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## A NEW SPECIES OF *ISOPHYA* (ORTHOPTERA: PHANEROPTERIDAE) FROM THE ROMANIAN CARPATHIAN MOUNTAINS

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**Abstract.** A new morphologically cryptic species from the genus *Isophya* Brunner von Wattenwyl is described from Ciucaș Mountains in Romania: *Isophya ciucasi* n. sp. Diagnosis, images and a bioacoustic analysis are presented for the new species and compared with *Isophya camptoxypha* (Fieber), the species with a very similar morphology.

**Résumé.** Une nouvelle espèce cryptique d'*Isophya* Brunner von Wattenwyl est décrite des monts Ciucaș en Roumanie: *Isophya ciucasi* n. sp. On présente la diagnose, des images et le comportement acoustique pour la nouvelle espèce, qui est comparée à *Isophya camptoxypha* (Fieber), une espèce très proche du point de vue morphologique.

**Key words:** *Isophya ciucasi* n. sp., morphology, bioacoustics.

### INTRODUCTION

*Isophya* Brunner von Wattenwyl, 1878 is one of the richest in species Orthoptera genera, with 91 currently recognized species (Eades & Otte, 2010). The distribution area of this genus expands from the Pyrenees Mountains in the West, Southern Germany and Poland in the North, the Caucasus Mountains in the East, the Balkan region in the South - West, following the Mediterranean shore to Israel and Asia Minor to Iran in the South - East (Sevgili et al., 2006; Warchalowska - Sliwa et al., 2008). A few species are reported from South America (Eades & Otte, 2010). The generic radiation center for this genus is considered Anatolia (La Greca, 1999) due to the fact that more than one third of the *Isophya* species are reported from this area (Sevgili et al., 2006). Several distinct *Isophya* species - groups were recognized and they include species with relatively uniform morphology (Heller et al., 2004; Sevgili et al., 2006; Warchalowska - Sliwa et al., 2008).

The *Isophya* species are phytophagous, with low dispersal ability (Bauer & Kenyeres, 2006). The wings are reduced in both sexes and only the proximal part of the tegmina, required for stridulation, is developed.

Genus *Isophya* is one of the most problematic groups of European Orthoptera from the taxonomical point of view (Sevgili et al., 2006). The identification of *Isophya* species is very difficult because of their high morphological similarity and the lack of male sclerotized genitalia (Heller et al., 2004; Sevgili et al., 2006). The identification criteria are present mainly on males, and consists of subtle differences in the shape and size of the pronotum and its correlation with the size and shape of tegminae, the length and the number of pegs on the stridulatory file, the size of the ovipositor etc. (Heller et al., 2004; Sevgili et al., 2006; Warchalowska - Sliwa et al., 2008). All known *Isophya* are stridulating species, male calling song being the most important tool for the identification and separation of new species. Also, in recent studies, the duet male - female has been recorded (Orci & Heller, 2004; Orci, 2007).

There are fifteen *Isophya* species in Romania, two of them being endemic: *Isophya harzi* Kis, 1960 and *Isophya dobrogensis* Kis, 1994 (Iorgu et al., 2008). In 1960, Kis shows that *Isophya camptoxypha* from Romania has a high morphological variability and Heller et al. (2004) hypothesized that there could be several new unidentified species within this genus in the Carpathian Mountains.

#### MATERIAL AND METHODS

In the summer of 2008, during authors' field work in Ciucaș Mountains, several Orthoptera species have been collected alive for bioacoustic studies. A surprising result was revealed by the oscillographic analysis of the song in 2 males of *Isophya* captured on some nettles' leaves (*Urtica*) from a valley near the old chalet Ciucaș. These are described here as *Isophya ciucasi* n. sp.

Males and females have been recorded indoors using the digital recorders SONY ICD SX56 and EDIROL R-09HR. The second recorder has a sound frequency response between 20 - 40000 Hz. The analysis of the sound was made with Audacity 1.2 and Batsound 4 software. All the studied adult specimens were caught in the wild, transported in plastic containers and recorded in laboratory at a temperature of 24 - 27°C.

