

# Biology of *Tetrix bolivari* (Orthoptera: Tetrigidae)

Research Article

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**Abstract:** The ecological requirements and biology of the Tetrigidae are almost unknown. The aim of the present work is to contribute to the knowledge of *Tetrix bolivari*, one of the least studied species of European Tetrigidae, by investigating its seasonal and daily activity, food biology, and vibratory communication. Adults of *T. bolivari* were found from March to September, with the greatest number of detections occurring between May and August. Based on the study of the daily activity patterns, most activities were positively correlated with temperature and negatively correlated with relative humidity. Detritus and mosses were the main components of the diet, with the most frequently consumed mosses being *Bryum caespiticium* and *Bryum argenteum*. Substrate-borne vibrational signals used in communication of *T. bolivari* are described here in detail for the first time. We distinguished four structural types of vibrational signals produced by males, including the signal produced by wing tremulation.

**Keywords:** Distribution • Daily activity • Food biology • Communication • Vibration • Bioacoustics • Central Europe

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## 1. Introduction

The Tetrigidae is an ancient group of Orthoptera with relatively uniform body structure [1,2]. Generally, the Tetrigidae is among the least-studied groups of Orthoptera, and their ecological requirements and biology are almost unknown [3-5]. Most Tetrigidae are small, inconspicuous orthopterans about one cm long. They are terricolous and inhabit humid places, and some species are even semi-aquatic [1,2].

In Europe, Tetrigidae are represented by only 12 species in three genera, including the genus *Tetrix*, which contains eight species [6]. The focus of the current research, *Tetrix bolivari* Saulcy in Azam, 1901, is a subpontic-Mediterranean species with a disjunct distribution [7]. The species is rare and occurs in southern Europe from Spain to the European part of Russia, and in Syria, Turkey, Iran, Caucasus, Turkmenia, and Uzbekistan [8]. *T. bolivari* is one of the least studied of the European

species of Tetrigidae, and knowledge of its ecology, biology, and distribution is very poor [7,9-11].

Food biology has been studied in some detail for only four European species: *T. tenuicornis* Sahlberg, 1891, *T. ceperoi* (Bolívar, 1887), *T. subulata* (Linnaeus, 1758) and *T. tuerki* (Krauss, 1876) [12-14]. Although Tetrigidae (Orthoptera) are known to feed on mosses, lichens, algae, small plants, and detritus, only a few published reports provide specific data about their food biology, and data about the proportions of food types and preferences are lacking [12]. The food biology of *T. bolivari* has not been reported [12,13].

Like food biology, behaviour of the Tetrigidae has received little research attention [2,7,10,14,15]. Knowledge about the diurnal activity of Tetrigidae can be found in only two studies, one on *Paratettix meridionalis* (Rambur 1838) [16] and one on *T. subulata* [17]. These studies, however, observed activity only during the light part of a 24-h day. According to the authors' observations, activity of these two species was positively correlated

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with temperature and negatively correlated with relative humidity.

The communicative behaviour of the genus *Tetrix* has also been studied infrequently. Visual courtship behaviour and partner selection were studied using mate-choice experiments by Hochkirch *et al.* [3,4,18,19] and Gröning *et al.* [5,20] for three *Tetrix* species: *T. subulata*, *T. undulata* (Sowerby, 1806), and *T. ceperoi*. The existence of vibrational signals in the genus *Tetrix* was first reported by Benediktov [21], and was based on recordings made with *T. subulata* and *T. tenuicornis*. Recently, vibrational signals of 10 species were described, but most were described incompletely [22]. Vibrational signalisation of *T. bolivari* was described by Benediktov [22], who included the characteristics of only one (the 3<sup>rd</sup>) structural type of vibration in the species.

The aim of this study was to summarize all previously published knowledge concerning the biology and ecology of *T. bolivari* and to add to that knowledge by obtaining new data. The new data are based on specimens collected on the northern edge of the *T. bolivari* range in central Europe. The paper presents the first data on the food biology of *T. bolivari* and a detailed description of the vibrational signals used in intraspecific communication.

## 2. Experimental Procedures

### 2.1 Distribution records

All published information about records of *T. bolivari* was gathered as part of this study, and specimens in all significant national museum collections (The Moravian Museum, the Silesian Museum, National Museum Prague, The Museum of Southeastern Moravia of Zlín, the Ostrava Museum and the Museum of East Bohemia in Hradec Králové) were reviewed. Localities with potential occurrence of *T. bolivari* in southern Moravia and Slovakia, as determined by the presence of potential habitat (lowland wetlands or fishponds with large, clayey littoral) were monitored from 2001–2010. Observations and analysis of food biology, daily activity, and communication behaviour were conducted on specimens collected in Pánov near Hodonín (GPS: N 48° 52' 30.00", E 17° 09' 05.00").

### 2.2 Food biology

Collected insects were immediately stored in ethanol (75%) to stop the digestion of food in the alimentary tract. For dissections of these specimens, the sternites of the thorax and abdomen were cut using thin forceps, and the oesophagus, crop, and proventriculus were removed. Permanent microscopic preparations of the

alimentary tract contents were made within Hoyer's solution [23]. These preparations were used for the comparison and determination of the moss tissue fragments in the alimentary tract. The percentage of food components within the alimentary tract was determined by image analysis software from micrographs of the permanent microscopic preparations in Hoyer's solution. Altogether, 14 males and 15 females (9 males and 8 females from 2009, 5 males and 7 females from 2010) were used for alimentary tract dissections and for determination of moss fragments. For the analysis of the relative food composition (percentage of food components), each preparation was photographed at 160x magnification 10 times with an Arsenal LC1601 LED microscope and a CANON PowerShot G9 camera. The relative amounts of mosses and detritus in each micrograph were determined with the image analyse software BaDra. At the same time that the insects were collected, terrestrial moss samples in the area were collected and later identified by a bryologist (V. Plášek, University of Ostrava). Statistical evaluation was made by comparison of two mixed linear models: the first determined how much of the variability in the amount of food consumed was random, and the second determined how much of this variability could be explained by sex.

The models were compared to clarify variability using ANOVA, and the software R 2.11.0 was used for statistical analyses. The significance level was  $\alpha=0.05$  throughout this study.

### 2.3 Daily activity

To determine the daily activity patterns of *T. bolivari*, we studied 30 specimens that were living and breeding in three well-ventilated insectaries (20 x 20 x 30 cm). The insectaries contained 5cm layer of fine sand, with one moss cushion on the sand surface, and one tussock of *Calamagrostis epigeios* in one of the corners. Five males and five females were placed in each insectary. The insectaries were located outside, but were protected from rain by a translucent plastic wrap. During the 3 days of observation, the light period of the day was 8 h. Air temperature and relative humidity were recorded every 10 min by DT-171 data loggers. Each insectary was visually monitored for three 24-h periods during one week. The observations were conducted for the first 2 min of each 30 minute interval. Night observations were conducted with lighting provided by 40-W tungsten bulbs (Philips) covered by red glass. We distinguished movements (change of location, rotation, cleaning, *etc.*) that were less than 1 cm, movements greater than 1 cm, defecation, and feeding.

Faecal pellets produced were collected with an automatic device. The apparatus consisted of a 27-cm

glue-covered disc that rotated with a period of 12 h. The faecal pellets fell down through the modified insectary with a wire netting floor, where they moved through a funnel and onto the surface of the disc that was covered by glue. Every 12 h, the disc was replaced and the removed disc was evaluated for the number of pellets produced during each hour. The specimens of *T. bolivari* were continually supplied with food by the moss cushion; the experiment lasted for 3 days.

## 2.4 Statistical analyses

Because the data were not normally distributed, the nonparametric Kruskal-Wallis and Mann-Whitney U-test nonparametric test was used to determine whether activities (number of specimens exhibiting a particular activity) differed with time in the case of daytime fecal pellet production, or with the light/dark period in the case in others daily activities. Regression analysis was used to determine how the intensity of specific activities was related to temperature or relative humidity. Statistica 9.0 was used for the analyses.

