

Article

Conservation Significance of Forest Remnants for Urban Biodiversity: Parks as a Refuge for the Wood Cricket, *Nemobius sylvestris* (Insecta: Orthoptera: Trigonidiidae)

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Abstract

Urban parks derived from historical forest fragments represent important refugia for biodiversity in rapidly expanding cities. The wood cricket, *Nemobius sylvestris*, was surprisingly found in a park in the northern part of Bucharest, Romania, an area under exponential residential development. The species was confirmed by calling song analysis and molecularly confirmed through DNA-barcoding. The acoustic analysis revealed substantial geographic variation in the signals of *N. sylvestris* across its European range, with the Romanian population exhibiting the most distinctive acoustic characteristics. A median joining network was constructed using available COI sequences from public databases, showing moderate genetic variability within European samples. This flightless, woodland-specialist cricket is highly sensitive to habitat fragmentation and its persistence in this urban park demonstrates the conservation value of retaining semi-natural forest structure within city green spaces. Our findings highlight the importance of urban parks as biodiversity refugia, particularly for habitat specialists with limited dispersal abilities. This discovery underscores the need for the integrative conservation management of urban forest remnants, emphasizing the retention of natural structural elements such as leaf litter and heterogeneous canopy cover to support diverse invertebrate communities.

Keywords: biodiversity refugia; urban ecology; bioacoustics; DNA-barcoding; Grylloidea



Received: 5 February 2026

Revised: 26 February 2026

Accepted: 3 March 2026

Published: 5 March 2026

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1. Introduction

The rapid expansion of urban areas represents one of the most significant threats to global biodiversity, resulting in habitat loss and the fragmentation and degradation of natural ecosystems [1,2].

Urban parks are defined as open spaces dominated by vegetation and water features, often larger than other urban green spaces, and reserved for public use. They are important for biodiversity as they offer diverse habitats and microhabitat heterogeneity, key factors in supporting a wide range of species. Despite the challenges posed by urbanization, such as habitat loss and fragmentation, urban parks have been identified as biodiversity hotspots

within cityscapes, providing opportunities for innovative conservation strategies [3]. Urban parks and green spaces, particularly those derived from historical forest remnants, can serve as refugia for native species in heavily modified landscapes [4]. These urban forest fragments often harbor surprisingly diverse communities and may represent the last remaining habitat for species with specific ecological requirements [5]. The conservation value of urban parks extends beyond their immediate ecological function. They provide essential ecosystem services, maintain connectivity within fragmented landscapes and serve as reservoirs of genetic diversity for populations isolated by urban development [6,7].

Bucharest, the capital of Romania, has undergone substantial urban transformation over the past century, with particularly rapid expansion during the post-socialist period [8,9]. This development has resulted in the conversion of extensive peri-urban forests and natural areas into residential, commercial and infrastructure zones [10]. The city's remaining parks often represent isolated fragments of formerly continuous forest ecosystems, with many retaining only limited structural characteristics of their original woodland habitats [10]. Despite these pressures, several urban parks in Bucharest continue to support notable biodiversity, as demonstrated by recent surveys documenting diverse arthropod communities [11]. However, the long-term viability of specialist species in these fragments depends on appropriate management practices to maintain the habitat [12].

One of the most species-rich infraorders within Orthoptera, the Grylloidea (crickets) comprises more than 5800 species that serve as a model group in the study of acoustic communication and neurobiology, and recently, as a source of alternative animal protein sources [13]. The Trigonidiidae, an extremely diversified family within the Grylloidea, consists of two heterogeneous subfamilies: Nemobiinae and Trigonidiinae. The members of these two subfamilies can be easily identified using a combination of homoplastic characters (body size, chaetotaxy, etc.) and apomorphies, such as foretibiae with one apical, medioventral spur, regularly serrated claws, indistinct lateral ocelli, longer than wide, diamond-shaped male mirror in Trigonidiinae, and the absence of ectophallic arc, the wider femur with a large, ventro-lateral gutter and shorter tibia in Nemobiinae [14].

