimitation of these species is mainly based on oscillographic song analysis of the male song in combination with morphological characters, such as the shape of male cerci, tegmina and ovipositor. In the present paper, we used an integrative taxonomic approach in order to resolve the status of four recently described *Isophya* taxa from Serbia. Based on our molecular and bioacoustic analyses, all analyzed taxa belong to a single species: *Isophya modestior*. The majority of the morphological characters used to differentiate the taxa showed strong intra- and interpopulational variability, proving that describing new species within the genus *Isophya* should not rely on morphological characters alone, but needs to consider bioacoustic analyses as the main tool and a larger series of specimens.

Keywords. Bioacoustics, bush crickets, *Isophya modestior*, phylogeography, synonym.

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Introduction

With 90 valid species, the genus *Isophya* Brunner von Wattenwyl, 1878 represents the second richest Orthoptera Latreille, 1793 genus in Europe after *Poecilimon* Fischer, 1853 (Cigliano *et al.* 2022). Although both genera belong to the family Tettigoniidae Krauss, 1902, they lack internal sclerotized genital organs in males (titillators), which are normally a very helpful taxonomic character used in identification of bush-crickets. Therefore, characters such as the shape of cerci, pronotum, tegmina and ovipositor have been used for identifying species within the genera *Poecilimon* and *Isophya* (e.g., Ramme 1933, 1951; Harz 1969). Due to a high intraspecific morphological variability, especially within species of *Isophya*, identification based upon morphological traits alone is almost impossible (Heller *et al.* 2004; Orci *et al.* 2005; Iorgu *et al.* 2017). Introducing male calling songs and stridulatory files as species-specific characters in the genus *Isophya* by Zhantiev & Dubrovin (1977) resolved the taxonomic status of many species and several new cryptic species have been discovered in recent years (e.g., Orci *et al.* 2010; Iorgu 2012; Iorgu *et al.* 2017). Therefore, this character nowadays represents one of the main tools for species identification and description.

In the past 15 years, increased research on Orthoptera from Serbia resulted in the discovery of several new species of Orthoptera for the country fauna, including five species of *Isophya* – *I. clara* Ingrisch & Pavićević 2010, *I. costata* Brunner von Wattenwyl, 1878, *I. bureschi* Peshev, 1959, *I. miksici* Peshev, 1985 and *I. rectipennis* Brunner von Wattenwyl, 1878 (Ingrisch & Pavićević 2010; Szövényi & Szekeres 2011; Pavićević *et al.* 2014; Chobanov *et al.* 2016; Ivković *et al.* 2021). Recently, Pavićević (2017, 2021) described four new *Isophya* taxa based mainly on their morphological characters, three of which were compared with *I. modestior* Brunner von Wattenwyl, 1882 (Fig. 1A) and one with *I. modesta modesta* (Frivaldszky, 1868). In 2017, two species of *Isophya* were described – *I. radmilae* Pavićević, 2017 from Radan Mt (1250 m a.s.l.) and *I. pancici* Pavićević, 2017 from Tara Mt (Račanska Šljivovica 1200 m a.s.l.) (Fig. 1B). *Isophya radmilae* differs from *I. modestior* by a smaller body size, shorter pronotum and tegmina, on average more teeth on stridulatory file, slender male cerci and shorter, moderately upcurved ovipositor. *Isophya pancici* has almost the same characters with *I. radmilae*, differing from *I. modestior* by smaller body size, shorter pronotum but longer tegmina, a different apex of cerci and shorter, moderately curved upside ovipositor. In 2021, two new subspecies were additionally described – *I. modestior grebenscikovi* Pavićević, 2021 from Avala Mt (Čarapićev Brest) and *I. modesta danubiensis* Pavićević, 2021 from Golubac (Tumane) (Fig. 1B). *Isophya modestior grebenscikovi* differs from *I. modestior modestior* by a smaller body, shorter postfemora and much shorter ovipositor in females. *I. modesta danubiensis* differs from *I. modesta modesta* by a smaller body, similar length of the tegmina and pronotum, a larger number of stridulatory files, shorter postfemora and much shorter ovipositor length.

Isophya modestior was described by Carl Brunner von Wattenwyl in 1882, the type locality being located between Niš and Bela Palanka in Serbia (“lichte Waldstelle an der Strasse zwischen Nisch und Ak Palanka in Serbien”) (Fig. 2A). In the same publication, the author described *I. fusconotata*, found in high grass on Rtanj Mt and Suva Planina Mt (Fig. 2B), initially comparing morphological characters with *I. camptoxypha* (Fieber, 1853). In his next publication (Brunner von Wattenwyl 1891), the author did not list *I. fusconotata* anymore as he considered it as a synonym of *I. modestior*, thereby expanding its distribution to the Serbian Balkan Mountains (“Montes Balcani Serbici”). Later, Ramme (1933) revised the type material and concluded that there are no specific differences between those two species, confirming the synonymy.

Previous studies on *I. modestior* showed high phylogenetic, karyotypic and bioacoustic variability across its distributional range (Ivković *et al.* 2022, 2023). Analyses of the male calling songs and morphology of the stridulatory file showed that the species is separated into two main groups – Group A present in the central part of the Balkan Peninsula (representing *Isophya modestior* sensu stricto), and Group B occurring in the Pannonian Basin, Dinarides, Slovenia and NE Italy. The most reliable difference between the groups is the duration of the main syllable, the number of stridulatory teeth and number of pulses in the main syllable, where all values are higher in specimens from the Balkan Peninsula. Additional analyses showed that within the second group, there are differences in analysed characters between specimens from the Pannonian Basin and specimens from the Dinaric area, the latter ones having intermediate song characteristics, closer to the group from the Balkan Peninsula. Further phylogenetic analyses using genetic markers revealed the existence of two well-supported clades within *I. modestior* – Clade A: present on the Balkan Peninsula, Slovenia (Inner Carniola), Italy, Pannonian Serbia (Vršac Mts and Deronje) and Austria (Burgenland and Lower Austria); Clade B: present in Slovenia (Upper Carniola), Croatia and Austria (Carinthia), Pannonian Serbia (Fruška Gora Mt) and Hungary.