## 2.5 Communication

The substrate-borne vibrations of pre-reproductive adults of *T. bolivari* (8 males and 3 females) were recorded. The insects were reared in insectaries at 22–24°C and with a photoperiod of 12/12 h; each sex was kept separately. Vibrations were recorded in the laboratory at 24–26°C and 50–60% relative humidity; temperature and relative humidity were monitored with R3121 data loggers (Comet Systems, The Czech Republic). The measurements of sound characteristics were presented as means  $\pm$ SD. Vibratory signals were studied with one insect or during manipulated contacts between two insects; the combinations were single male, single female, male–male, and male–female). The use of each signal in each combination or situation was evaluated for the first 10 min of each experiment. Contact experiments were repeated with different individuals. The recordings were performed in an arena made from a plastic, circular, half-opened container (10 cm diameter) with a sheet of grey, opaque cellophane paper fixed on the rim. In this method, which was modified from Kanmyia [24], the insects were placed on the cellophane paper, and a condenser microphone was placed beneath the paper. Insect-generated vibrations produced standing waves in the paper that in turn produced sound pressure causing greatly amplified oscillation of the diaphragm in the condenser microphone (type MCE-2500; response bandwidth 40–20 000 Hz). The microphone was attached to a recording device (Sony MZ-RH1, sampling rate 44.1 kHz, response bandwidth 20–20 000 Hz), and the recorded sounds were processed with a computer.

The temporal characteristics, oscillograms, and spectrograms were analysed and compared with the software Sound Ruler (version 0.941; Gridy-Papp M., <http://soundruler.sourceforge.net>). The classification and terminology of structural types of vibrations follow Kočárek [25].

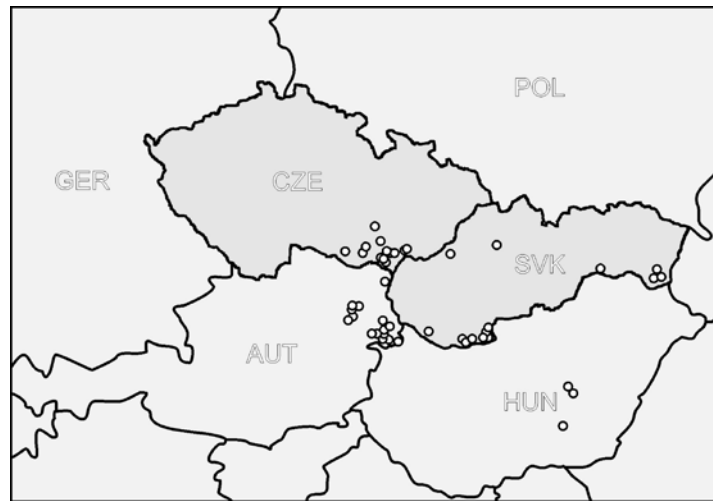
## 3. Results

### 3.1 Distribution and habitat preferences of *Tetrix bolivari* in Central Europe

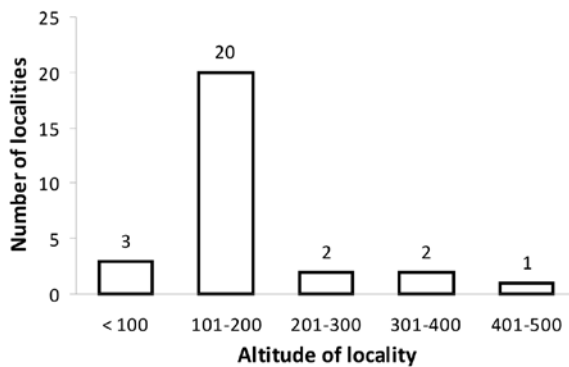
*T. bolivari* was found sporadically throughout southern Slovakia and the southeastern Czech Republic (Figure 1, Appendix). Most of the studied localities had altitudes < 200 m a.s.l. Only a few localities on the northern edge of the geographical district had altitudes as high as 400 m a.s.l. (Figure 2, Appendix), and exceptionally as high as 500 m (Mošovce, see Discussion). Among the 13 localities including representation by *T. bolivari*, eight were on clayey banks of ponds or salt marches, one was in a flooded gravel-pit, one was in a flooded and abandoned sand-pit, and one was on the gravelly bank of a river. Most specimens were found on the surface of wet ground near water bodies. Micro-localities were in the stage of initial succession and were sparsely vegetated. In a few cases, single, apparently migrant specimens were found in atypical habitats such as steppe (Velké Bílovice) or pasture (Mošovce). *T. subulata* was found with *T. bolivari* at all 13 localities, but *T. ceperoi* was not observed. Adults of *T. bolivari* were found from March to September, and most were recorded from May to August (Figure 3, Appendix).

### 3.2 Daily activity pattern of *Tetrix bolivari*

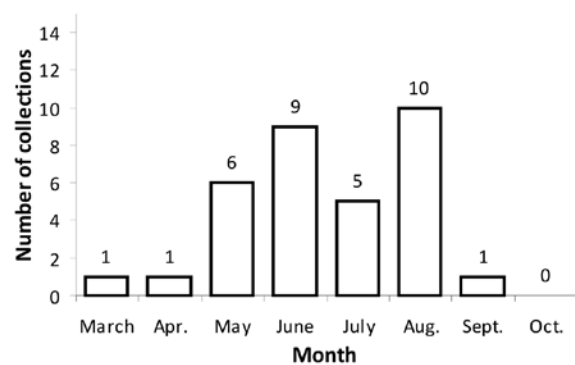
Specimens in insectaries were active throughout a 24-hour day. There was no significant difference in the time of day (light period vs. dark period) when specimens showed movement <1 cm (change of location, rotation, cleaning) (Mann-Whitney U-test;  $Z=-1.22$ ;  $P>0.1$ ) or movement >1 cm (Mann-Whitney U-test;  $Z=-0.75$ ;  $P>0.1$ ), feeding (Mann-Whitney U-test;  $Z=-1.95$ ;  $P>0.05$ ), and defecating (Mann-Whitney U-test;  $Z=-2.03$ ;  $P>0.01$ ). Faecal pellet production also did not differ with time of day (Kruskal-Wallis H test:  $H(23, N=192)=26.16$ ;  $P>0.1$ ). Most activities, including defecation, movement >1 cm, and body shaking, were positively correlated with air temperature (Table 1). The highest correlation coefficients for temperature were realized by movement >1 cm and feeding behaviour; movement <1 cm was not correlated with temperature. The correlations were the same for relative humidity except that they were



**Figure 1.** Distribution of *Tetrix bolivari* in eastern part of Central Europe (Germany, GER; Poland, POL; Czech Republic, CZE; Slovakia, SVK; Austria, AUT; Hungary, HUN). The distribution in the Czech Republic and Slovakia is based on the data presented in this study (Appendix), the distribution in Austria and Hungary has been extracted from published data [49,50].



**Figure 2.** Distribution of localities with occurrence of *T. bolivari* in Czech Republic and Slovakia (N=28) according to the altitude.



**Figure 3.** Seasonal occurrence of *T. bolivari* in Czech Republic and Slovakia displayed by the dates of individual findings (N=33).

	Movement (<1 cm)	Defecation	Walking activity (>1 cm)	Body shaking	Feeding activity
Temperature	0.0368 n.s.	0.4041***	0.6351***	0.4799***	0.5643***
Humidity	-0.0268 n.s.	-0.4378***	-0.5949***	-0.4423***	-0.5725***

**Table 1.** Correlation coefficients between individual activities and air temperature and relative humidity (n.s. - not significant, \*\*\* - highly significant,  $P < 0.001$ )

negative rather than positive (Table 1). Copulation was observed only once during the 3 days of observation.

### 3.3 Food biology of *Tetrix bolivari*

Based on specimens collected in the field, detritus (soil with unidentified decomposed organic matter) was the dominant component of the diet of *T. bolivari*, i.e., detritus represented  $88 \pm 18\%$  of the total amount of the food in the alimentary tract. Females consumed a significantly larger amount of mosses than did males in 2009 (one-

way ANOVA:  $df=4$ ,  $P > 0.001$ ), but males consumed a larger quantity of moss than females in 2008 (one-way ANOVA:  $df=4$ ,  $P=0.038$ ). Both sexes consumed the same amount of detritus in both 2008 and 2009.

The diet of *T. bolivari* is comprised of at least eight moss species (Table 2). Rhizoids and gemmae of these mosses were present in some samples. One gut sample from a male also contained gemmae of the moss *Bryum bicolor* Dicks., which grows epiphytically on the bark of trees. The most frequent mosses in the guts of

*T. bolivari* were *Bryum caespiticium* (in 65.5% of the guts) and *Bryum argenteum* (in 51.7% of the guts). The other moss species also occurred with relatively high frequency (Table 2). Besides mosses and detritus, some fragments of vascular plants (grasses), pollen grains, algae, hyphae of Basidiomycetes, and rarely fragments of arthropods (Arachnida, Insecta) were found. The total portion of these components in the food was <1.0%.