Genus *Nemobius* Serville, 1838 is currently represented by eight species: *N. australis* Walker, 1869 (*nomen dubium*); *N. grandis* Holdhaus, 1909; *N. interstitialis* Barranco, Gilgado & Ortuño, 2013; *N. karnyi* Chopard, 1925; *N. piracicabae* Piza Jr., 1968; *N. strigipennis* Chopard, 1928; *N. sylvestris* (Bosc, 1792) and *N. varius* (Afzelius & Brannius, 1804) (*nomen dubium*) [15,16].

The wood cricket, *Nemobius sylvestris* (Bosc, 1792) is the most widespread species within the genus, occurring in Western, Southern and Central Europe, North Africa and even North America, where it was recently introduced [17]. While the nominotypical subspecies is distributed across Europe, specimens recorded from Morocco and Algeria are considered to belong to a different subspecies, *Nemobius sylvestris tingitanus* Bolívar, 1914 [18,19]. *N. sylvestris* is a small (σ 7–10 mm, ϕ 9–12 mm), dark brown cricket, with reduced wings, preferring dry or semi-dry, warm tree habitats such as open deciduous forests, forest glades with shrubs and especially forest edges. The species was assessed as Least Concern for Europe in the IUCN Red List [20] and was not included in the Romanian Red Book of Invertebrates, due to data deficiency [21]. As a strict woodland specialist, this species exhibits strong microhabitat preferences and limited dispersal capacity, making it particularly vulnerable to habitat fragmentation [22,23]. *N. sylvestris* requires specific environmental conditions including continuous leaf litter layers, low ground vegetation cover, open understory with low canopy closure, and permanent mature forest edges [22,24]. Due to its ecological sensitivity and strong association with semi-natural woodland habitats, the species has been proposed as a useful bioindicator for assessing forest quality and the impacts of forest management practices [25].

Kis [26] mentions the species for the first time in Romania in Adâncata forest, close to Suceava, in the north-eastern part of the country. Later, Kis & Vasiliu [27] state that the species is very rare in Romania and can be encountered only in the area surrounding Suceava. After several years of intensively searching for the species in the northern and eastern forests, Iorgu et al. [28] marked *Nemobius sylvestris* as possibly extinct in Romania. In this paper, we report the discovery of *N. sylvestris* in an urban park in Bucharest, a remnant of a formerly extensive forest ecosystem.

2. Materials and Methods

2.1. Specimen Collection

The wood crickets were discovered on a warm autumn afternoon, when we heard a chorus of males singing and it was quite hard to track the individual singers. Two years later, we found the crickets thriving in the same area of Bordei park. The morphological identification of specimens followed Kis [29]. Photos were taken with Canon EOS R5 camera and Canon RF 100 mm f2.8 L IS USM macro lens (Canon Inc., Tokyo, Japan).

2.2. Acoustic Methods and Terminology

Acoustic recordings were taken with Tascam Portacapture X8 digital recorder (32-bit) and processed in Audacity 3.7. We recorded the calling song of the 14 males collected from Bucharest and compared them with data from the Xeno-canto database [30] and several published audio resources [31–34]. Table 1 contains a comprehensive list of the acoustic examined material. The bioacoustic terminology is adopted from Ragge and Reynolds [31]. We measured the syllable duration as the time elapsed from the beginning to the end of a syllable and the syllable repetition interval as the time elapsed from the beginning of a syllable to the beginning of the next syllable.

Table 1. Recorded material comprising calling songs used in the acoustic analysis.