Due to the high morphological similarity between newly described taxa and *I. modestior*, and the strong intraspecific variability found in previous research (Ivković *et al.* 2022), we decided to compare bioacoustic and genetic data from specimens collected at the type localities of the recently described taxa in order to verify their taxonomic validity. Furthermore, we provide new bioacoustic data from specimens collected in Romania and Italy that were missing in our previous study (Ivković *et al.* 2022). The morphological characters showing intra- and interpopulation variability within the species are discussed.

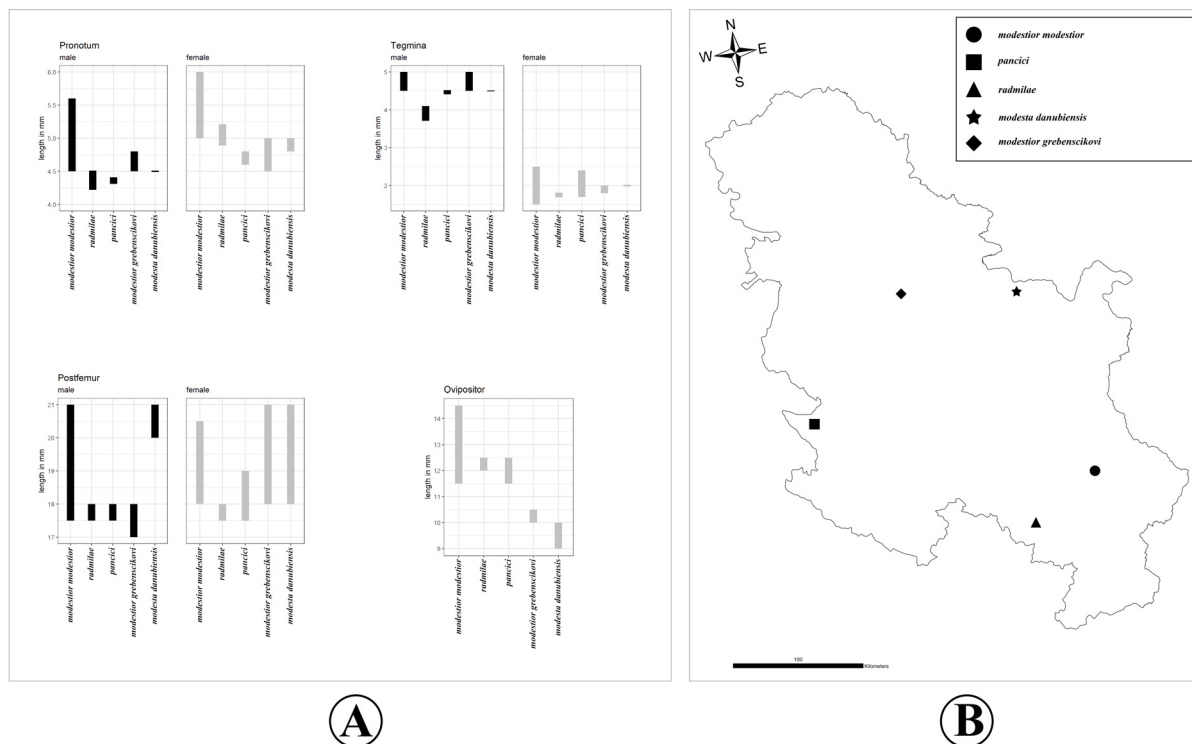


Fig. 1. A. Comparative measurements of recently described taxa of *Isophya* Brunner von Wattenwyl, 1878 and *Isophya modestior modestior* Brunner von Wattenwyl, 1882. Measurements presented in bar graph are used from original descriptions of type material. **B.** Map with localities of newly described *Isophya* taxa and *I. modestior modestior*.

Material and methods

All specimens were collected at the type localities provided by Brunner von Wattenwyl (1882) and Pavićević (2017, 2021). Part of the material was already bioacoustically analyzed and presented by Ivković (2014), while new material was collected for further bioacoustic and DNA analyses.