Song terminology follows Heller et al. (2004): *calling song* - song produced by an isolated male; *syllable*: the sound produced by one complete up (opening) and down (closing) stroke of the forewings; *impulse*: the highly damped sound impulse arising as the impact of one tooth of the stridulatory file; *after-click*: click produced with considerable delay after the main impulse group.

Photos were taken with a Canon EOS digital camera; a 100 mm 1:1 macro lens was used for habitus photos and a 65 mm 5:1 macro lens was mounted to camera in order to take photos of the morphological details. Subject lit was provided by a ring macro flash attached in front of the lenses. Insects' wing movements during the song have been video recorded with the same camera.

The type material of *Isophya ciucasi* n. sp. is deposited at "Grigore Antipa" National Museum of Natural History, Bucharest.

#### RESULTS AND DISCUSSION

Order Orthoptera  
Suborder Ensifera  
Family Phaneropteridae  
Subfamily Phaneropterinae

#### *Isophya ciucasi* nov. spec.

(Figs 1 B, C; 2; 3 A, C, E; 4 A, C; 5 A, C, E)

#### Material

*Holotype*: male, Romania, Ciucaș Mountains, 45°30'35"N, 25°56'43"E, 1585 m a.s.l., 27.06.2008, coll. "Grigore Antipa" National Museum of Natural History, Bucharest, No. 182511.

*Paratypes*: 1 ♂, 1 ♀, same data as holotype, ♂ No. 182512, ♀ No. 182516; 3 ♂♂, 3 ♀♀, Romania, Ciucaș Mountains, 45°30'39"N, 25°56'42"E, 1600 m a.s.l.,

17.07.2009, ♂♂ No. 182513, 182514, 182515, ♀♀ No. 182517, 182518, 182519. In the collection of "Grigore Antipa" National Museum of Natural History, Bucharest.

Audio recordings: 2 ♂♂, 27.06.2008 (wav files, 24 bits/96 kHz, temperature 24°C); 3 ♂♂, 3 ♀♀, 17.07.2009 (wav files, 24 bits/96 kHz, temperature 27°C).

#### *Description of the male*

Fastigium of vertex with lateral margins convergent towards tip, about 2 times narrower than scapus, with a dorsal groove. Pronotal disc 3.58 - 4.57 mm long, slightly constricted at midlength in the transverse sulcus area, with posterior area raised; lateral carinae slightly distinct; paranotum with ventral edge almost straight and posterior edge slightly rounded (Fig. 2 F). Wings with reticulate venation, reach the hind edge of first abdominal tergite;  $Cu_2$  vein about 2/3 times as long as posterior margin of pronotum and angle between cubital veins of about 90°; mirror large and quadrangular (Fig. 2 A); stridulatory file arcuate and counts 69 - 83 teeth on 1.69 - 1.88 mm length; distal teeth larger and rarer than proximal ones (Fig. 2 E). Subgenital plate elongated, with a triangular apical incision (Fig. 2 C). Cercus long, tapering towards tip, slightly curved in apical 1/4 (Fig. 2 I), with many small hairs; terminal denticle located in middle of cercus apex. Hind femur about 4 times pronotum length.

*Coloration:* body colour greenish, densely punctuated with dark green. Antennae brown or reddish-brown, with light brown or green scapus. Compound eyes usually bicolor, with upper part brownish-red and lower one green. A yellow or white narrow band starts from behind the eye and ends at the posterolateral angle of wing; in the distal third of pronotum above the white band there is a brown band which merges with wings colour. Wings brown, with apical area green and greenish - white costal margin. In some males and females, there are 2 parallel dorsal bands on pronotum and abdomen, of orange, red or yellow colour. Cerci brown or reddish - brown, green at base. In many individuals, tibiae and tarsi brownish or reddish. Ventral part yellowish (Fig. 1 B).

#### *Description of the female*

Fastigium as in male. Pronotum 3.72 - 4.71 mm long, with dorsal area slightly enlarged in posterior part; lateral carinae faded. Wings with dense reticulate venation, about 3 times shorter than pronotum (Fig. 2 B, G). Stridulatory apparatus formed by  $Cu_2$  vein on the left tegmen ventral side and denticles located on cubital veins from the right tegmen dorsal side (Fig. 2 J). Subgenital plate narrow, about 2 times as wide as long (Fig. 2 D). Ovipositor short, upcurved, 2.2 - 2.3 times longer than pronotum. The upper margin with 8 - 9 denticles, lower margin with 7 - 8 denticles (Fig. 2 H). Cercus hairy, 1.33 - 1.57 mm in length, conical, 1.2 times longer than epiproct (Fig. 2 K).