Xeno-Canto Code	Date	Locality	Author	Temperature
XC972100	5 September 2024	Průhonice, Praha-západ, Středočeský kraj, Czech Rep.	D. Bennett	22
XC968038	15 October 2022	Corrèze, Chanteix, France	J. Barataud	17
XC968037	20 August 2022	Creuse, Royère-de-Vassivière, France	J. Barataud	16
XC968026	15 September 2021	Corrèze, Noailles, France	J. Barataud	24
XC968025	15 September 2021	Corrèze, Noailles, France	J. Barataud	24
XC968017	17 December 2021	Corrèze, Chanteix, France	J. Barataud	12
XC780020	12 February 2023	Cordes-sur-Ciel, Tarn, Occitanie, France	C. Mroczko	16
XC756713	20 August 2022	Cordes-sur-Ciel, Tarn, Occitanie, France	C. Mroczko	-
-	19 September 1976	Hautes-Pyrénées, Vielle-Aure, Neste du Couplan, France	W.J. Reynolds	17
XC926500	31 July 2024	Kreisfreie Stadt Jena (near Jena), Thuringia, Germany	Leo	24
XC867749	25 September 1980	Bayern, Erlangen, Atzelsberg, Germany	K.-G. Heller	19
XC867748	25 September 1980	Bayern, Erlangen, Atzelsberg, Germany	K.-G. Heller	19

Table 1. *Cont.*

Xeno-Canto Code	Date	Locality	Author	Temperature
XC867747	25 September 1980	Bayern, Erlangen, Atzelsberg, Germany	K.-G. Heller	19
XC867746	25 September 1980	Bayern, Erlangen, Atzelsberg, Germany	K.-G. Heller	19
XC867745	25 September 1980	Bayern, Erlangen, Atzelsberg, Germany	K.-G. Heller	19
XC867744	25 September 1980	Bayern, Erlangen, Atzelsberg, Germany	K.-G. Heller	19
-	5 August 2008	Bayern, Bayreuth, Germany	C. Roesti	22
XC823886	20 August 2023	Kreisfreie Stadt Jena, Thuringia, Germany	Leo	23
XC828125	7 September 2023	Ober-Mörlen, Darmstadt, Hessen, Germany	B. Gickel	-
XC753807	22 July 2018	Monte Pigna, Bologna, Emilia-Romagna, Italy	C. Brizio	-
XC751419	1 October 2001	Appennino Tosco Emiliano (LU), Passo Radici, Italy	B. Odé	22
XC849167	6 October 2020	Plasmolen, The Netherlands	B. Odé	13
XC970387	8 September 1998	Ede, Lunteren, MeuLunteren, The Netherlands	P. Fontana	24.5
XC752034	28 August 1992	N.P.Veluwezoo, Zijpenberg, The Netherlands	B. Odé	-
XC752031	28 August 1992	N.P.Veluwezoo, Zijpenberg, The Netherlands	B. Odé	-
XC751474	27 July 1993	Bergherbos, The Netherlands	B. Odé	24.8
XC751473	21 July 1993	Bergherbos, The Netherlands	B. Odé	21
XC751471	21 July 1993	Bergherbos, The Netherlands	B. Odé	20.5
XC751469	21 July 1993	Bergherbos, The Netherlands	B. Odé	19.8
XC942520	23 October 2024	Xinzo, Brión, A Coruña, Galicia, Spain	J.R. Millarengo	19
XC927985	5 August 2024	Alvariza, Asturias, Principado de Asturias, Spain	T. Gris	-
-	24 October 2022	Bordei park, Bucharest, Romania (3 ♂♂)	I.Ş. Iorgu	22
-	11 October 2024	Bordei park, Bucharest, Romania (4 ♂♂)	I.Ş. Iorgu	22
-	17 September 2024	Bordei park, Bucharest, Romania (2 ♂♂)	I.Ş. Iorgu	22

2.3. Material Examined

Three ♂♂ and six ♀♀, Bordei Park, Bucharest, Romania, 44.47° N 26.09° E, 24 October 2022, leg. I.Ş. Iorgu, deposited in the collections of the “Grigore Antipa” National Museum of Natural History, Bucharest; 4 ♂♂, Bordei Park, Bucharest, Romania, 44.47° N 26.09° E, 17 September 2024, leg. I.Ş. Iorgu, deposited in the personal collection of E.I. Iorgu, Suceava;

7 ♂♂ and 2 ♀♀, Bordei Park, Bucharest, Romania, 44.47° N 26.09° E, 11 October 2024, leg. I.Ș. Iorgu, deposited in the personal collection of E.I. Iorgu, Suceava.