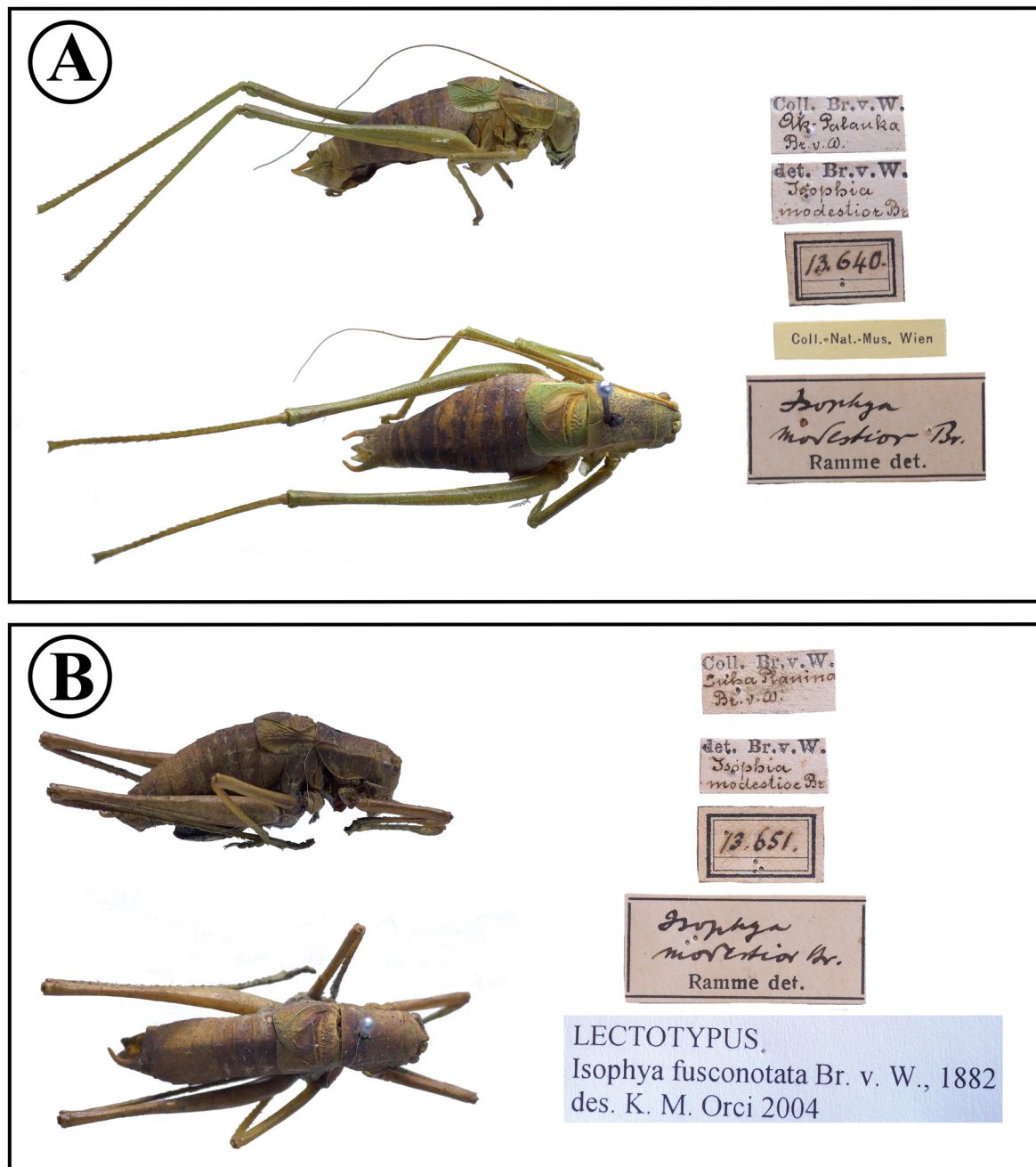


Fig. 2. Specimens of *Isophya modestior* Brunner von Wattenwyl, 1882 collected by Carl Brunner von Wattenwyl and deposited in the Natural History Museum Vienna. **A.** Syntype. **B.** Lectotype.

Table 1. Sequences of primers used in this study and references.

Primer name	F/R	Primer sequence	Reference
12S			
12Sai	F	AAA CTA GGA TTA GAT ACC CTA TTA T	Simon <i>et al.</i> (1994)
12Sbi	R	AAG AGC GAC GGG CGA TGT GT	
COI			
COBL	F	TYT CAA CAA AYC AYA ARG ATA TTG G	Huang <i>et al.</i> (2013)
COBU	R	TAA ACT TCW GGR TGW CCA AAR AAT CA	
ITS2			
ITS3	F	GCA TCG ATG AAG AAC GCA GC	Tautz <i>et al.</i> (1988)
ITS4	R	TCC TCC GCT TAT TGA TAT GC	
H3			
H3AF	F	ATG GCT CGT ACC AAG CAG ACV GC	Colgan <i>et al.</i> (1998)
H3AR	R	ATA TCC TTR GGC ATR ATR GTG AC	

Acoustic analysis

Specimens from Serbia were audio recorded with a Roland R-05 digital audio recorder (23–27°C) microphone frequency response of 0.02–40 kHz; sampling rate of 96 kHz), placed 15–30 cm from singing males. Insects from locations in Romania were recorded with a Tascam DR-100MKIII (25°C, microphone frequency response of 0.02–80 kHz; sampling rate of 192 kHz, 24 bit) and from Italy with Ultramic 250 (27°C). Most recordings were made during night when the males sing actively. Sound analyses and oscillograms were edited with Adobe Audition CC 2015 and Audacity.

For classification of bioacoustic traits, we used the terminology by Heller *et al.* (2004) and Ragge & Reynolds (1998):

- Calling song: spontaneous song produced by an isolated male.
- Main syllable duration: the sound produced by one complete up (opening) and down (closing) stroke of the forewings.
- Pulse: a simple, undivided, transient train of sound waves (here: the highly damped sound impulse arising as the impact of one tooth of the stridulatory file).
- After-click: pulse produced with considerable delay after the main pulse group.

DNA extraction and marker amplification

Total DNA was extracted from hind leg muscle tissue stored in 96% ethanol, using the Qiagen DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol for tissue samples. Four markers – two mitochondrial (COI and 12S) and two nuclear (ITS2 and H3) were amplified (for primers see Table 1). Two polymerases were used for performing PCR reactions – 5Prime HotMasterMix (ITS2, H3, 12S) and the Qiagen Multiplex Mastermix (COI). The reactions for HotMasterMix by 5Prime Master Mix polymerases were compiled as follows: 26 µl of diH₂O, 20 µl of HotMasterMix, 1.3 µl of each primer and 1.5 µl of DNA template (total vol. = 50 µl). The reactions for

Table 2. PCR protocols. Abbreviations: A = annealing; C = number of cycles of D–E; D = denaturation; E = extension; FE = final extension; ID = initial denaturation.

Primer	ID	C	D	A	E	FE
12S	94°C, 2 min	30	94°C, 30 sec	45°C, 35 sec	68°C, 1 min	68°C, 10 min
COI	95°C, 15 min	35	94°C, 30 sec	48.8°C, 90 sec	72°C, 90 sec	10°C, 20 min
ITS2	94°C, 2 min	37	94°C, 30 sec	57°C, 90 sec	65°C, 90 sec	65°C, 3 min
H3	94°C, 2 min	35	94°C, 30 sec	57.6°C, 90 sec	65°C, 90 sec	65°C, 3 min

Multiplex were compiled as follows: 5 µl of diH₂O, 3 µl of Multiplex, 0.5 µl of each primer and 1 µl of DNA template (total vol. = 10 µl). The PCR conditions are presented in Table 2.