### 3.4 Vibratory communication in *Tetrix bolivari*

The vibrations produced by *T. bolivari* are generated by two mechanisms and can be placed in two corresponding groups of signals. The first group of signals is produced by an undescribed mechanism that is probably based on muscle contractions (see Discussion). This first group of vibrational signals consists of well-defined single pulses or pulses united into rhythmically or irregularly repeated groups. Three structural types of these signals (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> type) were observed in males. The second group of signals is produced by wing tremulation, and only by males. Structural characteristics of all individual signals are summarised in Table 3 and illustrated on Figures 4-9. Females did not produce any vibrational signals.

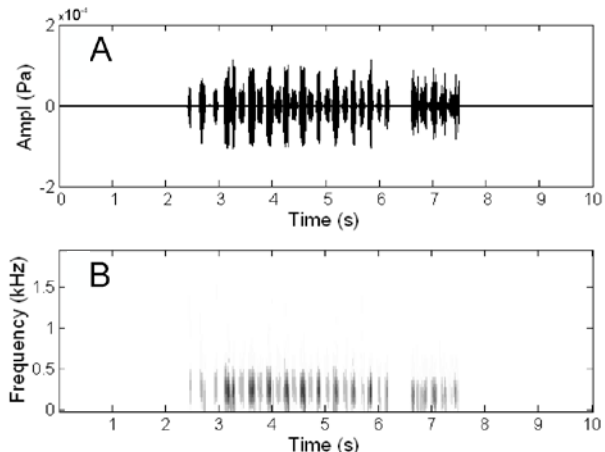
Moss species	Relative frequency in gut contents (%)		
	Males	Females	Total
<i>Amblystegium serpens</i> (Hedw.) B.S.G.	7.1	6.7	6.9
<i>Barbula unguiculata</i> Hedw.	7.1	13.3	10.3
<i>Bryum argenteum</i> Hedw.	50.0	53.3	51.7
<i>Bryum caespiticium</i> Hedw.	50.0	80.0	65.5
<i>Ceratodon purpureus</i> (Hedw.) Brid.	0.0	20.0	10.3
<i>Funaria hygrometrica</i> Hedw.	14.3	6.7	10.3
<i>Plagiomnium affine</i> (Blandow) T. J. Kop.	14.3	33.3	24.1
<i>Pottia intermedia</i> (Turner) Fűrnr.	0.0	6.7	3.4

**Table 2.** Relative frequencies of the presence of each moss species in the gut contents of *T. bolivari*. Number of dissected specimens 29 (14 males/15 females).

Structural type of signal	No of measured pulses	Mechanism of production	Pulse duration (ms±SD)	Number of pulses (range)	Interpulse interval (ms±SD)	Peak frequency (Hz±SD)	Frequency range (Hz)	Accompanied visual signalisation
1 <sup>st</sup> type, part a	25	unknown	115±32	22-24	53±28	229±34	40-2 200*	-
1 <sup>st</sup> type, part b	25	unknown	930±65	unrecognizable	unrecognizable	172±28	40-1 000	-
2 <sup>nd</sup> type	40	unknown	115±22	diverse	diverse	322±112	120-2 200	PB
3 <sup>rd</sup> type	1	unknown	678	unrecognizable	unrecognizable	206, 340, 501	80-760	S
5 <sup>th</sup> type	2	wing tremulation	196±45	diverse	diverse	1162±188	40-19 000	PB

**Table 3.** Characteristics of vibrational signals detected in males of *T. bolivari*. PB: pronotal bobbing; S: frontal and lateral swinging; \*: this part of the 1<sup>st</sup> type signal consists of broadband and narrowband pulses with different frequency characteristics.

The 1<sup>st</sup> structural type of male signal is the most complicated of all observed substrate-borne signals because it consists of two different parts, and each part can be used separately or in a complex scheme (Figures 4A,B, 8A,B, 9A). The first part (part a) of the signal (duration 3946±125 ms, N=25) consists of 22–24 regularly repeated pulses with a characteristic quick alternation between pulses of different frequency characteristics: one narrowband pulse (duration 129±37 Hz, peak frequency 236±35 Hz, N=25) followed by one broadband pulse (duration 99±16 ms, peak frequency 220±32 Hz, N=25). Part a) of this signal always starts with a narrowband pulse. In the middle of part a), pulses are quickly repeated with interpulse intervals of 53±28 ms. Part a) of the signal is usually followed by a second part (part b) that has irregular structure and consists of an unclear number of pulses (duration of part b: 930±65 ms, peak frequency 172±28 Hz, maximal frequency 800 Hz, N=25). The 1<sup>st</sup> type of male signal is especially produced by males during close contact with other conspecific males (Table 4). When producing this type of signal, the male does not move its body or any appendices.



**Figure 4.** Ten-second oscillograms (relative amplitude vs. time in seconds) and spectrograms (frequency in kHz vs. time in seconds) of 1<sup>st</sup> type of male signal produced by *T. bolivari*.

Sex	Situation	No. of repetitions	Type of vibrational signal				Visual signal	
			1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	5 <sup>th</sup>	Bobbing	Swinging
M	M	20	0	10	0	1	1	0
M	M-M	20	12	14	0	1	3	0
M	M-F	20	1	8	1	0	6	1

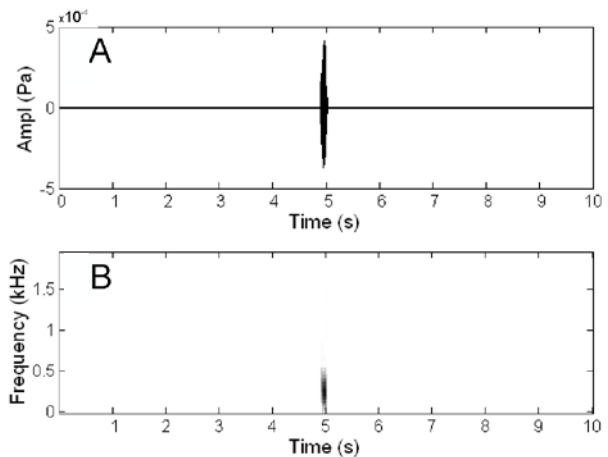
**Table 4.** Frequency of usage of individual vibrational and visual signals by *T. bolivari* recorded during contact experiments with two specimens and with single specimens. M – male, F – female.

The 2<sup>nd</sup> structural type of male signal (Figures 5A,B, 8A, 9B) consists of simple, irregularly repeated pulses (duration of pulse  $115 \pm 22$  ms, peak frequency  $322 \pm 112$  Hz,  $N=40$ ). Males produce this signal whether they are solitary or in the presence of a conspecific female or male (Table 2). When producing the 2<sup>nd</sup> structural type of signal, males usually produce isolated pulses or repeated pulses with long ( $>100$  ms) interpulse intervals. Sometimes males produce a series of two to three pulses with interpulse intervals of  $695 \pm 280$  ms. Production of this type of signal is not accompanied by movement of the insect body or any appendices.

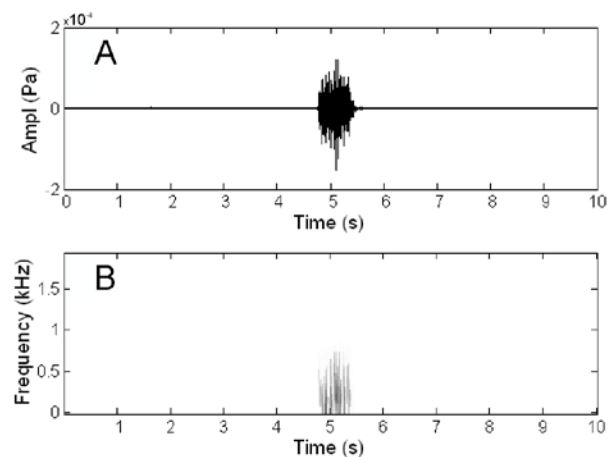
The 3<sup>rd</sup> type of male signal (Figures 6A,B, 8A, 9C) consists of quickly repeated pulses (duration of entire signal 678 ms; three frequency peaks of 206 Hz, 340 Hz, and 501 Hz; maximal frequency 780 Hz;  $N=1$ ). This signal was only produced once by a male that was in close contact with a conspecific female. Production of this signal is accompanied by quick, vertical, low amplitude shaking of the body (“swinging”—for details, see Discussion).

