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Cicadetta karandila sp. n. (Hemiptera: Auchenorrhyncha: Cicadidae) a new cryptic species of singing cicada from the Bulgarian mountains

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Abstract

In recent years, bioacoustic studies on *Cicadetta montana* s. l. have revealed the presence of morphologically cryptic species. This paper describes a new cicada species, *Cicadetta karandila* sp. n., discovered on the Stara Planina Mountain and the Sarnena Sredna Gora Mountain (Balkan Mountains, central Bulgaria). As with other species of the complex, no reliable morphological differences were found between the new species and the other species of the complex. Bioacoustically, the new species belongs to the »cerdaniensis« song group, species of which share double echemes with a first low-intensity part followed without interruption by a very short high-intensity part. The calling song comprises three phrases and is modulated to four power levels and three or probably four frequency ranges. Phrases 1 and 3 consist of evenly distributed (ungrouped) single echemes with different repetition rates, while phrase 2 contains double echemes that are very short at the beginning of the phrase and smoothly change into longer echemes after a few repetitions. This species is currently only known to be endemic to the central part of the Balkan Mountains in Bulgaria, where it is native to forests and ecotone habitats. Owing to this limited distribution, this species is probably vulnerable to habitat and climate change.

Key words *Cicadetta montana* species complex, description, new species, morphology, bioacoustics, Bulgaria, distribution, ecology.

Introduction

The Balkan Peninsula is a hotspot for the diversity and speciation of singing cicadas (Gogala *et al.* 2012). The discovery of another *Cicadetta* Kolenati, 1857 species with a very restricted distribution confirms the previous assumption that the Balkans is an area with active speciation of singing cicadas.

The use of bioacoustic methods in field studies has enabled cicada researchers in recent decades to map the diversity of cicada species and to search for new taxa much more efficiently than previously (e.g. Gogala & Trilar 1999, 2004; Gogala *et al.* 2009, 2011, 2012; Hertach 2011; Puissant & Sueur 2010; Hertach *et al.* 2015, 2016). This has been the case in recent years, when bioacoustic studies of the so-called mountain cicadas (*Cicadetta montana* sensu lato (Scopoli, 1772)) revealed the presence of morphologically cryptic species. Bioacoustics combined with molecular and morphological studies over the last 20 years have shown that *Cicadetta montana* s. l. represents at least 18 Palaearctic taxa that can be classified into four groups based on song characteristics, »montana«, »brevipennis«, »cerdaniensis« and »macedonica« (Gogala & Trilar 2004; Gogala 2013; Hertach *et al.* 2015, 2016; Wade *et al.* 2015). Most species from the »cerdaniensis« group have so far been found in Western and Central Europe; only the species *Cicadetta cantilatrix* Puissant & Sueur 2006 is widely distributed from Western and Central Europe; only the species *Cicadetta cantilatrix* Puissant & Sueur 2006 is widely distributed from Western and Central Europe; only

Recently, during our bioacoustic fieldwork in Bulgaria, we found another new species of this cicada group that resembles in some features, especially in bioacoustics, *Cicadetta cerdaniensis* s. str. Puissant & Boulard 2000, *Cicadetta sibillae* Hertach & Trilar 2015, *Cicadetta anapaistica anapaistica* Hertach 2011, *Cicadetta anapaistica lucana* Hertach 2015, and *C. cantilatrix*, sharing the double echemes with a first low-intensity part followed by a very short high-intensity part, without interruption.

Materials and Methods

We (IG, TT, MG) discovered this new species during the field excursion to Stara Planina, Karandila in Bulgaria on June 22 and 23, 2016. We recorded their songs and collected specimens at various localities at altitudes between 608 and 1400 metres above sea level (m a.s.l.). We first localized the cicadas acoustically and then, if possible, collected them with an entomological net. Collected and dry-prepared specimens, including type material, are deposited in the collections of the Slovenian Museum of Natural History, Ljubljana, Slovenia (PMSL), and the Zoological Collection of Sofia University, Sofia, Bulgaria (BFUS). Song recordings are deposited in the Slovenian Wildlife Sound Archive of the Slovenian Museum of Natural History (PMSL) and Cloud Storage of the Department of Zoology and Anthropology of Sofia University (BFUS). Samples of the selected recordings can be found on the website Songs of European singing cicadas (http://www.cicadasong.eu).

Only song-identified males were used for morphological analyses and are included in the type series. The taxonomy and morphological terminology of the dry-prepared specimens is based on Moulds (2005, 2012) and Sanborn (2013). Distribution maps were created using a GPS Visualizer (Schneider 2003–2024).

Because of the high-frequency range of the calls, we detected the acoustic signals of this cicada species using Pettersson D-200 ultrasonic detectors (heterodyne system) with electret microphones from the same manufacturer (frequency range 10–120 kHz \pm 0.15 kHz), mounted in front of a Telinga reflector (57 cm diameter), a Renault R-4 front light reflector (15 cm diameter), or a DYI epoxy parabola (40 cm diameter). The output from the detector was connected to a Marantz PMD-660 (sampling rate up to 48 kHz; frequency response 16 kHz \pm 0.5 dB; 16-bit digitization), Zoom H2 (sampling rate up to 96 kHz; 24-bit digitization), and a Tascam DR-60DmkII (sampling rate up to 96 kHz; frequency response 20 kHz \pm 0.5/-3 dB; 24-bit digitization) solid-state recorders (Gogala 2013). For the sound analyses, we used RAVEN PRO 1.6 (Cornell Lab of Ornithology). For sonography the Seewave package 4.3 (Sueur *et al.* 2008: oscillo, spectro, dfreq, dBscale) on the R platform (version 4.3.1, R Core Team 2023).

The frequency spectra were measured using a Hamming window (sample rate 48 kHz, window size 1024 points, frequency resolution 46.875 Hz, 87.5 % overlap). Because our microphones were not calibrated, and the distances of the microphone to the singing males were not constant during the field recordings, we standardized the maximal (peak) power per echeme for each recording to the average of the lowest echeme type. We then compared the delta power values of the different recordings.