Purification of PCR products followed the protocol provided by the manufacturer ((Roche Diagnostics GmbH), with minor modifications for COI (50 µl Binding Buffer, 25 µl Elution Buffer). Purified PCR products were sequenced in both directions using BigDye Terminator ver. 3.1 Cycle Sequencing Kit (Applied Biosystems) in 10 µl reactions as follows: 1 µl of PCR product (COI – 2 µl of PCR product) was mixed with 1 µl of primer, 2 µl of Big Dye and 6 µl of diH₂O (COI – 5 µl of diH₂O). The mixture was subjected to thermal cycling conditions consisting of an initial denaturation step at 96°C for 1 min, followed by 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min.

Cycle sequencing products were cleaned using BigDye XTerminator Purification kit (Life Technologies, Applied Biosystems) adding 10 µl XTermination solution and 45 µl Sam solution to each PCR sample well, reaching a final volume of 65 µl. The PCR plate was then sealed and vortexed for 30 min, after which the purified PCR products were sequenced in both directions on a 3500 Series Genetic Analyzer (The Applied Biosystems).

Sequence preparation and phylogenetic analysis

All sequences obtained were checked and aligned in Geneious ver. 10.0.9 (Kearse *et al.* 2012). Genetic distances between all samples per gene were extracted from Geneious and can be found in Supp. files 1–4. Partitionfinder ver. 2.1.1 (Lanfear *et al.* 2017) was used to select the correct partitioning frame and find the best fitting substitution model per fragment. A maximum likelihood tree was calculated using the IQ-Tree Web-Server ver. 1.6.12 (Nguyen *et al.* 2014). For this, a consensus tree was built using the default substitution model settings, Ultrafast Bootstrapping, 1000 maximum iterations and default IQ-Tree search parameters. Furthermore, a Bayesian inference tree was built using Beast2, with linked trees and clock models for 100 000 000 generations sampling every 1000 generation. Substitution models were set to: H3 – JC, ITS2 – TRN+I+G, COI – GTR+G and 12S – HKN+G. Tracer ver. 1.7.1 (Rambaut *et al.* 2018) was used to check the chain parameters to check for stationarity. Afterwards, TreeAnnotator integrated in the Beast2 package was used to calculate the consensus tree, discarding 10% of the trees. Consensus trees were visualized in Figtree and edited with Adobe Photoshop. Furthermore, Maximum Likelihood gene trees for every partition (by genes) were constructed with IQ-Tree Web-Server ver. 1.6.12 (Nguyen *et al.* 2014) and are shown in Supp. files 5–8. Based on the concatenated tree we used PTP species delimitation using the bPTP server (Zhang *et al.* 2013) with the options unrooted tree, 100 000 MCMC generations and a burn-in rate of 0.1. For the concatenated alignment we further constructed a TCS spanning network using the program POPART (Leigh & Bryant 2015).

Results

Phylogenetic analyses

Both phylogenetic analyses (Fig. 3, Supp. files 5–8) resulted in a similar topology in which the newly described *Isophya* (sub)species were placed together with *I. modestior* specimens from the Balkan Peninsula. *Isophya pancici* was placed within a clade from Dinaric area (Kosovo, Montenegro, south-western Serbia and Bosnia and Herzegovina). *Isophya modestior grebenscikovi* was placed together with specimens collected on localities near Belgrade (Pinosava and Kosmaj Mts), which are close to its type locality, while *I. radmilae* was placed together with specimens from north-western Bulgaria (Kanits) and eastern Serbia (Lazar's Gorge). Although we collected specimens in NE Serbia (RS_1BP2021, RS_2BP2021, RS_1VA2021, RS_2VA2021), close to the *I. modesta danubiensis* type locality, the analyzed specimens were not nested within the clade with NE Serbia samples.

Additionally, the network analysis shows a clustering in one big phylogenetic cluster without separation into several groups (Supp. file 9). The PTP species delimitation analysis also shows no separation into different molecular groups (Supp. file 10).

Taxonomic characters

The body coloration of all analyzed specimens was more or less uniform green with lighter colored lateral and ventral body parts, pronotum with two parallel white-light yellow stripes and a dorsal red, wide stripe, tegmina green with a dark brown field in the stridulatory area (Fig. 4). Rarely, lateral