*Coloration:* females are also green, usually paler than in males, with fine dark punctuation. As in males, antennae are reddish - brown and there is a white band between the bicoloured compound eyes and the posterolateral angle of wings. Wings green, with the basal area brownish and the costal margin white. Tip of cercus light brown; ovipositor green, with terminal denticles dark brown (Fig. 1 C).

#### *Measurements*

*Males* (n = 5): body length 22.21 - 27.39 mm (mean ± SD: 24.98 ± 2.09); head width 3.3 - 3.75 mm (mean ± SD: 3.51 ± 0.16); pronotum length 3.58 - 4.07 mm (mean ± SD: 3.77 ± 0.2); pronotum maximum width 3.65 - 4.28 mm (mean ± SD:

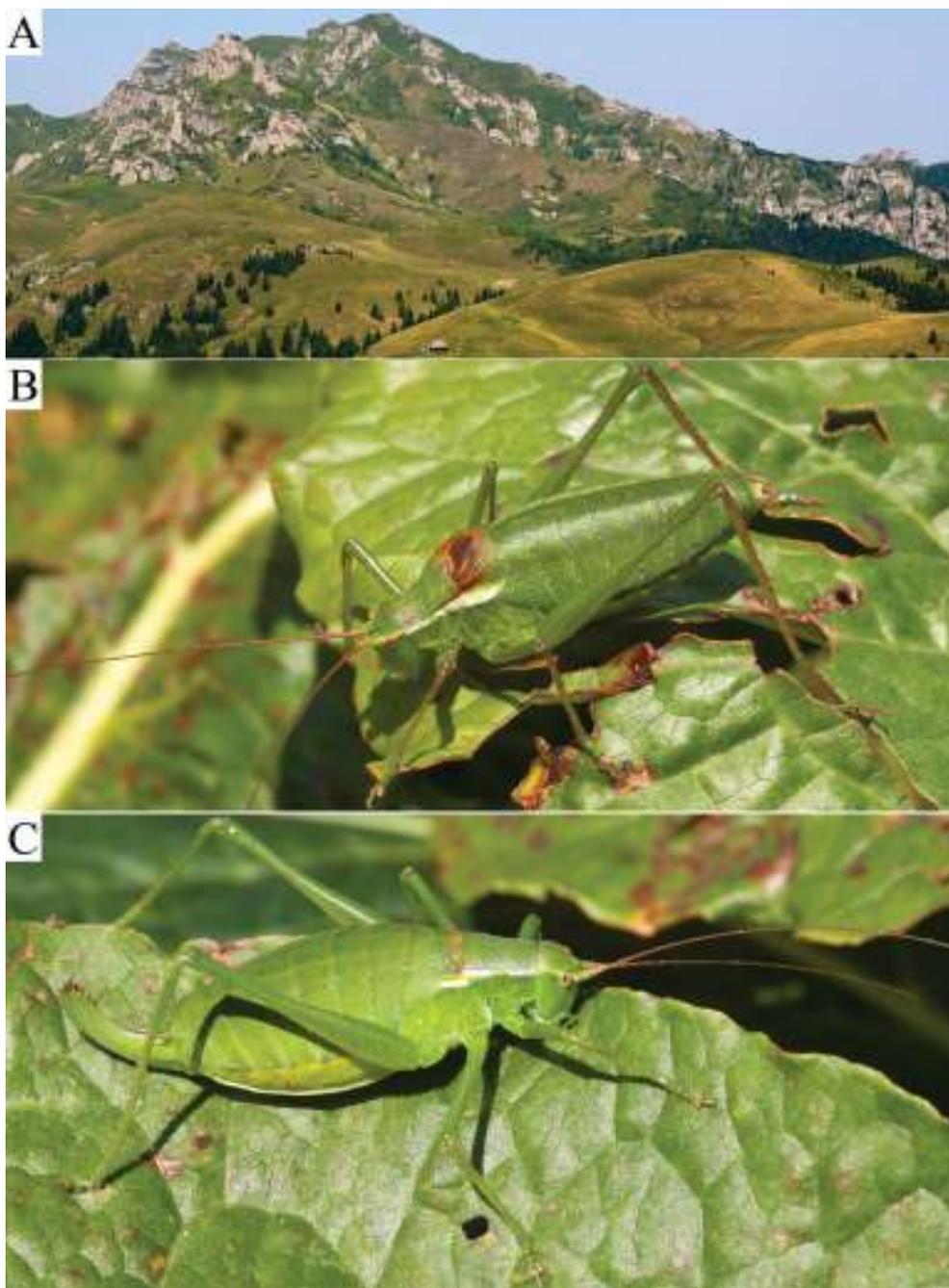


Fig. 1 - A, Ciucaș Mountains (Eastern Carpathians); B, *Isophya ciucasi* n. sp. ♂; C, *Isophya ciucasi* n. sp. ♀ (17.07.2009) (Photos: I. Șt. Iorgu).