Abbreviations and Acronyms CF – centre frequency D – duration DF – dominant frequency E – echeme ED – echeme duration F – frequency $F1Q - 1^{st}$ quartile frequency $F3Q - 3^{rd}$ quartile frequency FP – first part of the double echeme FPD - duration of the first part of the double echeme G – echeme/inter-echeme group GD - echeme/inter-echeme group duration IED - inter-echeme duration max – maximum min – minimum n – number of measured values NE – number of echemes in the phrase P – delta echeme power PH – phrase PHD - phrase duration RR – repetition rate SD - standard deviation SP – second part of the doble echeme SPD - duration of the second part of the double echeme index – represents the phrase number (1 – phrase 1, 2 – phrase 2, 3 – phrase 3) a.s.l. – above sea level

Results

Cicadetta karandila sp. n. Trilar, Gjonov et Gogala https://zoobank.org/urn:lsid:zoobank.org:act:6D492447-35C9-4EDE-AC6E-94F0ACE9204C

Description

Cicadetta karandila sp. n. appears to be very similar to the other species of the *Cicadetta montana* complex (Figs. 1 and 2). It has a medium body length: 17.6 mm in the holotype, 17.8 ± 0.6 mm in the paratypes (mean \pm SD).

This species (Figs. 1 and 2) was discovered due to its characteristic sound emissions in June 2016 on the Stara Planina Mountain between 608 and 1400 m altitude, where we collected a series of specimens (7 males and 2 females). In addition to the collected specimens, we also recorded *Cicadetta karandila* sp. n. acoustically (heard and/or recorded) at other localities on Stara Planina Mountain in June 2016, and in late June and early July 2020 on Stara Planina Mountain and Sarnena Sredna Gora Mountain (Figs. 3 and 4).

However, apart from Stara Planina Mountain and Sarnena Sredna Gora Mountain, we did not find *Cicadetta karandila* sp. n. anywhere in Bulgaria or in neighboring countries Romania, Serbia, North Macedonia, Albania, Greece and Turkey.

Morphology

Measurements. Body length (from the head to the tip of the abdomen): 18.5 mm in holotype specimen; 17.6 ± 0.5 mm (mean \pm SD) in male paratypes (n=6, min=16.9 mm, max=18.3 mm); 20.9 ± 0.6 mm in female paratypes (n=2, min=20.6 mm, max=21.4 mm). Body width (abdomen, tergite 2): 6.1 mm in holotype specimen; 5.8 ± 0.1 mm in male paratypes (n=6, min=5.7 mm, max=6.1 mm); 6.3 ± 0.2 mm in female paratypes (n=2, min=6.1 mm, max=6.4 mm). Forewing length: 22.3 mm in holotype specimen; 20.0 ± 0.8 mm in male paratypes (n=6, min=19.2 mm, max=21.2 mm); 23.6 ± 0.9 mm in female paratypes (n=2, min=22.9 mm, max=24.2 mm). Forewing width: 7.8 mm in holotype specimen; 7.7 ± 0.2 mm in male paratypes (n=6, min=7.4 mm, max=7.9 mm); 8.5 ± 0.1 mm in female paratypes (n=2, min=8.5 mm, max=8.6 mm). Hindwing length: 13.0 mm in holotype specimen; 12.2 ± 0.5 mm in male paratypes (n=6, min=11.7 mm, max=13.0 mm); 14.3 ± 0.1 mm in female paratypes (n=2, min=14.3 mm, max=14.4 mm).



Figure 1. Cicadetta karandila sp. n.: live male (a) and live female (b) from Karandila, upper chairlift station.



Figure 2. *Cicadetta karandila* sp. n.: Habitus of a male holotype (a) and a female paratype (b). Scale bar is 10 mm.

Type material examined. Holotype \Diamond : Bulgaria: Sliven Province: Sliven Municipality, Stara Planina, Karandila, upper chairlift station; 42.71803°N, 26.36090°E; 982 m; 22.6.2016; leg. T.Trilar, I.Gjonov, M.Gogala (karandila holotype – deposited in PMSL). Paratype \Diamond : data as the holotype (karandila paratype 1 \Diamond , karandila paratype 2 \Diamond and karandila paratype 6 \Diamond – PMSL). Bulgaria: Tvarditsa Province: Tvarditsa Municipality, Eleno-Tvarditshka Planina, Tvarditshki prohod, poljana Kuriata; 42.76575°N, 25.91995°E; 1042 m; 27.6.2016; leg. T.Trilar, I.Gjonov, M.Gogala (karandila paratype 3 \Diamond and karandila paratype 4 \Diamond – PMSL, karandila paratype 5 \Diamond – BFUS, karandila paratype 6 \Diamond – PMSL). Paratype \wp : data as the holotype (karandila paratype 7 \wp – BFUS). Bulgaria: Tvarditsa Province: Tvarditsa Municipality, Eleno-Tvarditshka Planina, Tvarditshki prohod; 42.78966°N, 25.89392°E; 1045 m; 26.6.2016; leg. I.Gjonov, T.Trilar, M.Gogala (karandila paratype 8 \wp – PMSL).