Production of the 3<sup>rd</sup> type of male signal is often accompanied by specific visual-communication behaviour (movement of the pronotum and wings in so-called “pronotal bobbing”—for details, see Discussion). Part of this “pronotal bobbing” visual communication behaviour is the quick opening of the wings accompanied by specific vibrations produced by wing tremulation—the 5<sup>th</sup> structural type of signal (Figures 6A,B, 8A, 9D). This tremulation signal was observed only in males (duration  $196 \pm 45$  ms, peak frequency  $1162 \pm 188$  Hz, maximal frequency 19 000 Hz,  $N=2$ ). A single tremulation pulse consists of a series of sinusoidal oscillations executed by rapid succession with intervals of  $16 \pm 6$  ms. The pulse is composed of a median of five (range four to six) oscillations. Substrate-borne signals and visual signals (including associated wing tremulation) are not mutually synchronised.

The 4<sup>th</sup> type of signal was not registered in *T. bolivari* in this study.



**Figure 5.** Ten-second oscillograms (relative amplitude vs. time in seconds) and spectrograms (frequency in kHz vs. time in seconds) of 2<sup>nd</sup> type of male signal produced by *T. bolivari*.



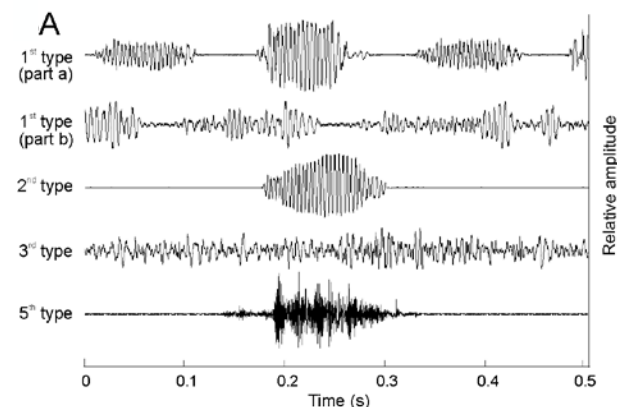
**Figure 6.** Ten-second oscillograms (relative amplitude vs. time in seconds) and spectrograms (frequency in kHz vs. time in seconds) of 3<sup>rd</sup> type of male signal produced by *T. bolivari*.



## 4. Discussion

### 4.1 Distribution and habitat preferences

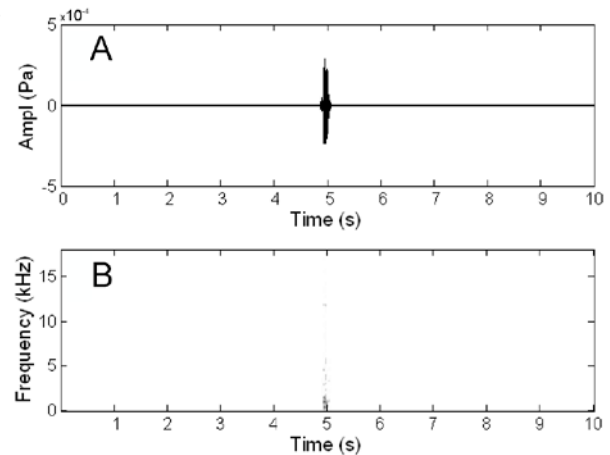
*T. bolivari* is a species with a disjunct distribution extending from Spain to the European part of Russia, Syria, Turkey, North Iran, Caucasus, Turkmenia and Uzbekistan [8]. In Europe, the northern border of its range does not extend beyond France [11], and probably lies along the southern foothills of the Alps to lower Austria [26-30], the Czech Republic, Slovakia [31], Ukraine, and Central Russia [32,33, <http://www.faunaeur.org>]. Surprisingly, this species has not been detected in Luxemburg, Belgium, or Germany, especially given that it has been detected in France [11]. *T. bolivari* had not been reported in Hungary for many years [34], but was recently detected there [35]. The failure to detect *T. bolivari* in these countries can probably be explained by the fact that i) groundhoppers are rarely studied, and ii) *T. bolivari* can be overlooked because it is difficult to distinguish from other similar species. The reported low frequency of occurrence (detected in 0.005% of the sampled sites) of this species in Hungary [32] might be a substantial underestimate given that *T. bolivari* occurs in half of the bioregions in neighbouring Rumania [36]. As a consequence of its ecological requirements, occurrence of *T. bolivari* is strongly fragmented on the edge of Pannonia. Efficient detection of this species requires precise selection and extensive study of localities with suitable habitats, as demonstrated by the detection of *T. bolivari* in many localities in France [11]. All known localities are within the territory of the Pannonian geographical subprovince, or sometimes on the border of this subprovince (localities Přímětice, Jehnice, Beckov, Turnianske podhradie; see Appendix). Only one female was found outside of Pannonia (locality Mošovce), and it probably represents a migrant, suggesting that *T. bolivari* has substantial migratory ability.



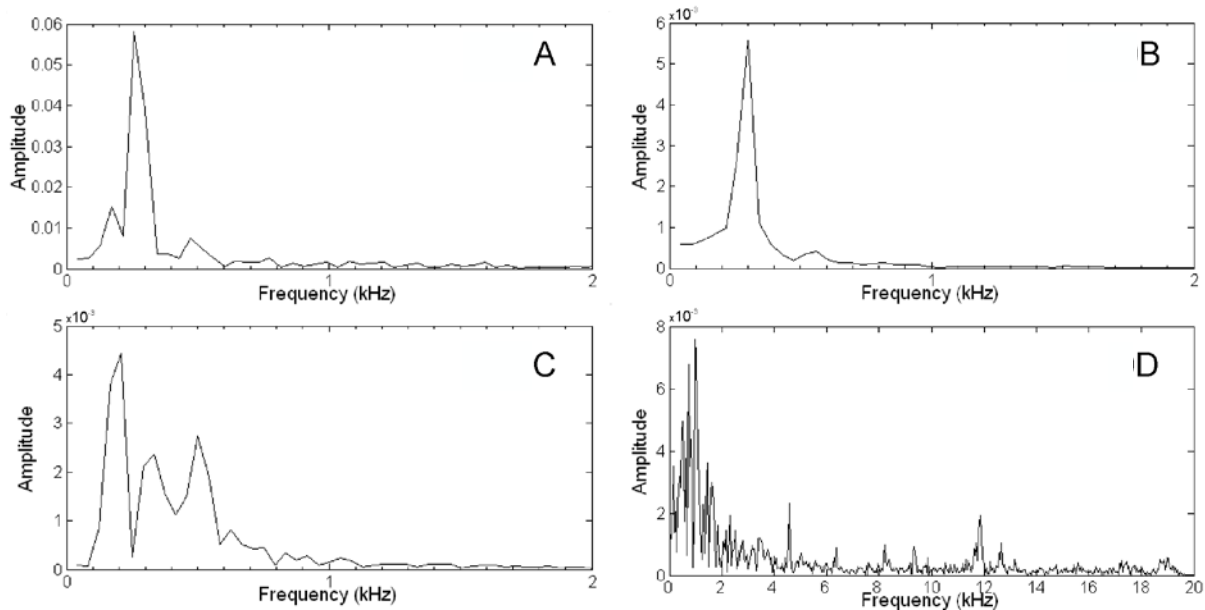
**Figure 8.** Male vibrational signals of *T. bolivari*. of the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> structural types. (A) Pulse structures of 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> structural types; 0.5-s samples. (B) Oscillogram and spectrogram of a 1-s sample of part a of the male signal of the 1<sup>st</sup> type. The spectrogram shows alternation of broadband and narrowband pulses.

Most *T. bolivari* localities on the edge of Pannonia are below 200 m a.s.l., with 0 to 200 m a.s.l. being considered the species' optimal altitude [11]. All localities are damp, which is in accordance with other studies [1,9,11,37]. Although *T. bolivari* is more common and abundant in clayey and muddy parts of marshlands and river banks [9,11,37], it also occurs in sandy places [9,38] as we confirmed in this study. In contrast to the reported detection in France [11], it was observed in gravel pits in the current study. *T. bolivari* was not observed occurring together with *T. ceperoi*, another species with a highly fragmented distribution in Europe [5], as well as on the border of Pannonia [39,40]. *T. bolivari*, however, is commonly found with *T. subulata* [9,11].