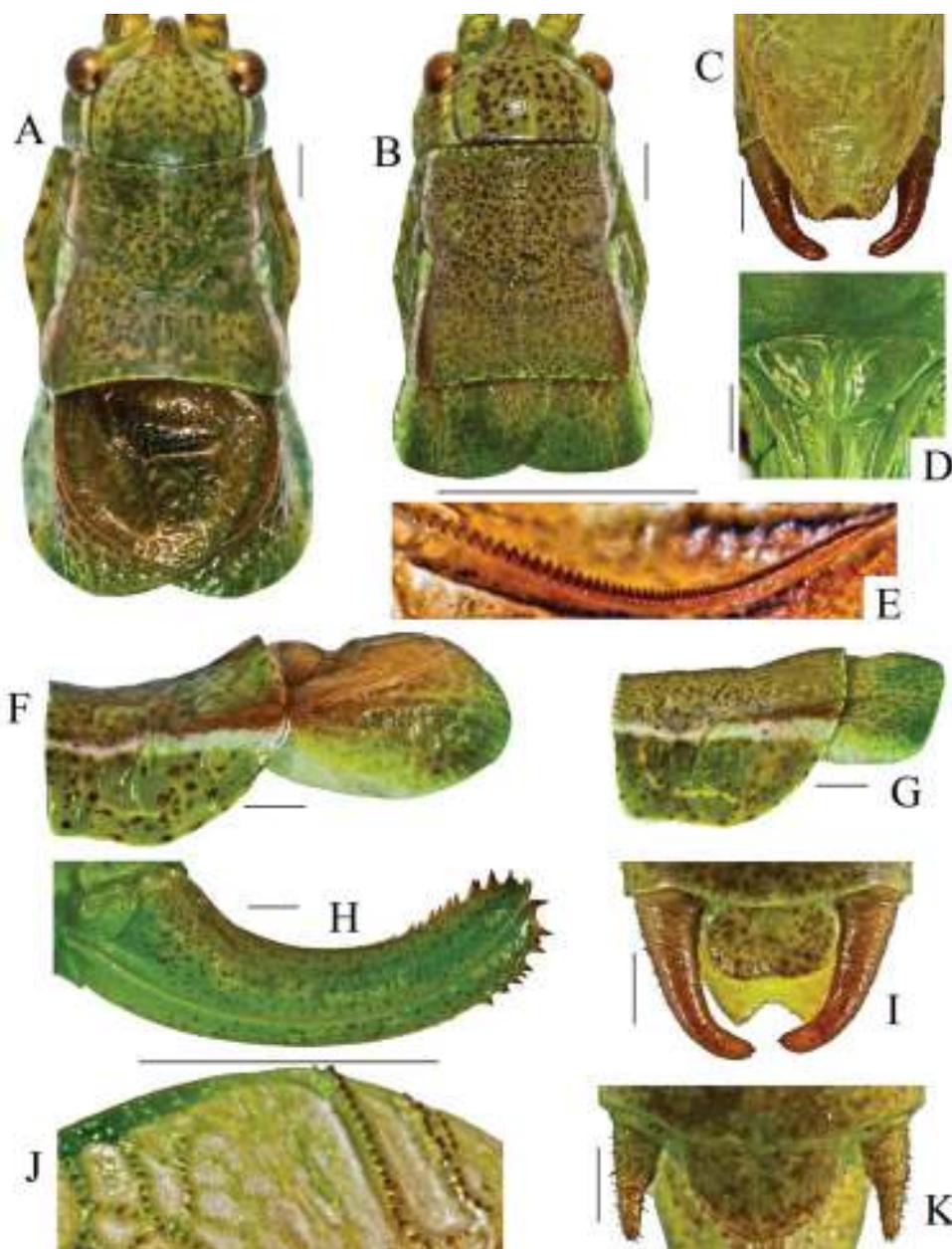


Fig. 2 - *Isophya ciucasi* n. sp.: A, male head, pronotum and tegminae (dorsal view); B, female head, pronotum and tegminae (dorsal view); C, male subgenital plate; D, female subgenital plate; E, male stridulatory file; F, male paranotum and wing (lateral view); G, female paranotum and wing (lateral view); H, ovipositor; I, male cerci; J, female stridulatory pegs; K, female cerci. Scale: 1 mm. (Photos: I. Șt. Iorgu).