Acoustically detected: Bulgaria: Sliven Province: Sliven Municipality, Stara Planina, Karandila, Ichera; 42.75388°N, 26.39801°E; 929 m; 22.6.2016; recorded T.Trilar, I.Gjonov, M.Gogala. Bulgaria: Stara Zagora Province: Kazanlak Municipality, Shipka, Shipchenska Planina; 42.74611°N, 25.32791°E; 1118 m; 27.6.2016; recorded T.Trilar, I.Gjonov, M.Gogala. Bulgaria: Stara Zagora Province: Kazanlak Municipality, Kran, Shipchenska Planina, Buzludzha (Buzludja); 42.73406°N, 25.40036°E; 1400 m; 27.6.2016; heard T.Trilar, I.Gjonov, M.Gogala. Bulgaria: Stara Zagora Province:

Pavel Banya Municipality: Tazha, Dzhendema reserve, gorge of the river Tazha; 42.66867°N, 25.04638°E; 608 m; 29.6.2020; recorded T.Trilar, I.Gjonov. Bulgaria: Sliven Province: Sliven Municipality: Stara Planina, Karandila, upper chairlift station; 42.71797°N, 26.36002°E; 1037 m; 30.6.2020 and 1.7.2020; recorded T.Trilar, I.Gjonov. Bulgaria: Stara Zagora Province: Kazanlak Municipality: Kran, Shipchenska Planina, Buzludzha (Buzludja); 42.73347°N, 25.40324°E; 1400 m; 1.7.2020; recorded T.Trilar, I.Gjonov. Bulgaria: Stara Zagora Province: Kazanlak Municipality: Kran, Shipchenski prohod; 42.75622°N, 25.31451°E; 1250 m; 2.7.2020; recorded T.Trilar, I.Gjonov. Bulgaria: Stara Zagora Province: Pavel Banya Municipality: Tazha, Dzhendema reserve, gorge of the river Tazha; 42.66867°N, 25.04638°E; 608 m; 2.7.2020; recorded T.Trilar, I.Gjonov. Bulgaria: Plovdiv Province: Brezovo Municipality: Svezhen, Sarnena Sredna Gora; 42.51334°N, 25.05534°E; 1138 m; 2.7.2020; recorded T.Trilar, I.Gjonov.

In all localities where the type material was collected, the male calling song was also recorded, including the *type locality*.



Figure 3. Map with known localities of *Cicadetta karandila* sp. n. (blue). The known localities of *Cicadetta cantilatrix* in Bulgaria are marked in red.

Head: Black with inconspicuous brownish patch on epicranial suture and central suture of dorsal plate of postclypeus. Postclypeus frontally black with ochre-coloured lateral margin. Rostrum reaching middle trochanter, anteclypeus black, labrum light ochre, mentum, and labium black. Compound eyes brownish, ocelli honey-brown. Supra-antennal plates black, scapes, pedicels and flagellum of antennae black, ochre-coloured rings basally on scapes and first segment of pedicels.

Thorax: Pronotum and pronotal collar black. Lateral angles of pronotal collar pronounced and black in colour. Pronotal collar frontal to lateral angles with straight margin. Median, paramedian, and lateral fissure distinct and typically shaped. Mesonotum, cruciform elevation and metanotum black, the latter with thin brownish margins. Parapsidal sutures black (5 of 9 specimens (including males and females)) or slightly brownish in colour (3 of 9 specimens), or with small ochraceous spot at rearmost end (2 of 9 specimens). The paratype male with most pronounced thin brownish margins on metanotum and small ochraceous spot at rearmost end of parapsidal suture also has pronotal collar with thin brownish margin.



Figure 4. Altitudinal distribution of *Cicadetta karandila* sp. n. The percentage of recorded localities is shown in 200-metre altitude zones.

Ventral side black, with the exception of membranes on leg bases brown, and ochraceous spot on anepisternum 2 (5 of 9 specimens (including males and females)) or ochraceous margin centrally on anepisternum 2 (4 of 9 specimens).

Male opercula not overlapping, broadly rounded with concave margin all around, with black to dark brown base and ochraceous distal two-thirds (Fig. 5). Female operculum is ilustrated at Fig. 6. Meracanthus centrally black, with ochre-coloured margin and spike; relatively long, with broad, rounded spike slightly curved and directed laterally (Fig. 7).



Figure 5. *Cicadetta karandila* sp. n.: Male opercula (holotype). The black tergite 1 with orange-coloured ridges anterior to the timbals is also visible.



Figure 6. *Cicadetta karandila* sp. n.: Female operculum (paratype $\stackrel{\bigcirc}{\rightarrow}$).



Figure 7. *Cicadetta karandila* sp. n.: Meracanthus (paratype 3).

Abdomen: Abdomen triangular in cross-section. Tergite 1 in females black and in males black with ridges marked orange anterior to timbals (Fig. 5). Timbals with two long ribs, one half-long rib, and one very short rib (Fig. 8). Tergite 2 black (6 of 9 specimens (including males and females)) or black with a red mediolateral line (3 of 9 specimens). In living specimens, there is a shiny, waxy exudation on the dorsal ridge of tergite 2, which is particularly extensive in fresh specimens and usually more prominent in males than in females. Tergites 3 through 7 black with broad red caudal margins. The red caudal margins are interrupted or almost interrupted at dorsal ridge. Tergite 8 orange with black frontal margin of half to one-third of the width.



Figure 8. Cicadetta karandila sp. n.: Timbal with two long ribs, one half-long and one very short rib (holotype).

Abdominal sternites I and II black, sternite III black with dark orange caudal margin, sternites IV to VI dark orange with basal black square spots with rounded caudal corners that become smaller towards the posterior abdomen (except for one male paratype orange with small basal black triangular spots). Sternite VII dark orange with basal black triangular spot tapering caudally; sternite VIII dark orange. Epipleurites III to VI basally black, caudally dark orange, or completely orange in some paratypes.

Legs: (Fig. 9). Front legs coxa black with dark orange dorsal margins. Trochanter black with dark orange lateral margin. Femora dark orange with black longitudinal stripes laterally on medial surface and on superior and inferior margin, with 3 black spines on inferior margin. The longest is the primary spine, whereas the last distal spine is small and sometimes less pronounced (Fig. 9). Tibiae proximally and dorsally black, distally and ventrally dark orange. Tarsi dark orange, third tarsomeres darkened distally, as are the claws.

Mid- and hindleg coxae and femora black with dark orange longitudinal stripes. Trochanter black. Tibiae brown to dark orange, as are tarsi; third tarsomere darkened distally, as are claws. On the ventral margin of hind tibiae, 3 equally long dark orange coloured tibial spurs with blackish tip; tibial combs dark orange with blackish tip.

The legs of females are generally darker in colour.



Figure 9. Cicadetta karandila sp. n.: Front leg with 3 spines on inferiror margin (holotype).