Like other groundhopper species, adults of *T. bolivari* can be found during almost any time of the year [11]. Overwintering specimens mature in spring [11], and the most abundant stage occurs in May and June



**Figure 7.** Ten-second oscillograms (relative amplitude vs. time in seconds) and spectrograms (frequency in kHz vs. time in seconds) of 5<sup>th</sup> type of male signal produced by *T. bolivari*. (A) Oscillograms, (B) Spectrograms.



**Figure 9.** Representative power spectra (portion of a signal's power within given frequency bins) of vibrational signals produced by *T. bolivari*. (A) 1<sup>st</sup> type of male signal, (B) 2<sup>nd</sup> type of male signal, (C) 3<sup>rd</sup> type of male signal, (D) 5<sup>th</sup> type of male signal. FFT size: 1024.

on the edge of Pannonia. On the northern border of Pannonia, the next generation matures in late summer, when nymphs are also present [11]. While the reported abundance in late summer might be lower than in spring/early summer, it is possible that abundance only seems lower because the groundhoppers may move into denser vegetation where they are difficult to detect [11]. Published phenological observations correspond with results of our study.

#### 4.2 Daily activity pattern

*T. bolivari* specimens were active during the entire day and they performed the same activities in the night as in the day. Most activities were positively correlated with temperature and negatively correlated with relative humidity. Similar results were reported by Lock and Durwael [41] for *Paratettix meridionalis*. Temperature is known to have profound effects on the activity patterns of insects and other ectothermic animals [42]. Forsman [43] documented strong thermal sensitivity for behaviour and locomotor performance of *T. bipunctata* (L., 1758) and *T. subulata*: the reaction distance, the propensity to fly, and jumping capacity all increased with ambient temperature.

#### 4.3 Food biology

The dominant component of *T. bolivari* diet was found to be detritus, which represented  $88 \pm 18\%$  of the total amount of the food in the alimentary tract. The diet of *T. bolivari* contained at least eight moss species, with

the most frequently consumed mosses being *Bryum caespitium* and *Bryum argenteum*. The total portion of the other components of the food (fragments of vascular plants, pollen grains, algae, hyphae of Basidiomycetes, and fragments of arthropods) was  $<1.0\%$ .

Comparable study of the feeding biology based on the dissection of *T. ceperoi* gut contents was conducted by Kočárek *et al.* [12], who found that *T. ceperoi* guts contained at least four mosses species: *Bryum argenteum*, *B. caespiticum*, *Ceratodon purpureus*, and *Barbula sp.* (*B. convoluta* or/and *B. unguiculata*). As reported for *T. ceperoi* [12], *Bryum caespiticum* was the most common moss species found in the *T. bolivari* alimentary tract in the current study. Of the *T. ceperoi* specimens examined in that study, 89.7% (94.4% of females and 85.7% of males) contained at least one fragment of one moss species, 20% (only females) contained fragments of vascular plants (grasses), and 25% contained algae. Detritus was present in all specimens, and some animal fragments (sclerotised parts of an unidentified beetle larva) were occasionally found.

Only a few species of groundhoppers have been reported as moss consumers. According to Verdcourt [44], 80% of the faecal pellets of *Tetrix undulata* (Sowerby, 1806) and *T. subulata* L., 1758 contained the leaves of *Hypnum* and rhizoids and protonemata of a variety of mosses. Lock [17] found algae, detritus, and grains of sand, but no traces of mosses or higher plants in the crops of three specimens of *T. subulata*, but his sample size was insufficient to support generalization.



Hodgson [45] observed *T. undulata* feeding on grasses (*Festuca rubra* L. and *Brachypodium sylvaticum* (Huds.) P. Beauv.), mosses, lichens and algae, and humus. He also observed *T. undulata* feeding on a dead bush-cricket *Pholidoptera griseocaptera* (De Geer, 1773) in an insectarium. The diet and feeding behaviour of *T. subulata* were studied by Hochkirch *et al.* [14] in a sand-pit near Bremen, Germany. They found that males fed exclusively on algae and mosses (*Brychythecium* cf. *rutabulum* (Hedw.) B.S.G.), while females also fed on grasses and forbs. Lock [17] observed *T. subulata* feeding on algae (*Nostoc* sp., *Pennales*, *Scenedesmus* sp., and *Spirogyra* sp.).

#### 4.4 Vibratory communication

The first information about the existence of vibrational signals in the genus *Tetrix* was published by Benediktov [21] and was based on recordings made with *T. subulata* and *T. tenuicornis* Sahlberg, 1891. More recently, Benediktov [22,46] described vibrational signals in seven other *Tetrix* species, including *T. bolivari*. Recently, Kočárek [25] presented a detailed description of the vibrational signals in *T. ceperoi* Bolivar, 1887. Benediktov [22,46] distinguished vibrational signals of three structural types (the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> type). Kočárek [25] adopted his classification but extended it by the addition of newly recognised signals of the 4<sup>th</sup> and 5<sup>th</sup> structural types. This latter classification was used for the present study. Benediktov [21] experimentally demonstrated that the vibrational signals are transmitted to the substrate through the middle legs, without observable motion of the body or its parts. Although production of these vibrations is probably associated with contractions of some muscles [47], how these substrate-born vibrations are produced remains unknown.

Vibrational signalisation by *T. bolivari* was described by Benediktov [22], but his documentation was incomplete and the recordings were based on only four males. Benediktov [22] only described the 3<sup>rd</sup> structural type of vibration; this consisted of quickly repeated pulses with a signal duration of 230–500 ms, but frequency characteristics were not reported. We recorded a signal with duration of 678 ms, but this was based on only one observation. Benediktov [22] also mentioned the presence of the 1<sup>st</sup> type of signal for *T. bolivari*, but he did not record or analyse this signal.

The vibratory repertoire of *T. bolivari* is similar that of *T. ceperoi*, as described in detail by Kočárek [25]. The 1<sup>st</sup> type of male signal consists of two parts in both species. The first part (part a) consists of a similar number of pulses in both species, but individual pulses are longer for *T. bolivari* than for *T. ceperoi*, and interpulse intervals are considerably shorter for *T. bolivari* than for *T. ceperoi*.

The second part (part b) of the signal differs considerably between the two species. In *T. ceperoi*, part b) has a regular structure and consists of well-defined individual pulses. In *T. bolivari*, part b) has an irregular structure and consists of poorly defined individual pulses. Frequency ranges are also very different for these two species (as high as 11 kHz for *T. ceperoi* vs. as high as 1 kHz for *T. bolivari*). The 2<sup>nd</sup> type of male signals are very similar for both species, especially in their duration and peak frequency. The 3<sup>rd</sup> type of signal, however, differs greatly between *T. ceperoi* and *T. bolivari*. This signal consists of quickly repeated, short, well-defined pulses in *T. ceperoi*, but the signal is compact without recognizable internal structure in *T. bolivari*. The 4<sup>th</sup> type of signal was not observed with *T. bolivari*. Wings tremulations (the 5<sup>th</sup> type of signal) were observed in both species, but in *T. bolivari* it was only observed in males. The signal is shorter and with higher peak frequency in *T. bolivari* than in *T. ceperoi*.

In this study of *T. bolivari*, the production of the 1<sup>st</sup> type of male signal was induced by contact with other conspecific males (12 cases) or females (1 case). Isolated males produced no signal of this type. These observations of *T. bolivari* agree with those of *T. ceperoi* by Kočárek [25]. The 1<sup>st</sup> type of signal seems to be a rivalry signal, according to the functional classification of Ragge and Reynolds [48]. The significance of the 2<sup>nd</sup> type of signal is unclear, but because males use this signal when alone, it might function as a calling signal in the sense of Ragge and Reynolds [48], and might also function as part of a mate-recognition system. This signal is species-specific in that it consists of a single pulse in *T. bolivari* but usually consists of four to 10 pulses in one phrase in other species [22]). The significance of this signal should be clarified in the future by use of contact experiments with other species of *Tetrix*. In *T. ceperoi*, the production the 3<sup>rd</sup> type of signal is exclusively associated with close male–female and male–male body contact and copulation [25]. This agrees with our single recording for *T. bolivari*. This specific contact signal could not be easily associated with a functional type of signal in the sense of Ragge and Reynolds [48]. *T. bolivari* production of the female signal of the 4<sup>th</sup> structural type was not detected in this study.