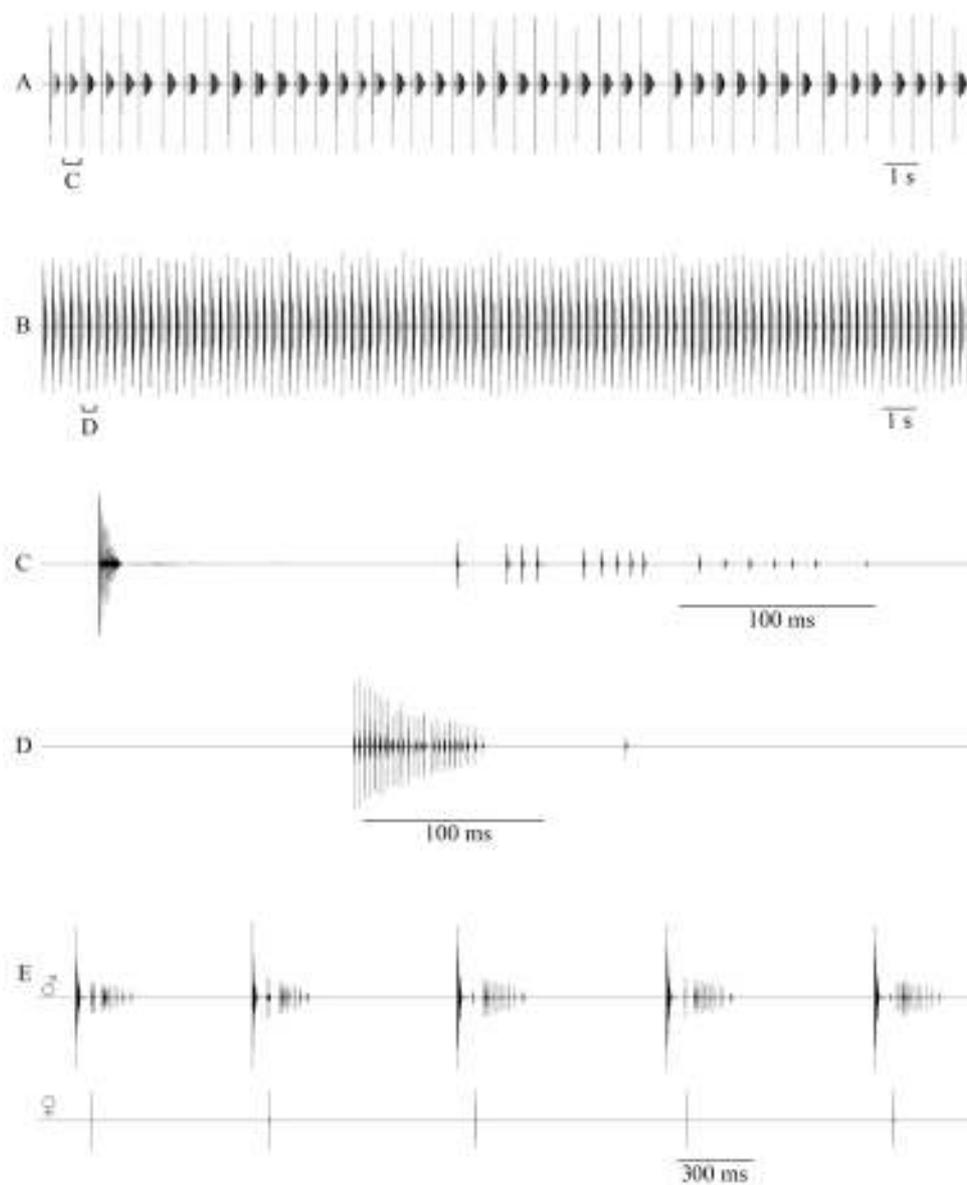


Fig. 3 - Oscillographic analysis of the song: A, *Isophya ciucasi* n. sp.; B, *Isophya camptoxypha* (Muntele Roșu, 18.07.2009); C, detailed syllable in *Isophya ciucasi* n. sp.; D, detailed syllable in *Isophya camptoxypha*; E, *Isophya