Wings: (Fig. 2). Forewings transparent, without markings. Basal cell of forewings slightly golden yellowish, pterostigma ochraceous, basal membrane bright orange. Median and anterior cubitus veins originate at one point in the basal cell (one paratype with very short arculus between them). Basal junction of anal veins dark (black) (5 of 9 specimens, 55% (including males and females)), medium coloured (2 of 9 specimens, 22.5%), or light (yellowish) (2 of 9 specimens, 22.5%).

Veins with characteristic colouration: costal vein, radius, and subcostal vein medium brown; posterior cubitus vein and first anal vein whitish basally, apically turning black (4 of 9 specimens (including males and females)) or dark brown (5 of 9 specimens); third anal vein ochre; second anal vein black. Anterior cubitus vein black, proximal median veins whitish to nodal line (6 of 9 specimens (including males and females)) or brown (3 of 9 specimens); anterior cubitus vein, anal veins, apical median veins, and radius veins black. Irregularities in the venation can be observed in some specimens: in one paratype male on the right forewing apical cell 5 is very small and grows out of median vein 3, which is why median vein 2 is missing, and in one paratype female there is an additional ellipsoid cell on median vein 3+4 on the right forewing (Fig. 2).

Hindwings transparent except for bright orange inner corner of costal cell; black shaded apical margin of vannus (anal cell 2) and dirty orange framed jugum and plaga. Veins black; in one male paratype veins are brown.

The number of apical cells is 8 on forewings and 6 on hindwings, but in some specimens on one side, there may also be 9 forewing apical cells (2 of 9 specimens (including males and females)) and 5 on hindwing (1 of 9 specimens).

Male genitalia: (Figs. 10, 11). The pygofere follows the *Cicadetta*-type morphology, characterized by the absence of a tooth on the basal lobe. Pygofer with pronounced, triangularly pointed dorsal beak, which greatly exceeds the height of the anal tube. Upper lobes flat; moderately developed; distant from dorsal beak; rounded and completely blunt; the line connecting the highest points of upper lobes reaches dorsal rim of anal tubes. Basal lobe undivided, narrow, and pointed tip is narrowly



rounded; inclined towards main capsule. Pygofer basally and anteriorly (rostrally) black; middle part, upper lobes and basal lobes of pygofer, anal styles, and anal tube ochre coloured.

Figure 10. Cicadetta karandila sp. n.: Lateral view of male genital capsule (holotype).

Median lobe of uncus shiny black basally, muted black apically, dark reddish in between; duckbill-shaped; horizontally orientated and very slightly curved upwards (paratype) or curved upwards, therefore concave (5 paratype males); triangular, rounded, and broad.

Pseudoparamere long, oblong and flattened, with sharp tips (Fig. 10, 11), more resembling elogate ones in *C. sibillae* than the expanding ones as in *C. cerdaniensis* (cf. Fig. 5 in Puissant & Gurcel 2018). There is no fine denticulation along the outer edge of the pseudoparamere. Gonopore half as long as the pseudoparamere. Pseudoparamere and gonopore amber coloured. Basal plate of aedeagus T-shaped. Claspers dark brown and flat, in shape of decreasing or increasing crescent with pointed, laterally directed tip.



Figure 11. *Cicadetta karandila* sp. n.: View of the male genital capsule from behind (holotype), aedeagus with pseudoparameres, uncus and claspers can be seen, also a dark basal plate in the background.

Female terminalia (genitalia): (Fig. 12, 13). Tergites 3 to 7 black with red caudal margins, reduced in width on dorsal ridge. Tergite 8 ochre-coloured with black frontal margin occupying half-width. Tergite 9 is black anteriorly and dorsally, ochre-coloured ventrally and posteriorly; dorsal beak black; stigma covered with black spot. Anal tube and anal styles light ochre-coloured. Gonocoxite VIII (valvifer 1) ochre with black line along ventral margin (grey in 1 paratype of 2 females); gonapophyse IX dark brown; gonapophyse X black; ovipositor dark reddish-brown.

The song pattern: Time parameters

The song of *C. karandila* sp. n. resembles that of *C. cerdaniensis* s. str., *C. sibillae*, *C. anapaistica anapaistica*, *C. a. lucana* and also *C. cantilatrix*, all of which have the double echemes in common, with the first low-intensity part followed without interruption by a very short high-intensity part. Based on these acoustic features, we categorize all these species into the »cerdaniensis« acoustic group of the Cicadetta montana species complex (Sueur & Puissant 2007; Hertach 2011; Hertach et al. 2015).

Because of similar elements in the songs of all these species, we tried to use the same or similar acoustic terminology as that used by other authors (Sueur & Puissant 2007; Hertach 2011; Hertach *et al.* 2015) and not to introduce new terms.



Figure 12. Cicadetta karandila sp. n.: Lateral view of female terminalia (paratype female).



Figure 13. Cicadetta karandila sp. n.: Ventral view of female terminalia (paratype female).

The calling song of *Cicadetta karandila* sp. n. consists of three phrases (Fig. 14). Phrases 1 (PH1) and 3 (PH3) are built up by equidistant short single echemes (E_1, E_3) . PH1 and PH3 differ in phrase duration (PH_1D, PH_3D) , echeme duration (ED_1, ED_3) , number of echemes in the phrase (NE_1, NE_3) and repetition rate (RR_1, RR_3) (Table 1). The duration of PH1 (PH_1D) is 8.96 ± 8.06 s and of PH3 (PH₃D) 7.32 ± 3.49 s, the number of echemes in PH1 (NE₁) is 16 ± 12 and in PH3 (NE₃) 32 ± 14 , the repetition rate of PH1 (RR₁) is 2.05 ± 0.56 and PH3 (RR₃) is 4.41 ± 0.59 echems per second, the echem duration in PH1 (ED₁) is 30.4 ± 6.8 ms, while in PH3 (ED₃) it is 33.3 ± 7.7 ms. Two-phase structure of echemes is also indicated in PH1 and PH3, but the first low-intensity part is so short and difficult to separate from the second high-intensity part, so we did not measure the parts separately.