Visual courtship behaviour in *T. ceperoi* was described by Hochkirch *et al.* [3], who characterized it as “pronotal bobbing”, *i.e.*, synchronous movement of hind legs and pronotum and with “frontal and lateral swinging”—horizontal movements of the insect's body with minor spatial dimension. These visual signals are relatively slow and have an average duration of 0.8 s [3]. “Pronotal bobbing” of *T. bolivari* is ended by the

quick opening of its wings. This component of “pronotal bobbing” produces low frequency vibrations (vibration described as the 5<sup>th</sup> structural type) that propagate through the insect’s legs to the substrate. The production of this signal is attributed to a tremulation mechanism and was first described by Kočárek [25] in *T. ceperoi*. There is no evidence as to whether the opening of the wings is entirely a visual signal and the vibrations are only an artefact, or whether the vibrations are an important part of a complex visual and vibrational signal. The visual and vibrational signals are not synchronised but are usually used together (but not simultaneously) in certain situations [25]. “Pronotal bobbing”, including the opening of wings and the associated vibrational signal, probably helps in the recognition of conspecific individuals. Until now, this signal was known only in *T. ceperoi* [3]. “Pronotal bobbing” is more simple in *T. bolivari* than in *T. ceperoi*, and the behaviour in *T. bolivari* lacks specific movement of hind legs as documented in *T. ceperoi*. Further study based on videographic analysis is needed

to describe detailed differences in this visual behaviour between these two species. Another kind of visual signal, “swinging”, was observed only during the production of the 3<sup>rd</sup> type of signal in the current study. This type of visual signal is found also in *T. subulata*, *T. undulata*, and *T. ceperoi* [3,25]. It follows that swinging probably has an insignificant role in the specific mate-recognition system.

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## References

- [1] Podgornaya L.I., Pryamokrylye nasyekomye semeystva Tetrigidae (Orthoptera) fauny SSSR (Orthopteroid insects of the family Tetrigidae (Orthoptera) of the Soviet Union), Trudy Zoologicheskogo Instituta AN SSSR (Annales of the Institute of Zoology of Academy of Sciences of USSR) 112, Moskva, 1983, (in Russian)
- [2] Paranjape S.Y., Bhalerao A.M., Naidu N.M., On etho-ecological characteristics and phylogeny of Tetrigidae, In: Bacetti B.M., (Ed.), Evolutionary biology of Orthopteroid insects, Ellis Horwood, New York, 1987, 386-395
- [3] Hochkirch A., Deppermann J., Gröning J., Visual communication behaviour as a mechanism behind reproductive interference in three pygmy grasshoppers (genus *Tetrix*, Tetrigidae, Orthoptera), J. Insect Behav., 2006, 19, 559-571
- [4] Hochkirch A., Gröning J., Krause S., Intersexual niche segregation in Cepero’s Ground-hopper, *Tetrix ceperoi*, Evol. Ecol., 2007, 21, 727-738
- [5] Gröning J., Krause S., Hochkirch A., Habitat preferences of an endangered insect species, Cepero’s ground-hopper (*Tetrix ceperoi*), Ecol. Res., 2007, 22, 767-773
- [6] Heller K.-G., Korsunovskaya O., Ragge D.R., Vedenina V., Willeme F, Zhantiev R.D., et al., Check-list of European Orthoptera, Articulata, 1998, Supplement 7, 1-61
- [7] Devriese H., Bijdrage tot de systematik, morfologie en biologie van de West-Palearktische Tetrigidae (Contribution of systematics, morphology and biology of Westpaleartic Tetrigidae), Saltabel, 1996, 15, 2-38, (in Dutch)
- [8] Storozhenko S.Y., Ichikawa A., Uchida M., Review of Orthoptera of the Eastern Palearctica: Genus *Tetrix* Latreille (Tetrigidae, Tetriginae), Part 1, New Entomol., 1994, 43, 6-19
- [9] Adamovic Z.R., Habitat relationship of some closely related species of Tetrigidae, Orthoptera, Ekologia, 1969, 4, 165-184
- [10] Ingrish S., Köhler G., Die Heuschrecken Mitteleuropas (Grashoppers and crickets of Central Europe), Westarp Wissenschaften, Magdeburg, 1998, (in German)
- [11] Sardet E., *Tetrix bolivari* Saulcy in Azam, 1901, espèce mythique ou cryptique? (Caelifera, Tetrigoidea, Tetrigidae) (Is *Tetrix bolivari* Saulcy in Azam, 1901, mystic or cryptic species? (Caelifera, Tetrigoidea, Tetrigidae)), Matériaux Orthoptériques et Entomocénétiques, 2007, 12, 45-54, (in French)
- [12] Kočárek P., Grucmanová Š., Filipcová Z., Bradová L., Plášek V., Holuša J., Bryophagy in the groundhopper *Tetrix ceperoi* (Orthoptera: Tetrigidae): Analysis of alimentary tract contents, In: Kočárek P., Plášek V., Malachová K., Cimalová Š., (Eds.), Environmental changes and biological assessment IV, Scripta Facultatis Rerum Naturalium Universitas Ostraviensis 186, Ostrava, 2008, 348-352

- [13] Grucmanová Š., Filipcová Z., Kuřavová K., Bradová L., Kočárek P., Potravní biologie ohrožených druhů maršů (Orthoptera: Tetrigidae) (Food biology of endangered species of groundhoppers (Orthoptera: Tetrigidae)), In: Tuf I.H., Kostkan V., (Eds.), Využití výzkumu a monitoringu pro ochranný management (Using of research and monitoring results in conservation management), Proceedings of 2<sup>nd</sup> conference on conservation biology (14-17 September 2010, Olomouc, Czech Republic), Tribun EU, Brno, 2010, 46-46, (in Czech)
- [14] Hochkirch A., Gröning J., Loos T., Metzger C., Reichelt M., Specialized diet and feeding habits as key factors for the habitat requirements of the grasshopper species *Tetrix subulata* (Orthoptera: Tetrigidae), *Entomol. Gen.*, 2000, 25, 39-51
- [15] Hochkirch A., Folger M., Länder S., Meyer C., Papen M., Zimmermann M., Habitatpräferenzen von *Tetrix subulata* (Linnaeus, 1758) und *Tetrix tenuicornis* (Sahlberg, 1893) in einer Sandkuhle bei Bremen (Orthoptera: Tetrigidae) (Preference of habitat in *Tetrix subulata* (Linnaeus, 1758) and *Tetrix tenuicornis* (Sahlberg, 1893) in the sand depression at Bremen), *Articulata*, 1999, 14 31-43, (in German)
- [16] Lock K., Durwael L., One Day Activity Pattern of the Grasshopper Species *Paratettix meridionalis* (Orthoptera: Tetrigidae), *Entomol. Gen.*, 1998, 24, 177-183
- [17] Lock K., Ecologische studie over het activiteitenpatroon en de voeding van *Tetrix subulata* (L.) (Ecological studies of activity pattern in *Tetrix subulata* (L.)), *Saltabel*, 1997, 16, 30-36, (in Dutch)
- [18] Hochkirch A., Bücken A., Gröning J., Reproductive interference between the Common Groundhopper *Tetrix undulata* and the Slender Groundhopper *Tetrix subulata* (Orthoptera, Tetrigidae), *B. Entomol. Res.*, 2008, 98, 605-612
- [19] Hochkirch A., Gröning J., Bücken A., Sympatry with the devil - Reproductive interference could hamper species coexistence, *J. Anim. Ecol.*, 2007, 76, 633-642
- [20] Gröning J., Lücke N., Finger A., Hochkirch A., Reproductive interference in two ground-hopper species: Testing hypotheses of coexistence in the field, *Oikos*, 2007, 116, 1449-1460
- [21] Benediktov A.A., Akusticheskaya signalizatsiya pryamokrylykh roda *Tetrix* (Orthoptera, Tetrigidae) (Acoustical communication in groundhoppers of the genus *Tetrix* (Orthoptera, Tetrigidae)), *Zool. Zhurnal.*, 1998, 77, 1021-1025, (in Russian)
- [22] Benediktov A.A., Vibracionnye signaly pryamokrylykh nasyekomykh semeystva Tetrigidae (Orthoptera) (Vibrational signals in groundhoppers of the family Tetrigidae (Orthoptera)), *Proc. Rus. Ent. Sci.*, 2005, 76, 131-140, (in Russian)
- [23] Anderson L.E., Hoyer's Solution as a Rapid Permanent Mounting Medium for Bryophytes, *The Bryologist*, 1954, 57, 242-244
- [24] Kanmyia K., Mating behaviour and vibratory signals in whiteflies (Hemiptera: Aleyrodidae), In: Drosopoulos S., Claridge M.F., (Eds.), *Insect sounds and communication, Physiology, Behaviour, Ecology and Evolution*, Taylor and Francis, Boca Raton, 2006, 365-379
- [25] Kočárek P., Substrate-borne Vibrations as a Component of Intraspecific Communication in the Groundhopper *Tetrix ceperoveperou*, *J. Insect Behav.*, 2010, 23, 348-363
- [26] Ebner R., Kritisches Verzeichnis der orthopteroiden Insecten von Österreich (Critical check-list of orthopteroid insect of Austria), *Verh. Zool.-Bot. Ges. Wien*, 1951, 92, 143-165, (in German)
- [27] Ebner R., Saltatoria, Dermaptera, Blattodea, Mantodea, In: Strouhal H., (Ed.), *Catalogus Faunae Austriae, Teil 13a, Österreichische Akademie der Wissenschaften*, Wien, 1953, (in German)
- [28] Ebner R., Die Orthopteroiden (Geradflügler) des Burgenlandes (Orthopteroid insects of Burgenland), *Burgenländ. Heimatbl.*, 1955, 17, 56-62, (in German)
- [29] Franz H., Höfler K., Scherf E., Zur Biosoziologie des Salzlachengebietes am Ostufer des Neusiedlersees (On the biology of inhabitants of salt areas on eastern bank of Neusiedlersee lake), *Ver. Zool.-Bot. Ges. Wien*, 1937, 86/87, 297-364, (in German)
- [30] Kaltenbach A., Zur Soziologie, Ethologie und Phänologie der Saltatoria und Dictyoptera des Neusiedlerseegebietes (On the communities, ethology, and phenology of Saltatoria and Dictyoptera of the area of the Neusiedlersee lake), *Wiss. Arb. Burgenland*, 1962, 29, 78-102, (in German)
- [31] Holuša J., Kočárek P., Occurrence of groundhopper *Tetrix bolivari* (Orthoptera: Tetrigidae) in the Czech Republic and Slovakia, *Articulata*, 2000, 15, 251-256
- [32] Pushkar T.I., Pygmy locusts (Orthoptera, Tetrigidae) of the Wood-and-Steppe Zone of Ukraine, *The Kharkov Entomological Society Gazette*, 2005, 13, 9-18
- [33] Pushkar T.I., *Tetrix tuerki* (Orthoptera, Tetrigidae): Distribution in Ukraine, Ecological characteristic and features of biology, *Vestnik Zoologii*, 2009, 43, 1-14
- [34] Rácz I.A., Biogeographical survey of the Orthoptera Fauna in Central Part of the Carpathian Basin