ciucasi* n. sp. female response as mating acceptance with male (all recordings at 27°C).

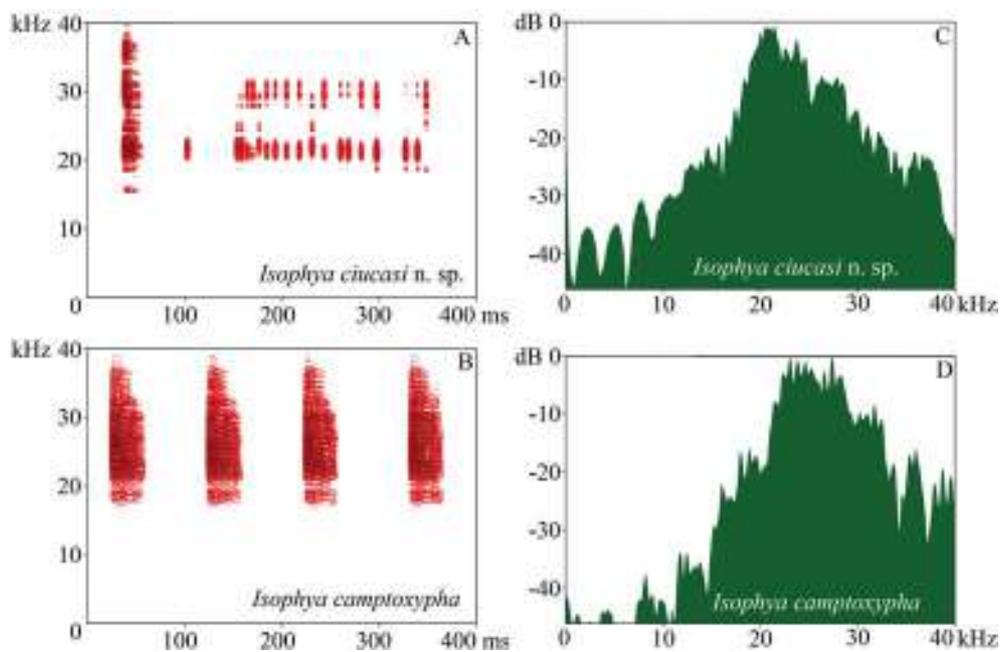


Fig. 4 - Spectrographic analysis of the song frequencies between 0 - 40 kHz: A, B, spectrogram (Batsound); C, D, plot spectrum (Audacity) (FFT: Hanning window 512).