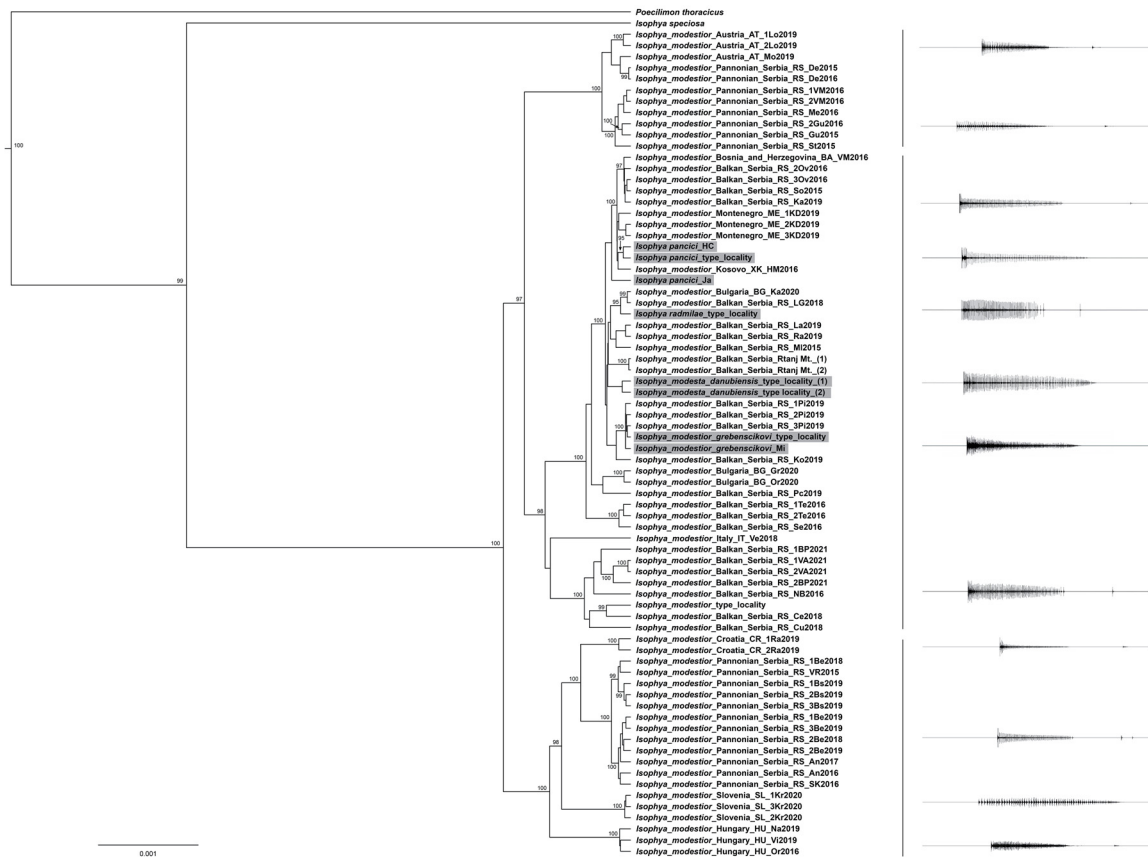


Fig. 3. Species tree generated with BEAST analyzing four sequenced loci (H3, ITS2, COI, 12S) simultaneously.

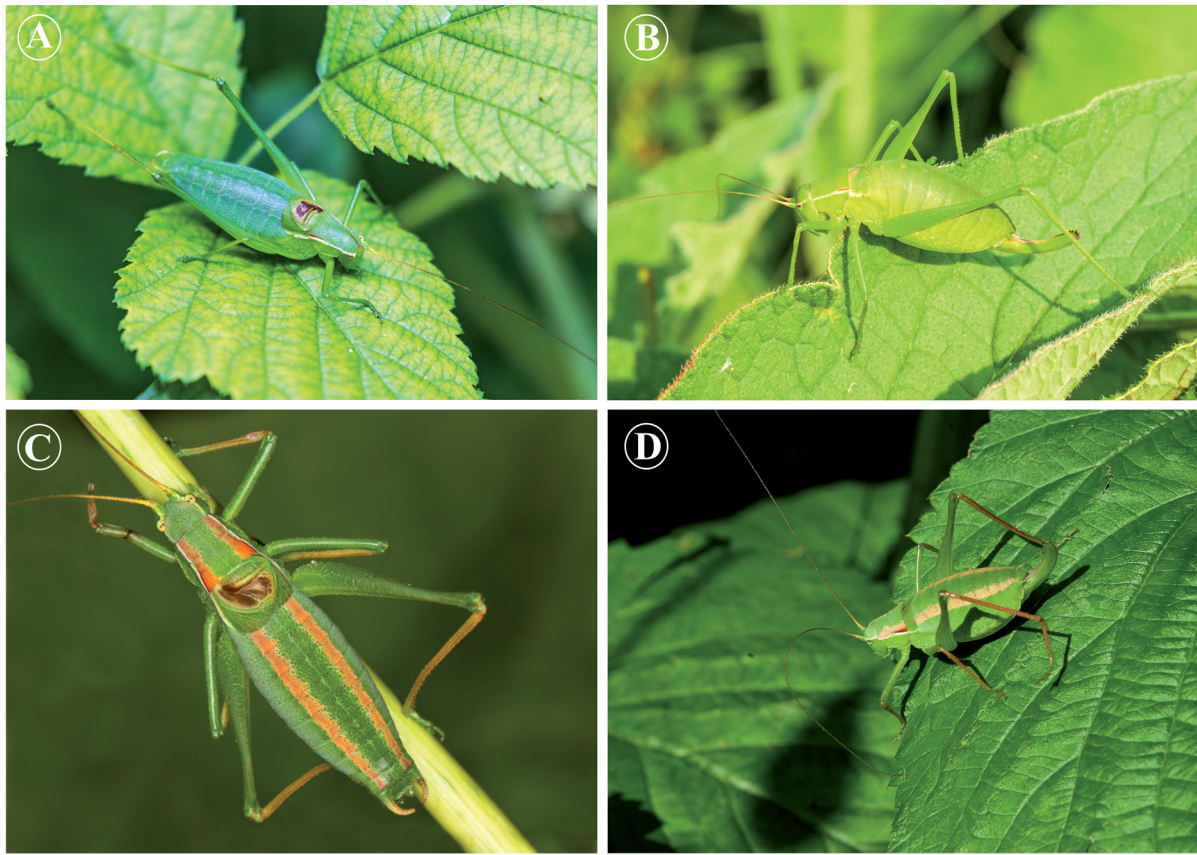


Fig. 4. General appearance of *Isophya modestior* Brunner von Wattenwyl, 1882. **A–B.** Green form (A: male, B: female). **C–D.** Melanistic form (C: male, D: female). Photos: A–B: Ionuț Ștefan Iorgu; C: Dragișa Savić; D: Laslo Horvat.