Figure 14. *Cicadetta karandila* sp. n.: Selected part of the typical calling songs. (A) Spectrogram (time versus frequency versus amplitude) (the amplitude scale is indicated on the right) and oscillogram (time versus amplitude); (B) oscillogram (time versus amplitude) of the last 4 double echemes of phrase 2, followed by 12 echemes of phrase 3 (the song section corresponds to the inverted window in (A)). The triangles indicate the beginning of the phrases: black – beginning of phrase 1, grey – beginning of phrase 2, outlined and white – beginning of phrase 3. The terms of the temporal variables of phrase 2 are indicated: ED_2 – echeme duration, FP_2 – first part of the double echeme, G_2 – echeme/inter-echeme group, IED_2 – inter-echeme duration, SP_2 – second part of the echeme. The black arrow indicates the discuntiniuty (probably a failure in the nevron network that generates the song pattern).

The duration of PH2 (PH₂D) is 22.69 ± 11.88 s, the number of echemes in PH2 (NE₂) is 38 ± 19 , the PH2 repetition rate (RR₂) is 1.76 ± 0.51 echems per second (Table 1, Fig. 15). Phrase 2 (PH2) consists of double echemes, where the first low-intensity echeme part (FP₂) is followed without interruption (fully connected) by a very short high-intensity echeme part (SP₂). While the duration of SP₂ is more or less constant, namely 22.3 ± 5.4 ms, FP₂ are very short at the beginning of PH2 and

smoothly transitions into a longer ones after a few repetitions. The changes in FPD₂ are best shown by a generalized logistic function that follows S-shaped curves (Fig. 16). The FPD₂ at the beginning of PH2 (beg-FPD₂) is 26.0 ± 13.6 ms, taking into account the parts shorter of 50 ms. At the end of the PH2, the duration of the FPD₂ (end-FPD₂) is 383.3 ± 57.8 ms, taking into account the parts longer than 300 ms.



Figure 15. *Cicadetta karandila* sp. n.: Frequency pattern of a typical double echeme in phrase 2. (A) Dominant frequency variation along the echeme; (B) spectrogram (time versus frequency versus amplitude, coloured amplitude scale on the right) and mean spectrum (frequency versus amplitude) of the first low-intensity echeme part, followed without interruption by a second, very short high-intensity echeme part; (C) oscillogram (time versus amplitude).



Figure 16. *Cicadetta karandila* sp. n.: Phrase 2 duratoin. (A) Phrase 2 structure in time shown by a generalized logistic function following S-shaped curves; (B) oscillogram (time versus amplitude).

Table 1. Time and frequency parameters of the song of *Cicadetta karandila* sp. n.:

PHD – phrase duration; NE – number of echemes in phrase; RR – repetition rate of the echemes; ED – echeme duration; FPD – duration of the first part of double echeme; SPD – duration of the second part of double echeme; beg-FPD₂ – duration of the first part of double echeme at the beginning of phrase 2; end-FPD₂ – duration of te first part of double echeme at the beginning of phrase 2; end-FPD₂ – duration of te first part of double echeme at the beginning of phrase 2; end-FPD₂ – duration of te first part of double echeme at the end of phrase 2; IED – inter-echeme duration; GD – echeme/inter-echeme group duration; DF – dominant frequency; CF – centre frequency; F1Q – 1st quartile frequency; F3Q – 3rd quartile frequency; EP – echeme delta power; FPP – delta power of the first part of the double echeme; SPP – delta power of the second part of the double echeme; n \pm SD – mean values between individuals \pm standard deviation; n – number of measured values; min – minimum; max – maximum; index – represents the phrase number (1 – phrase 1, 2 – phrase 2, 3 – phrase 3).