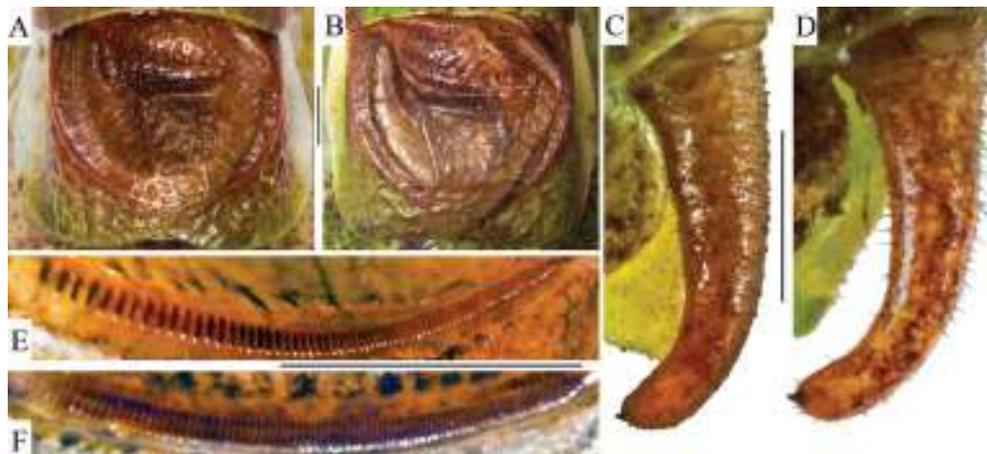


Fig. 5 - *Isophya ciucasi* n. sp. (A, C, E) and the closely related *Isophya camptoxypha* (B, D, F), males: A, B - tegminae (dorsal view); C, D - right cercus; E, F - stridulatory file. Scale: 1mm. (Photos: I. Șt. Iorgu).

3.98 ± 0.22); wing length 4.01 - 4.58 mm (mean ± SD: 4.2 ± 0.23); wing maximum width 3.38 - 3.82 mm (mean ± SD: 3.59 ± 0.19); number of stridulatory pegs 69 - 83 (mean ± SD: 76.2 ± 6.06); stridulatory file length (area with stridulatory pegs) 1.69 - 1.88 mm (mean ± SD: 1.79 ± 0.08); cercus length 2.06 - 2.52 mm (mean ± SD: 2.37 ± 0.19); hind femur 14.45 - 17.01 mm (mean ± SD: 15.64 ± 0.94).

*Females* (n = 4): body length 26.77 - 31.86 mm (mean ± SD: 28.42 ± 2.36); head width 3.38 - 3.99 mm (mean ± SD: 3.64 ± 0.26); pronotum length 3.72 - 4.71 mm (mean ± SD: 4.15 ± 0.41); pronotum maximum width 3.68 - 4.58 mm (mean ± SD: 4.05 ± 0.44); wing length 1.9 - 2.3 mm (mean ± SD: 2.11 ± 0.17); wing maximum width 2.96 - 3.82 mm (mean ± SD: 3.33 ± 0.37); subgenital plate length 1.07 - 1.14 mm (mean ± SD: 1.12 ± 0.03); subgenital plate width (proximally) 2.32 - 2.89 mm (mean ± SD: 2.69 ± 0.26); cercus length 1.33 - 1.57 mm (mean ± SD: 1.45 ± 0.11); ovipositor 8.77 - 9.2 mm (mean ± SD: 8.98 ± 0.21); hind femur 13.99 - 16.4 mm (mean ± SD: 15.12 ± 1.01).

#### *Bioacoustics*

If undisturbed, males can sing for more than 3 minutes a song that consists of a long series of syllables (Fig. 3 A), but smaller groups of 8 - 23 syllables have also been recorded. Each syllable is formed by a compact series of 10 - 22 impulses (mean ± SD: 18.4 ± 4.82), lasting for 11 - 22 ms (mean ± SD: 18.2 ± 4.32). After about 52 - 73 ms (mean ± SD: 63.8 ± 9.44), the syllable is always followed by 13 - 23 after - clicks (mean ± SD: 17.6 ± 3.71), which last for 200 - 314 ms (mean ± SD: 228 ± 25.31). The time interval between the successive syllables is about 347 - 516 ms (mean ± SD: 423.6 ± 44.16). The signal amplitude in a syllable decreases fast and it is much higher in the first impulses than in the last ones. Also in the series of after - clicks the amplitude decreases from the first to the last impulse; the first after - clicks have the same signal amplitude as impulses from the middle part of the syllable (Fig. 3 C). Female response as mating acceptance with the singing male is a single impulse produced after the first part of male's syllable, sometimes overlapping with the after - clicks (Fig. 3 E). This impulse is emitted about 44 - 54 ms (mean ± SD: 51 ± 2.82) after the main part of male syllable. We have recorded duets male - female that lasted for more than 2 minutes in which females replied to all male's syllables. Nevertheless, female response is not always constant, so she doesn't reply for each male syllable.