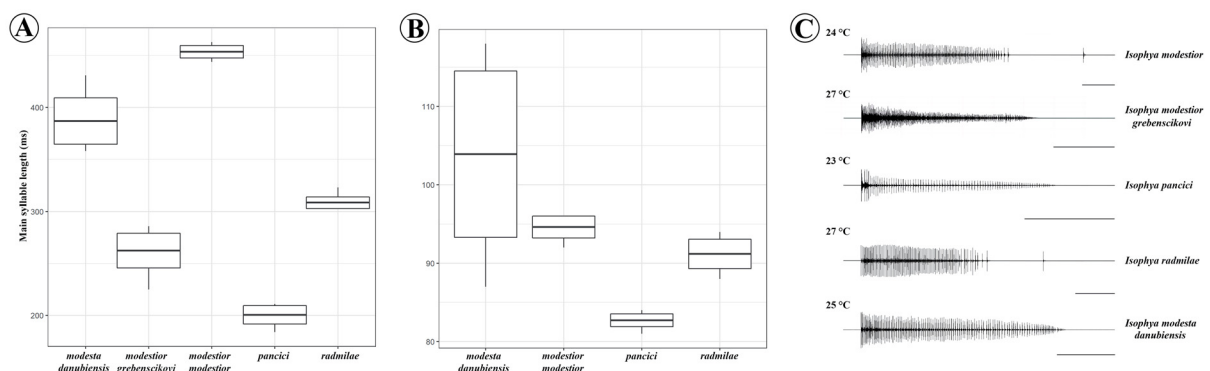


Fig. 5. Comparative measurements of male calling song characters in recently described taxa of *Isophya* Brunner von Wattenwyl, 1878 and *Isophya modestior modestior* Brunner von Wattenwyl, 1882. **A.** Main syllable length (ms). **B.** Number of pulses in main syllable. **C.** Oscillograms of male calling songs of the species of *Isophya* (the line under oscillogram indicates 100 ms).

margins of pronotum have wide pink stripes, extending beyond the pronotum across the whole abdomen (Fig. 4C–D).

The majority of characters used to identify the taxa show strong intra- and interpopulational variability (Supp. files 9–13). However, throughout their distribution, specimens from the Balkan populations have longer ovipositors (10–13 mm; mean 11.8 ± 1.08 mm; Supp. file 11A–H) compared to females from the Pannonian region (9–12 mm; mean 9.78 ± 0.97 mm; Supp. file 11I–N). They also have a higher number of teeth on the stridulatory file than populations from the Pannonian region (Ivković *et al.* 2022; Supp. file 12), but in both cases exceptions occur. The cerci are gradually narrowing towards the apex and are typically curved in the apical third showing a variability in thickness, especially in the straight, basal part (Supp. file 13A–L). The position of apical tooth also shows high variability between different populations (Supp. file 13a–l). The fastigium verticis is approximately half as wide as the scapus with no high variability between populations (Supp. file 14A–V).

In most of the analysed specimens, the tegmen reaches the second abdominal tergite. The hind cubital vein (the vein bearing the stridulatory file) is visibly bulged and thick, positioned parallel and close to the fore cubital vein (Supp. file 15A–M), except for a specimen from Croatia (Supp. file 15J), where these are separated by a smaller distance.

Bioacoustics

The calling songs in all new analysed specimens were similar to those described in Ivković *et al.* (2022), single diminuending syllables, with one after-click in some specimens (Fig. 5).

In *Isophya modestior* individuals from the type locality, the duration of the main syllable lasted for 453.7 ± 5.9 ms, with 94.6 ± 1.4 pulses, in *I. radmilae* 308.4 ± 5.5 ms, with 91.2 ± 1.9 pulses, in *I. pancici* 200.5 ± 8.9 ms, with 82.7 ± 0.8 pulses, in *I. modesta danubiensis* 386.9 ± 22.3 ms with 103.9 ± 10.6 pulses, and in *I. modestior grebenschikovi* 262.5 ± 16.7 ms (in the latter case we were not able to count the number of pulses). From Romania, we analyzed specimens from three populations – Potoc (23 Jun. 2022, 25°C), Comănești (27 Jun. 2022, 25°C) and Dobraia (28 Jun. 2022, 25°C). The duration of the main syllables from Potoc lasted for 353.3 ± 11.8 ms, with 66.4 ± 2.7 pulses, in Comănești 280.1 ± 10.1 ms, with 67.4 ± 4.1 pulses, and in Dobraia 263.3 ± 22.7 ms, with 70.9 ± 2.8 pulses. In a male recorded from Italy (Dolomiti Bellunesi National Park, 18 Jul. 2022, 27°C) we were not able to count the number of pulses, while the duration of main syllable lasted for 149 ± 9.4 ms.

Although the majority of males were captured as fresh adults, several specimens were captured as nymphs and reared. Those specimens were recorded on the same day after final molting, and we noticed that the song pattern slightly differed from typical adults of *I. modestior* (Fig. 6), which, due to a little knowledge on phenological variation of bioacoustic behavior, we first presumed that they might belong to a new species. In a specimen from Tuman (Fig. 6A) the syllable consisted of a main part (40.1 ± 3 ms) and a second part (273.1 ± 32.4 ms), followed by 3–8 after-clicks. The song of an individual from Vajuga (Fig. 6B) was characterized by 1–2 distinct pulses at the beginning of the syllable, lasting for 212.2 ± 15.9 ms.

Discussion

Our study shows that all recently described species and subspecies of *Isophya* from Serbia are phylogenetically not distinct from *I. modestior*. Specimens analyzed in this study confirmed the considerable morphological and bioacoustic variation found among different populations, which correspond with the results presented in previous publications (Ivković *et al.* 2022, 2023). None of the recently described taxa of *Isophya* are morphologically, bioacoustically or genetically distinct. Therefore, we synonymize *I. radmilae* Pavičević, 2017 syn. nov., *I. pancici* Pavičević, 2017 syn. nov., *I.*

modestior grebenscikovi Pavićević, 2021 syn. nov. and *I. modesta danubiensis* Pavićević, 2021 syn. nov. with *I. modestior* Brunner von Wattenwyl, 1882. Although the species is missing in some parts of its wider distribution (Fig. 7; Supp. file 16), such as the area around Velika Morava, Mačva and Kolubara districts in Serbia and a large part of Bosnia and Herzegovina, this is likely to be influenced by a lack of the field research in these areas.