Phrase 1				
PH_1D	8.96 ± 8.06 s (n=33, min=1.25 s, max=38.07 s)			
NE_1	16 ± 12 (n=33, min=2, max=53)			
RR_1	$2.05 \pm 0.56 E_1$ /s (n=33, min=1.18 E ₁ /s, max=4.05 E ₁ /s)			
ED_1	$30.4 \pm 6.8 \text{ ms} (n=405, min=11.9 \text{ ms}, max=49.3 \text{ ms})$			
IED ₁	$311.3 \pm 167.0 \text{ ms} (n=405, min=13.17 \text{ ms}, max=98.86 \text{ ms})$			
GD_1	$341.7 \pm 167.4 \text{ ms} (n=405, min=158.0 \text{ ms}, max=1014.8 \text{ ms})$			
DF_1	13.45 ± 1.00 kHz (n=403, min=11.44 kHz, max=12.75 kHz)			
CF_1	13.44 ± 0.67 kHz (n=403, min=11.81 kHz, max=12.94 kHz)			
$F1Q_1$	13.44 ± 0.67 kHz (n=403, min=11.81 kHz, max=12.94 kHz)			
$F3Q_1$	14.38 ± 0.75 kHz (n=403, min=12.75 kHz, max=13.78 kHz)			
EP ₁	5.84 ± 5.80 dB (n=385, min=-12.18 dB, max=19.18 dB)			
Phrase 2				
PH_2D	22.69 ± 11.88 s (n=92, min=3.99 s, max=63.65 s)			
NE_2	38.53 ± 19.20 (n=92, min=8, max=106)			
RR_2	$1.76 \pm 0.51 \text{ E}_2/\text{s}$ (n=92, min=1.08 E ₁ /s, max=5.46 E ₁ /s)			
FPD ₂	$235.5 \pm 148.0 \text{ ms} (n=5721, min=1.3 \text{ ms}, max=584.3 \text{ ms})$			
SPD_2	22.3 ± 5.4 ms (n=5653, min=0.2 ms, max=46.8 ms)			
beg-FPD ₂	$26.0 \pm 13.6 \text{ ms} (n=1251, min=1.3 \text{ ms}, max=50.0 \text{ ms})$			
end-FPD ₂	$383.3 \pm 57.8 \text{ ms} (n=2257, min=300.1 \text{ ms}, max=584.3 \text{ ms})$			
ED_2	258.1 ± 150.8 ms (n=5653, min=0.5 ms, max=634.6 ms)			
IED_2	$325.1 \pm 194.2 \text{ ms} (n=5653, min=84.0 \text{ ms}, max=2899.9 \text{ ms})$			
GD_2	$583.2 \pm 185.6 \text{ ms} (n=5653, min=146.2 \text{ ms}, max=3402.5 \text{ ms})$			
FPDF ₂	13.11 ± 1.27 kHz (n=5659, min=9.19 kHz, max=12.19 kHz)			
FPCF ₂	13.11 ± 0.88 kHz (n=5659, min=11.06 kHz, max=12.47 kHz)			
FPF1Q ₂	13.11 ± 0.88 kHz (n=5659, min=11.06 kHz, max=12.47 kHz)			
FPF3Q ₂	14.09 ± 0.94 kHz (n=5659, min=11.91 kHz, max=13.41 kHz)			
FPP ₂	0.00 ± 4.48 dB (n=5528, min=-18.16 dB, max=20.05 dB)			
SPDF ₂	13.53 ± 1.15 kHz (n=5614, min=10.22 kHz, max=12.66 kHz)			
SPCF ₂	13.58 ± 0.86 kHz (n=5614, min=11.25 kHz, max=12.94 kHz)			
SPF1Q ₂	13.58 ± 0.86 kHz (n=5614, min=11.25 kHz, max=12.94 kHz)			
SPF3Q ₂	14.58 ± 1.02 kHz (n=5614, min=12.38 kHz, max=13.78 kHz)			
SPP ₂	9.49 ± 4.42 dB (n=5486, min=-14.84 dB, max=23.38 dB)			
Phrase 3				
PH ₃ D	7.32 ± 3.49 s (n=76, min=1.66 s, max=21.73 s)			
NE_3	32 ± 14 (n=76, min=10, max=77)			
RR_3	4.41 ± 0.59 nE ₃ /s (n=76, min=3.50 E ₃ /s, max=6.02 E ₃ /s)			
ED ₃	33.3 ± 7.7 ms (n=2710, min=15.7 ms, max=80.9 ms)			
IED ₃	$199.0 \pm 50.7 \text{ ms} (n=2710, min=102.6 \text{ ms}, max=652.2 \text{ ms})$			
GD ₃	231.0 ± 53.1 ms (n=2710, min=113.5 ms, max=689.7 ms)			
DF ₃	13.36 ± 1.19 kHz (n=2700, min=10.78 kHz, max=12.47 kHz)			
CF ₃	13.38 ± 0.95 kHz (n=2700, min=10.97 kHz, max=12.75 kHz)			
$F1Q_3$	13.38 ± 0.95 kHz (n=2700, min=10.97 kHz, max=12.75 kHz)			
F3Q ₃	14.32 ± 1.12 kHz (n=2700, min=12.09 kHz, max=13.5 kHz)			
EP ₃	6.88 ± 5.37 dB (n=2833, min=-15.58 dB, max=20.22 dB)			

We could observe the discontinuity (gaps, missing one or two pulses) in the low-intensity parts (FP2) of phrase 2 (PH2) (arrow in Fig. 14), which look more like a failure in the neural network generating the song pattern than a regular song pattern.

The song always begins with PH1, followed by PH2. This first sequence appears to be an activation and introduction of the song. It is also present even if the complete singing of the previous song has been interrupted for only a few seconds. The singing then continues with various repetitive patterns: PH1-PH2-PH1-PH2-PH1-PH2... or PH2-PH3-PH2-PH3-PH2-PH3... and rarely PH1-PH2-PH3-PH1-PH2-PH3...

The song pattern: Frequency range

The frequency spectrum of the calling song shows a broad maximum of intensity (at -20 dB range) between 10.5 and 15.0 kHz (Table 1, Figs. 14, 15).

The dominant frequency is not constant in all phrases but shows no clear modulation pattern (Figs. 14, 15). The dominant frequency (DF) of the echemes in phrase 1 (DF₁) is 13.44 ± 0.67 kHz and in phrase 3 (DF₃) 13.38 ± 0.95 kHz. In phrase 2, the dominant frequency of first low-intensity part of double echeme (FPDF₂) is 13.11 ± 0.88 kH and is slightly but significantly lower then in second very short high-intensity part of double echeme (SPDF₂) where is 13.58 ± 0.86 kHz (Table 1, Fig. 15).

The song pattern: Power range

The calling males modulate the echeme power (EP) at four levels, with differences between the levels of 1, 2.5, and 5 dB (Table 1, Fig. 17).



Figure 17. *Cicadetta karandila* sp. n.: Power modulation between different echeme types, shown in chronological order for mean values of 29 singers. The delta maximal echeme power (EP) is normalised to the average of first part of the double echeme (FP_2) for each calling male.

Ecology

Cicadetta karandila sp. n. occurs in mountains at altitudes between 900 and 1,400 m a.s.l., with the exception of the Dzhendema Reserve in the Tazha River gorge at 600 m a.s.l. Males often stay and sing on deciduous trees or higher shrubs and on Norway Fir (*Picea abies* (L.) Karsten), but they also visit meadows or forest clearings (Figs. 18, 19), where they sit and call for a while on low vegetation, such

as grasses and ferns. *Cicadetta karandila* sp. n. is active during the day, and we measured pearch temperatures between 23.4 and 25.0 $^{\circ}$ C.



Figure 18. Cicadetta karandila sp. n.: Habitat of the type locality on Karandila, upper chairlift station.



Figure 19. Cicadetta karandila sp. n.: Type locality and typical habitat in Tvarditshki prohod, poljana Kuriata.