In both males and females, sounds are produced only when the insect closes the tegminae. Spectrogram analysis shows the main frequency in a syllable between 15 - 40 kHz, with a maximum at about 22 kHz. Due to recording conditions, it is very probable that the frequency highest limit is above 40 kHz. The sound frequency is lower in the after - clicks, between 18 - 33 kHz (Fig. 4).

#### *Etymology*

The species is named after the type locality: Ciucaș Mountains, in the Southern part of the Eastern Carpathians, Romania.

#### *Type locality, distribution, habitat*

The species is known only from the subalpine plateau of the Ciucaș Mountains, Carpathian Mountains, Romania (Fig. 1 A). In the high altitude meadows of the type locality, at about 1600 m above sea level, the newly discovered *Isophya* species occurs on different plant species, usually *Urtica*, *Rumex*, *Rubus*, *Juniperus*, *Senecio*, *Vaccinium* etc. Most of the individuals are adults in mid July.

The other Orthoptera species that occur sympatrically with *Isophya ciucasi* n. sp. are: *Poecilimon affinis* (Fivaldszky, 1867), *Polysarcus denticauda* (Charpentier, 1825), *Pholidoptera transsylvanica* (Fischer, 1853), *Metrioptera brachyptera* (Linnaeus, 1761), *Miramella ebneri* Galvagni, 1953, *Euthystira brachyptera* (Ocskay, 1826), *Stenobothrus stigmaticus* (Rambur, 1838), *Omocestus viridulus* (Linnaeus, 1758), *Chorthippus parallelus* (Zetterstedt, 1821) etc.

#### Remarks

*Isophya ciucasi* n. sp. resembles very much *Isophya camptoxypha* (Fieber, 1853) but by comparing the bioacoustics of these two species with similar morphology, their identity is easily verified. The songs of the males in these two species follow the same pattern - a long series of short syllables. While in *Isophya camptoxypha* the syllable is usually followed by only one after - click (Fig. 3 B, D), in *Isophya ciucasi* n. sp. the syllable is followed by a series of 13 - 23 after - clicks (Fig. 3 A, C). Even if in both species the syllables have almost the same number of impulses, the syllable duration is shorter in *Isophya ciucasi* n. sp., 11 - 22 ms, compared with 25 - 42 ms in *Isophya camptoxypha*. The frequency spectrum is similar in both species, from about 15 to more than 40 kHz, with the highest peak between 20 - 30 kHz (Fig. 4).

Comparing some morphological details in males of these two species, only small differences could be noticed: angle between cubital veins has almost 90° in *I. ciucasi* n. sp. and about 70° in *I. camptoxypha* (Fig. 5 A, B), cercus slightly more curved in *I. camptoxypha* than in the new species (Fig. 5 C, D) and teeth are rarer in the distal part of stridulatory file in *I. ciucasi* n. sp. (Fig. 5 E, F). In order to separate these two species, the mentioned morphological characters are not very reliable due to the high intra - and interpopulation high variability. As an already known fact, the combination between morphological description, bioacoustics and DNA analysis is the only correct method to decide on the real status of the Orthopteran populations.

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#### O NOUĂ SPECIE DE *ISOPHYA* (ORTHOPTERA: PHANEROPTERIDAE) DIN CARPAȚII ROMÂNEȘTI

##### REZUMAT

O nouă specie criptică morfologic din genul *Isophya* Brunner von Wattenwyl este descrisă din Munții Ciucaș: *Isophya ciucasi* n. sp. Diagnoză, imagini și o analiză a bioacusticii sunt prezentate pentru această specie care este comparată cu *Isophya camptoxypha* (Fieber), specia cea mai apropiată morfologic de *Isophya ciucasi* n. sp. Stridulația celor două specii înrudite diferă prin tipul de silabă specific. Comparând unele elemente de morfologie la mascul, am observat că unghiul dintre nervurile cubitale, curbura cercilor și poziționarea dințișorilor în regiunea distală a carenei stridulante diferă foarte puțin la cele două specii.

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