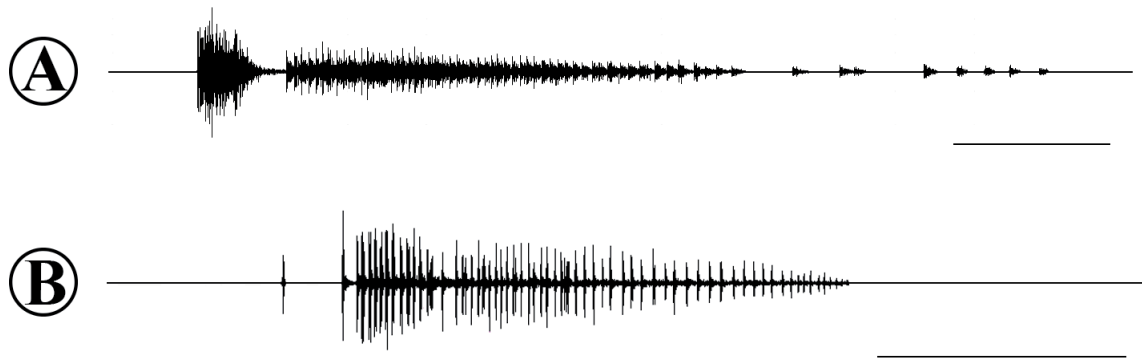


Fig. 6. Syllables of *Isophya modestior* Brunner von Wattenwyl, 1882 showing different characteristics compared to the typical song. **A.** Tumane 25°C. **B.** Vajuga 24°C (the line under oscillogram indicates 100 ms).

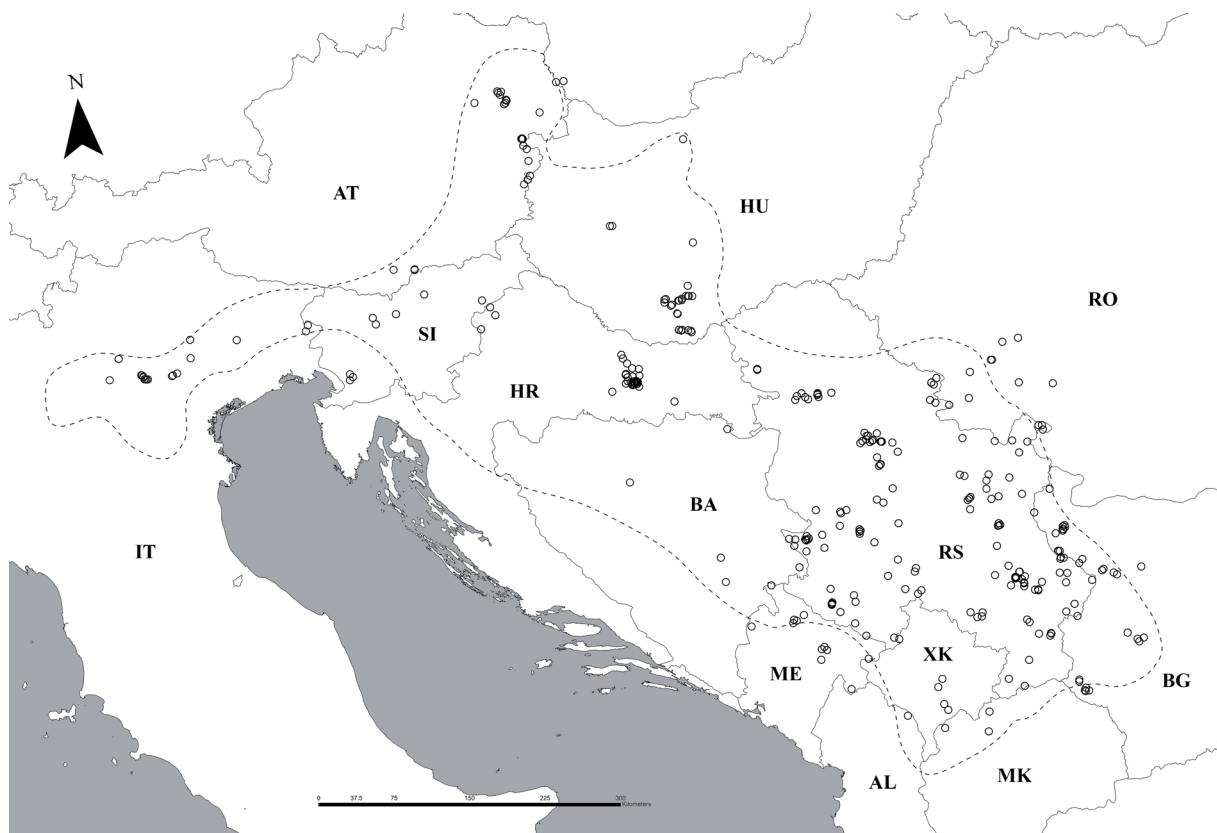


Fig. 7. Distribution of *Isophya modestior* Brunner von Wattenwyl, 1882 based on unpublished and literature data (see Supp. file 9). Dashed line represents distribution by Hochkirch *et al.* (2016).

Bioacoustic analyses of the additional material from Romania and Italy presented in this paper confirm conclusions published in Ivković *et al.* (2022), placing them in a group with lower values of bioacoustical details (Group B). Only one studied individual from Romania (Potoc), showed song characters of both groups – length of main syllable as in Group A, but number of pulses fits to Group B.

Although the species is missing in some parts of its wider distribution (Fig. 7), such as the area around Velika Morava, Mačva and Kolubara districts in Serbia and a large part of Bosnia and Herzegovina, this is likely to be influenced by a lack of the field research in these areas. Contrary to this, populations located north from Sava and Danube rivers are indeed rather scattered, mainly in the Pannonian region, where the species, for the most part, occurs only on hills and mountains, such as Vršac Mts, Fruška Gora, Villány Mts, Mecsek and Papuk Mt. Although physical barriers such as large rivers, mountain ranges between the populations are present, so far, our analyses do not support the existence of cryptic species within *I. modestior* populations.