Together with *C. karandila* sp. n., we regularly acoustically detected *Cicadetta montana* s. str. (Scopoli, 1772) and *Cicadetta macedonica* Schedl, 1999 at all localities, except for the Dzhendema Reserve in the Tazha River gorge, the lowest locality with *C. karandila* sp. n., where *Cicada orni* Linnaeus, 1758 and *Lyristes plebejus* (Scopoli, 1763) also occurred. In the Tvarditshki prohod, the three *Cicadetta* species were also accompanied by *Cicadetta brevipennis* Fieber, 1876. Only once (30.6.2020) in Karandila near the upper chairlift station we also recorded *Dimissalna dimissa* (Hagen, 1856), which was also present during our only visit (2.7.2020) in Sarnena Sredna Gora.

Etymology (Derivatio nominis): The name of the species reflects the name of the locality where we first heard and recorded the characteristic sound emissions near the upper chairlift station on Karandila on Stara Planina Mountain.

Discussion

Singing cicadas (Hemiptera: Auchenorrhyncha: Cicadidae) produce species-specific song during pair formation (Sueur 2006) (i.e. prereproductive barrier), which is very useful for their taxonomy. Several studies have demonstrated that the use of bioacoustic methods and sound analysis enables the recognition and discovery of species, confirmation or verification, and inventorying of species (Sueur & Puissant 2007). This also applies to the mountain cicadas complex (*Cicadetta montana* sensu lato) (Gogala & Trilar 2004; Sueur & Puissant 2007; Trilar & Gogala 2007; Gogala *et al.* 2008, 2009, 2011; Hertach *2*011; Hertach *et al.* 2015, 2016; Trilar *et al.* 2020).

The song group »cerdaniensis« of the mountain cicada complex (*Cicadetta montana* sensu lato) now comprises five species and one subspecies: *C. cantilatrix*, *C. sibillae*, *C. cerdaniensis* s. str., *C. anapaistica anapaistica*, *C. a. lucana* and *C. karandila* sp. n. The species share the double echemes with the first low-intensity part followed without interruption by a very short high-intensity part.

Comparison of the acoustic behaviour

The calling songs of these species differ mainly in their temporal patterns and repetition rates of echemes but not radically in their power and frequency characteristics. This homogeneity in frequencies can be attributed to their morphological similarities. All species are in the same body length range (approximately 20–30 mm). Because the body size and frequency of the emitted sound are usually negatively correlated (Bennet-Clark 1998; Bennet-Clark & Young 1994), they are all subject to the same mechanical constraints and therefore produce sound in the same frequency bandwidth (12–18 kHz). Probably because of these internal constraints, sexual selection, in principle, affects temporal and amplitude variations (Sueur & Puissant 2007).

The calling song of all the species comprises three phrases, where phrase 3 (PH3) is important for distinguishing each species. The exception is C. cantilatrix, where a song pattern with a fast repetition rate is not part of the calling song, but part of a special, rarely emitted courtship song (Trilar et al. 2006; Hertach 2007, 2011; Hertach et al. 2015). The calling song of C. anapaistica (both subspecies C. a. anapaistica and C. a. lucana) is characteristic of grouped echemes in phrase 3 (PH3) (Hertach 2011; Hertach et al. 2015). Phrases 3 (PH3) in C. sibillae, C. cerdaniensis s. str. and C. karandila sp. n. consists of evenly distributed (ungrouped) single echemes without exceptions (Sueur & Puissant 2007; Hertach 2011; Hertach et al. 2015). Hertach et al. (2015) suggested that species-specific information must be encoded in the time domain of phrase 3, which plays a key role in the speciation process. The most distinguishing parameter is the repetition rate of the echemes (RR₃), which is the highest in C. sibillae with 7.8 echemes per second, followed by C. cerdaniensis s. str. with 6.7 and C. karandila sp. n. with 4.4 (Table 2). In addition, the duration of the echeme/inter-echeme group in phrase 2 (GD₂) separates the species. The duration of GD₂ in C. karandila sp. n. is 0.583 ± 0.186 s, followed by C. cerdaniensis s. str. with 1.06 ± 0.29 s and C. sibillae with 1.34 ± 0.26 s (Table 2). Consequently, there are also differences in the double echeme duration (ED_2) , duration of the first part of the double echeme (FPD₂), and inter-echeme duration (IED) of phrase 2 (Table 2), while the duration of the second part of the double echeme (SPD₂) is more or less of equal lenght in all three species (Table 2).

Table 2. Temporal and frequency parameters in *Cicadetta cerdaniensis* s. str. (according to Hertach *et al.* 2015), *Cicadetta sibillae* (according to Hertach *et al.* 2015) and *Cicadetta karandila* sp. n. Variables: ED – echeme duration; FPD – duration of first part of double echeme; SPD – duration of second part of double echeme; IED – inter-echeme duration; GD – echeme/inter-echeme group duration; PHD – phrase duration; RR – repetition rate; CF – center frequency; F1Q – 1st quartile frequency; F3Q – 3rd quartile frequency; FPF – frequency of the first part of double echeme; SPF – frequency of the second part of double echeme; EF – echeme frequency; Index – indication of the song phrase. Data are presented as the mean values between individuals \pm SD.

	Variable	C. cerdaniensis s. str.	C. sibillae	C. karandila sp. n.
Phrase 2	ED_2	0.097 ± 0.038	0.213 ± 0.083	0.258 ± 0.151
duration (s)	FPD ₂	0.065 ± 0.031	0.169 ± 0.075	0.235 ± 0.148
	SPD_2	0.036 ± 0.007	0.046 ± 0.008	0.022 ± 0.005
	IED_2	0.967 ± 0.204	1.131 ± 0.218	0.325 ± 0.194
	GD_2	1.064 ± 0.238	1.344 ± 0.261	0.583 ± 0.186
	PHD_2	33.9 ± 11.5	23.1 ± 7.1	22.7 ± 11.9
	RR_2			1.76 ± 0.51
Phrase 3	ED_3	0.034 ± 0.004	0.050 ± 0.009	0.033 ± 0.008
duration (s)	IED ₃	0.208 ± 0.023	0.236 ± 0.035	0.199 ± 0.051
	GD ₃	0.242 ± 0.027	0.286 ± 0.043	0.231 ± 0.053
	PHD ₃	7.4 ± 1.9	7.1 ± 2.5	7.3 ± 3.5
	RR ₃	6.7	7.8	4.4
CF (kHz)	FPF ₂	13.0 ± 0.7	13.3 ± 0.8	13.11 ± 0.88
	SPF_2	14.0 ± 1.2	14.2 ± 1.0	13.58 ± 0.86
	EF ₃	13.8 ± 1.2	14.0 ± 1.1	13.38 ± 0.95
F1Q (kHz)	FPF ₂	12.3 ± 0.7	12.5 ± 0.6	13.11 ± 0.88
	SPF_2	13.1 ± 1.1	13.3 ± 0.8	13.58 ± 0.86
	EF ₃	13.0 ± 1.0	13.1 ± 0.8	13.38 ± 0.95
F3Q (kHz)	FPF ₂	14.0 ± 1.0	14.4 ± 1.0	14.09 ± 0.94
	SPF ₂	15.1 ± 1.4	15.2 ± 1.1	14.58 ± 1.02
	EF ₃	14.8 ± 1.3	15.0 ± 1.2	14.32 ± 1.12