- (Hungary). Fauna types and community types, *Articulata*, 1998, 13, 53-69
- [35] Nagy A., Rácz I.A., A hazai orthoptera fauna 10 x 10 km-es utm alapú adatbázisa 189-198, In: Kóvics G.J., Dávid I., (Eds.), 12 Tiszántúli Növényvédelmi Fórum (12 Tiszántúli Plant Forum) (17 18 October, Debrecen, Hungary), Debreceni Egyetem, Debrecen, 2007, 189-198, (in Hungarian)
- [36] Iorgu I., Pisciă E., Păi L., Lupu G., Iusan C., Checklist of Romanian Orthoptera (Insecta) and their distribution by eco-regions, *Travaux du Muséum National d'Histoire Naturelle «Grigore Antipa»*, 2007, 51, 119-135
- [37] Olmo-Vidal J.M., Atlas de los ortópteros de Cataluña y libro rojo (Atlas of the Orthoptera of Catalonia and the red book), Edition 2a, Generalitat de Catalunya, Departamente de Mediambient i Habitatge, Barcelona, 2006, (in Catalanian)
- [38] Krištín A., Kaňuch P., Sárosy M., Grasshoppers and crickets (Orthoptera) and mantids (Mantodea) of sand dunes in the Danube lowland (S-Slovakia), *Linzer biol. Beitr.*, 2004, 36, 273-286
- [39] Holuša J., Chládek F., Faunistické správy zo Slovenska (Faunistic reports from Slovakia). Orthoptera: Caelifera. Tetrigidae. *Tetrix* (*Tetrix*) *ceperoi* (Bolivar 1887), *Entomofauna carpathica*, 1998, 10, 126
- [40] Holuša J., Holuša O., First record of *Tetrix ceperoi ceperoi* (Bolivar, 1887) in Moravia (Czech Republic), *Entomol. Probl.*, 2003, 33, 54
- [41] Lock K., Durwael L., One day activity pattern of the grasshopper species *Paratettix meridionalis* (Orthoptera: Tetrigidae), *Entomol. Gen.*, 1999, 24, 177-183
- [42] Chapman R.F., *The insects: structure and function*, Cambridge University Press, Cambridge, 1998
- [43] Forsman A., Temperature influence on escape behaviour in two species of pygmy grasshoppers, *Ecoscience*, 1999, 6, 35-40
- [44] Verdcourt B., A note on the food of *Acridium* Geoff. (Orthopt.), *The Entomologist's Monthly Magazine*, 1947, 83, 190
- [45] Hodgson C.J., Some observations on the habits and life history of *Tetrix undulata* (Swrb.) (Orthoptera: Tetrigidae), *Proc. R. Entomol. Soc. London (A)*, 1963, 38, 200-205
- [46] Benediktov A.A., Novye dannye o vibrosignalizatsiyi pryamokrylykh semeystva Tetrigidae (Orthoptera) (New records on the vibrational signalization in orthopteroid insects of the family Tetrigidae), *Proceedings of the VII Ubsunurski conference (20 24 September 2001, Kyzyl, Russia)*, M. Slovo, Kyzyl, 2002, 97-106, (in Russian)
- [47] Benediktov A.A., Vibrational communication in orthopteroid insects (Orthoptera) from suborder Caelifera, *Moscow Univ. Biol. Sci. Bull.*, 2009, 64, 126-128
- [48] Ragge D.R., Reynolds W.J., *The Songs of the Grasshoppers and Crickets of Western Europe*, Harley Books, Colchester, 1998
- [49] Zuna-Kratky T., Karner-Raner E., Lederer E., Braun B., Berg H.-M., Denner M., et al., *Verbreitungsatlas der Heuschrecken und Fangschrecken Ostosterreichs (Distribution maps of grasshoppers and mantis of Austria)*, Verlag NMW, Wien, 2009, (in German)
- [50] Nagy A., Bozsó M., Kisfali M., Rácz I.A., Data on the Orthoptera fauna of the Tisza District, *Tiscia Monograph Series*, 2008, 8, 1-22
- [51] Čejchan A., K poznání orthopteroidního hmyzu (s. I.) ČSSR IV. (Dermaptera, Grylloptera, Orthoptera s. str.) (On the Orthopteroid insects (s. I.) of Czechoslovakia. IV. (Dermaptera, Grylloptera, Orthoptera s. str.)), *Čas. Nár. Mus. Praha, Řada přírodověd.*, 1985, 154, 133-144, (in Czech)
- [52] Chládek F., Zajímavé nálezy rovnokřídých (Insecta, Orthoptera) v ČR. (Interesting findings of Orthoptera in Czech Republic), *Terix*, 2006, 2, 7
- [53] Čejchan A., A Contribution to the Knowledge of the Orthoptera of Czechoslovakia and Poland, *Acta Faun. Entomol. Mus. Nat. Pragae*, 1963 9, 149-153

**Appendix 1** . List of published and new localities of *Tetrix bolivari* in the Czech Republic and Slovakia. Localities studied by the authors are in bold.