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Supplementary files

Supp. file 1. Genetic distances between all samples – 12S. <https://doi.org/10.5852/ejt.2024.935.2559.11571>

Supp. file 2. Genetic distances between all samples – COI. <https://doi.org/10.5852/ejt.2024.935.2559.11573>

Supp. file 3. Genetic distances between all samples – H3.
<https://doi.org/10.5852/ejt.2024.935.2559.11575>

Supp. file 4. Genetic distances between all samples – ITS2.
<https://doi.org/10.5852/ejt.2024.935.2559.11577>

Supp. file 5. ML tree deduced from COI sequences. <https://doi.org/10.5852/ejt.2024.935.2559.11579>

Supp. file 6. ML tree deduced from 12S sequences. <https://doi.org/10.5852/ejt.2024.935.2559.11581>

Supp. file 7. ML tree deduced from H3 sequences. <https://doi.org/10.5852/ejt.2024.935.2559.11583>

Supp. file 8. ML tree deduced from ITS2 sequences. <https://doi.org/10.5852/ejt.2024.935.2559.11585>

Supp. file 9. Network joining analysis in *Isophya* Brunner von Wattenwyl, 1878 considered in this study. <https://doi.org/10.5852/ejt.2024.935.2559.11587>

Supp. file 10. Species-delimitation using the PTP model. <https://doi.org/10.5852/ejt.2024.935.2559.11589>

Supp. file 11. Ovipositor in *Isophya modestior* Brunner von Wattenwyl, 1882 from different regions. A: SERBIA, Temska; B: SERBIA, Senokos; C: SERBIA, Čukljenik; D: SERBIA, Mlava Gorge; E: SERBIA, Miljakovac; F: SERBIA, Ovčar Spa; G: SERBIA, Sopotnica; H: ITALY, Veneto; I: PANNONIAN SERBIA, Mesić; J: PANNONIAN SERBIA, Fruška Gora, Beočin; K: PANNONIAN SERBIA, Deronje; L: HUNGARY, Villany Mt; M: CROATIA, Papuk Mt, Radovanci; N: SLOVENIA, Kranj. <https://doi.org/10.5852/ejt.2024.935.2559.11591>

Supp. file 12. SEM photos of male stridulatory file of *Isophya modestior* Brunner von Wattenwyl, 1882 from different regions. A: PANNONIAN SERBIA, Fruška Gora, Andrevlje; B: PANNONIAN SERBIA, Deronje; C: PANNONIAN SERBIA, Fruška Gora, Beška; D: PANNONIAN SERBIA, Fruška Gora, Beočin; E: SERBIA, Avala Mt, Čarapićev Brest; F: SLOVENIA, Kranj; G: AUSTRIA, Loipersbach; H: PANNONIAN SERBIA, Vršac Mts; I: SERBIA, Đerekare; J: HUNGARY, Villany Mt; K: CROATIA, Papuk Mt, Radovanci; L: SERBIA, Šljivovica. <https://doi.org/10.5852/ejt.2024.935.2559.11593>

Supp. file 13. SEM photos of male left cercus (A–L) and apex of male cercus (a–l) of *Isophya modestior* Brunner von Wattenwyl, 1882 from different regions. A/a: PANNONIAN SERBIA, Fruška Gora, Andrevlje; B/b: PANNONIAN SERBIA, Deronje; C/c: PANNONIAN SERBIA, Fruška Gora,

Beška; D/d: PANNONIAN SERBIA, Fruška Gora, Beočin; E/e: SERBIA, Avala Mt, Čarapićev Brest; F/f: CROATIA, Papuk Mt, Radovanci; G/g: HUNGARY, Villany Mt; H/h: AUSTRIA, Loipersbach; I/i: SERBIA, Đerekare; J/j: SLOVENIA, Kranj; K/k: SERBIA, Šljivovica; L/l: PANNONIAN SERBIA, Vršac Mts. <https://doi.org/10.5852/ejt.2024.935.2559.11595>

Supp. file 14. Morphology of male head in *Isophya modestior* Brunner von Wattenwyl, 1882. A: SERBIA, Temska; B: SERBIA, Senokos; C: SERBIA, Čukljenik; D: SERBIA, Lazar's Gorge; E: SERBIA, Mlava Gorge; F: SERBIA, Miljakovac; G: SERBIA, Ovčar Spa; H: SERBIA, Sopotnica; I: PANNONIAN SERBIA, Vršac Mts; J: PANNONIAN SERBIA, Mesić; K: PANNONIAN SERBIA, Vršac Mts; L: PANNONIAN SERBIA, Fruška Gora, Andrevlje; M: PANNONIAN SERBIA, Fruška Gora, Andrevlje; N: PANNONIAN SERBIA, Sremska Kamenica; O: PANNONIAN SERBIA, Fruška Gora, Beočin; P: PANNONIAN SERBIA, Deronje; Q: PANNONIAN SERBIA, Deronje; R: CROATIA, Papuk Mt, Radovanci; S: HUNGARY, Villany Mt; T: HUNGARY, Fekete-hegy; U: AUSTRIA, Mödling; V: SLOVENIA, Kranj. <https://doi.org/10.5852/ejt.2024.935.2559.11597>

Supp. file 15. Morphology of male tegmina in *Isophya modestior* Brunner von Wattenwyl, 1882. A: SERBIA, Temska; B: SERBIA, Senokos; C: SERBIA, Lalinac; D: SERBIA, Niška Banja; E: SERBIA, Miljakovac; F: SERBIA, Pinosava; G: SERBIA, Istočni Mojstir; H: PANNONIAN SERBIA, Vršac Mts; I: PANNONIAN SERBIA, Deronje; J: CROATIA, Papuk Mt, Radovanci; K: HUNGARY, Nagyárpád; L: AUSTRIA, Loipersbach; M: SLOVENIA, Kranj. <https://doi.org/10.5852/ejt.2024.935.2559.11599>

Supp. file 16. The list of all published and unpublished localities of the *Isophya modestior* Brunner von Wattenwyl, 1882. <https://doi.org/10.5852/ejt.2024.935.2559.11601>

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