We measured and compared also power values of the echemes (Table 1), but according to our opinion, this character has too many uncertanties to use it to separate the species.

Distribution

The song group »cerdaniensis« of the mountain cicada complex (*Cicadetta montana* sensu lato) now comprises five species and one subspecies: *C. cantilatrix*, *C. sibillae*, *C. cerdaniensis* s. str., *C. anapaistica anapaistica*, *C. a. lucana* and *C. karandila* sp. n. It is widely distributed in Europe, from Germany and Poland in the north (Trilar *et al.* 2006; Meineke 2012), to North Macedonia and Sicily in the south (Gogala *et al.* 2005; Hertach 2011), from the Pyrenees in the west (Puissant 2006), and Hungary, Romania, Bulgaria, and Russia in the east (Trilar & Gogala 2008, 2012; Trilar *et al.* 2020; Benediktov & Mikhailenko 2017). The eastern border is the least explored.

In contrast to the two widespread species (*C. cantilatrix* and *C. sibillae*), the other three species have only very small ranges or are even endemic mainly to mountainous habitats (*C. cerdaniensis* s. str., *C. anapaistica* and *C. karandila* sp. n.).

The most widespread species is *C. cantilatrix*, which occurs in France, Switzerland, Italy, Slovenia, Austria, Germany, the Czech Republic (Malenovský & Lauterer 2017), Poland, Hungary, Montenegro, North Macedonia, Serbia, Bulgaria, Romania and Russia (Benediktov & Mikhailenko 2017) (all data except the countries with the citation in brackets were summarised by Hertach *et al.* 2015 and Hertach *et al.* 2016). Despite our very intensive cicada survey, the species was not found in Greece and Albania, while Croatia and Bosnia and Herzegovina are not sufficiently surveyed (Gjonov, Gogala, Trilar, unpublished data).

The distribution area of *C. sibillae* extends from southern Switzerland (Ticino) to central Italy and eastern France (Alpes-Maritimes) and is the most common cicada in the northern Apennines (Hertach *et al.* 2015; Puissant & Gurcel 2018).

Cicadetta cerdaniensis s. str. is endemic to the Pyrenees and is the only member of the group that is currently clearly allopatric (Puissant & Boulard 2000), whereas the other five taxa are parapatric (or at least close to allopatric) (Hertach *et al.* 2015).

The distribution of *C. a. anapaistica* is restricted to the mountains of Sicily and to smaller local populations on the mainland in Calabria (Hertach 2011; Hertach *et al.* 2015), while *C. a. lucana* is also restricted to southern Italy, mainly to the Basilicata region, but also touches Calabria, Apulia and Campania (Hertach *et al.* 2015). *Cicadetta karandila* sp. n., on the other hand, is endemic to the central part of the Balkan Mountains in Bulgaria.

The altitudinal distribution of *C. cantilatrix* is very broad, ranging from 219 to 1642 m a.s.l. (Trilar & Gogala, unpublished data), similar to that of *C. sibillae* from 20 to 1430 m a.s.l. (Hertach *et al.* 2015). The altitudinal range of *C. a. lucana* is also broad, ranging from 250 to 1960 m a.s.l. (Hertach *et al.* 2015). The other three species are restricted to the highlands up to the tree line: *C. a. anapaistica* from 920 m a.s.l. to 1750 m a.s.l. (Hertach 2011; Hertach *et al.* 2015), *C. cerdaniensis* s. str. at approximately 1350 m a.s.l. (Puissant & Boulard 2000) and *C. karandila* sp. n. from 600 m a.s.l. to 1400 m a.s.l.

Origin and postglacial evolution

During the Pleistocene ice ages, European cicadas were probably displaced to classic southern refugia, as they are thermophilic and most of them are associated with temperate areas. Regarding the »cerdaniensis« song group, Hertach *et al.* (2016) propose an Iberian refugium for *C. cerdaniensis*, a central Apennine for *C. sibillae*, a southern Apennine for *C. a. anapaistica* and *C. a. lucana*, and a Balkan refugium for *C. cantilatrix*.

In the Balkan refugium, the species of the »cerdaniensis« song group probably have a common ancestor that has evolved acoustically in at least two directions. In the first, the courtship song is only emitted in the presence of the female in close proximity and was not integrated into the regular calling song, as is the case in *C. cantilatrix*. In the second, the courtship song is integrated into the calling song as PH3 and is regularly emitted naturally, as is the case in *C. karandila* sp. n. We can also observe the same process in other refugia in *C. cerdaniensis*, *C. sibillae*, *C. a. anapaistica* and *C. a. lucana* as is described in Hertach *et al.* (2016).

The discovery of the highly endemic *C. karandila* sp. n. in the central Balkan Mountains proves once again that the evolutionary development of a new species is driven by mechanisms including prereproductive barrier based on acoustic communication and that morphological characters alone cannot be used to separate closely related species. The patterns of hidden species diversity are distributed throughout the entire range of the genus *Cicadetta*.

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