Locality	Location	Geographical coordinates	Altitude	Date	Males/Females	Collector	Determinator	Collection	Reference
<b>Beckov</b>	<b>Průdčie</b>	<b>48°49'5.79"N, 17°55'54.19"E</b>	<b>195</b>	<b>18 May 2003</b>	<b>1/5</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	
<b>Božice</b>	Jurský chlm	<b>48°49'5.38"N, 16°17'23.127"E</b>	<b>185</b>	<b>10 May 2009</b>	<b>4/2</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	
Čenkov		47°48'5.944"N, 18°32'2.151"E	107	unlisted	unlisted	unlisted	unlisted	unlisted	[38]
Gabčíkovo		47°53'34.744"N, 17°34'30.295"E	116	4 September 1953	1/1	Mařan	Čejchan	National Museum in Prague	[51]
<b>Hodonin</b>	<b>Pánov</b>	<b>48°52'29.161"N, 17°9'8.87"E</b>	<b>190</b>	<b>5 June 2008</b>	<b>1/1</b>	<b>Kočárek</b>	<b>Kočárek</b>	<b>Kočárek</b>	
<b>Hodonin</b>	<b>Pánov</b>	<b>48°52'29.161"N, 17°9'8.87"E</b>	<b>190</b>	<b>10 May 2010</b>	<b>5/4</b>	<b>Kočárek</b>	<b>Kočárek</b>	<b>Kočárek</b>	
<b>Hodonin</b>	<b>Pánov</b>	<b>48°52'29.161"N, 17°9'8.87"E</b>	<b>190</b>	<b>29 June 2010</b>	<b>1/0</b>	<b>Kočárek</b>	<b>Kočárek</b>	<b>Kočárek</b>	
<b>Hodonin</b>	<b>Pánov</b>	<b>48°52'29.161"N, 17°9'8.87"E</b>	<b>190</b>	<b>10 August 2009</b>	<b>1/1</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	[33]
<b>Chlába</b>		<b>47°49'21.811"N, 18°49'41.779"E</b>	<b>114</b>	<b>18 May 1995</b>	<b>1/1</b>	<b>Kočárek</b>	<b>Kočárek</b>	<b>Kočárek</b>	[33]
<b>Chlába</b>		<b>47°49'21.811"N, 18°49'41.779"E</b>	<b>114</b>	<b>2 July 1996</b>	<b>3/2</b>	<b>Kočárek</b>	<b>Kočárek</b>	<b>Kočárek</b>	[33]
<b>Chlába</b>		<b>47°49'21.811"N, 18°49'41.779"E</b>	<b>114</b>	<b>2 July 1996</b>	<b>1/1</b>	<b>Kočárek</b>	<b>Kočárek</b>	<b>Kočárek</b>	[33]
Chotín		47°48'28.168"N, 18°11'45.636"E	191	4 August 1952	1/0	Mařan	Čejchan	National Museum in Prague	[51]
Chotín		47°48'28.168"N, 18°11'45.636"E	191	22 July 1960	3/0	Čejchan	Čejchan	Čejchan	[51]
Chotín		47°48'28.168"N, 18°11'45.636"E	191	8 August 1962	4/2	Čejchan	Čejchan	Čejchan	[51]
Jehnice		49°16'21.094"N, 16°35'51.587"E	310	22 May 2000	1/1	Chládek	Chládek	Chládek	[52]
Kamenica	= Kamenica nad Hronom	47°49'37.308"N, 18°43'25.737"E	113	2 August 1946	1/0	Dobšik	Holuša	The Moravian Museum	
Kamenica nad Hronom		47°49'56.918"N, 18°41'36.286"E	113	July 1952	0/1	unlisted	Čejchan	National Museum in Prague	[53]
Kamenica nad Hronom		47°49'56.918"N, 18°41'36.286"E	113	20 March 1954	0/1	Pospíšil	Čejchan	Pecina	[53]
Kamenín		47°53'20.321"N, 18°38'31.543"E	126	26 June 1959	0/1	Smetana	Čejchan	Čejchan	[51]
Kamenný Most		47°51'9.373"N, 18°39'30.515"E	200	August 1952	1/2	Mařan	Čejchan	National Museum in Prague	[51]
Lednice		48°47'53.084"N, 16°48'8.183"E	170	1 July 1952	0/2	unlisted	unlisted	unlisted	[51]
Lednice		48°47'53.084"N, 16°48'8.183"E	170	10 July 1954	2/0	unlisted	unlisted	unlisted	[51]
Leles		48°27'42.384"N, 22°1'36.944"E	99	5 June 1960	0/1	Pecina	Čejchan	Pecina	[53]
Malé Kosihy		47°55'17.702"N, 18°45'30.048"E	108	6 June 1967	1/1	Olejníček	Holuša	MZM	
<b>Miřoslav</b>	<b>Miřoslavský rybník</b>	<b>48°56'20.219"N, 16°21'7.188"E</b>	<b>215</b>	<b>1 June 2010</b>	<b>2/2</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	
<b>Mořavice</b>	<b>Nedožerska dolina</b>	<b>48°54'16.195"N, 18°54'28.356"E</b>	<b>511</b>	<b>20 June 2003</b>	<b>0/1</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	
<b>Mušlov</b>	<b>Mušlovský dolní rybník</b>	<b>48°47'40.535"N, 16°40'56.705"E</b>	<b>205</b>	<b>17 June 2010</b>	<b>1/0</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	
Pouzďřany		48°55'43.842"N, 16°37'24.315"E	175	August 1959	0/2	Mařan	Čejchan	National Museum in Prague	[51]

**Continued Appendix 1** . List of published and new localities of *Tetrix bolivari* in the Czech Republic and Slovakia. Localities studied by the authors are in bold.

Locality	Location	Geographical coordinates	Altitude	Date	Males/Females	Collector	Determinator	Collection	Reference
Pouzdířany		48°55'43.842"N, 16°37'24.315"E	175	1954	0/1	Dobšík	Holuša	Museum Ostravian	
<b>Prímětice</b>		<b>48°53'14.371"N, 16°37'85"E</b>	<b>170</b>	<b>8 May 2000</b>	<b>3/4</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	[33]
<b>Prímětice</b>		<b>48°53'14.371"N, 16°37'85"E</b>	<b>170</b>	<b>19 August 2000</b>	<b>2/1</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	[33]
<b>Prímětice</b>		<b>48°53'14.371"N, 16°37'85"E</b>	<b>170</b>	<b>19 August 2000</b>	<b>0/2</b>	<b>Kočárek</b>	<b>Kočárek</b>	<b>Kočárek</b>	[33]
Radvan nad Dunajom		47°44'48.522"N, 18°21'56.986"E	120	unlisted	unlisted	unlisted	unlisted	unlisted	[38]
Sedlec	Nesyt	48°46'32.587"N, 16°41'51.707"E	170	25 April 1962	1/2	Lauterer	Holuša	The Moravian Muzeum	
<b>Sedlec</b>	<b>NPR Slanisko u Nesytu</b>	<b>48°46'32.587"N, 16°41'51.707"E</b>	<b>170</b>	<b>17 June 2010</b>	<b>3/5</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	
Svätá Mária		48°25'59.678"N, 21°50'28.982"E	97	22 August 1960	0/1	Čejchan	Čejchan	Regional Museum of Eastern Bohemia in Hradec Králové	[51]
Štúrovo		47°47'53.111"N, 18°43'25.973"E	103	24 August 1950	1/0	Trakal	Čejchan	Trakal	[53]
Štúrovo	Kováčovské kopce hills	47°49'27.783"N, 18°46'57.498"E	?	17 June 1949	1/0	Hoffer	Čejchan	Čejchan	[51]
<b>Turňa nad Bodvou</b>	<b>Turňanský rybník</b>	<b>48°36'20.302"N, 20°52'5.223"E</b>	<b>185</b>	<b>1 May 2001</b>	<b>1/2</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	
<b>Velké Bílovice</b>	<b>Trkmanský dvůr</b>	<b>48°51'56.814"N, 16°50'16.42"E</b>	<b>165</b>	<b>6 August 2008</b>	<b>5/4</b>	<b>Holuša &amp; Kočárek</b>	<b>Holuša &amp; Kočárek</b>	<b>Holuša &amp; Kočárek</b>	
<b>Velké Bílovice</b>	<b>Úzké Úlehle</b>	<b>48°52'12.368"N, 16°55'39.152"E</b>	<b>195</b>	<b>12 September 2010</b>	<b>1/0</b>	<b>Kočárek</b>	<b>Kočárek</b>	<b>Kočárek</b>	
<b>Velké Raškovce</b>		<b>48°33'6.944"N, 21°54'41.334"E</b>	<b>98</b>	<b>21 August 1996</b>	<b>2/5</b>	<b>Holuša</b>	<b>Holuša</b>	<b>Holuša</b>	[33]
Véstonice	probably Dolní Véstonice	48°53'18.15"N, 16°38'25.013"E	175	2 July 1952	0/1	unlisted	unlisted	unlisted	[51]