2.4. Statistical Analysis

All statistical analyses were performed using R v.4.4.2 and RStudio v.2024.12.1 [35]. Data cleaning and manipulation were conducted using the dplyr package. Prior to analysis, the dataset was filtered to include only records with complete observations for median syllable length (MEDL), median repetition interval (MEDR), and geographic origin (Country). To assess regional variations in bioacoustic parameters, we employed the Kruskal–Wallis H test, a non-parametric alternative to one-way ANOVA, as it is robust to unequal sample sizes and potential non-normality in acoustic data. This test was used to compare both MEDL and MEDR across all recorded countries. The relationship between syllable length and repetition rate was evaluated using Spearman’s correlation coefficient (r). A linear regression model was fitted to the data to visualize the overall trend across the entire sample set. For data visualization, boxplots were generated with individual data points overlaid as jittered points to show the full distribution. Romania was highlighted using a distinct color palette to emphasize its unique acoustic signature. All figures were produced using the ggplot2 and ggpubr packages and exported as SVG. Statistical significance was defined at $p < 0.05$.

2.5. Genetic Sampling and Sequence Analysis

The DNA barcoding technique was used on two samples in order to confirm the morphological identification of the individuals. DNA barcoding is a method of molecular species identification using a short fragment from a specific gene that can be used to uniquely identify an organism to the species level through comparison with a reference library. For this, total genomic DNA was extracted from one hind leg using Genomic II DNA kit (Bioline, London, UK), as per manufacturers’ instructions. A fragment of the cytochrome oxidase subunit I was amplified (COI—GenBank reference number OQ352853), following the protocols of Hebert et al. [36]. The obtained sequences were edited using CodonCode Aligner 3.7.1 (CodonCode Corporation, Centerville, MA, USA) and then compared with other sequences available in BOLD databases [37] (Table 2). Together with seven other available sequences, we constructed a haplotype network, using a median-joining algorithm [38] implemented in PopArt v.1.7 [39]. Genetic variability indices were calculated using DnaSP v.5.10 [40].

Table 2. Geographic locations, BOLD sequence id-s and COI haplotypes of the samples used in the network analysis.

Crt. No.	Location	Latitude	Longitude	Bold Sequence ID	Haplotype
1.	Bavaria, Unterfranken, Germany	50.4831	10.1152	FBORT161-09	H1
2.	Bavaria, Unterfranken, Germany	50.5323	10.1437	FBORT179-09	H1
3.	Bavaria, Unterfranken, Germany	50.5323	10.1437	FBORT185-09	H1
4.	Portocamino, Spain	42.932	−8.816	GBMIX3139-16	H2
5.	Mauvron, France	47.12	3.242	GBMIX3166-16	H3
6.	Vienna, Austria	48.2436	16.2908	TDAOE290-21	H1
7.	Geneva, Switzerland	46.185	6.077	UGEAA034-16	H4
8.	Bucharest, Romania	44.4715	26.0921	OQ352853	H3

3. Results

3.1. DNA Barcoding and Genetic Diversity

Two 521 bp sequences of the COI marker were obtained from the individuals of Bordei Park. Seven other COI sequences of *Nemobius* from Europe were downloaded from the BOLD database (Table 2). From the total of nine *Nemobius sylvestris* sequences analyzed, we obtained four haplotypes (H1–H4), which grouped in a network with a star-like topology, with H3 as a central haplotype, at only one substitution apart from the other three haplotypes (Figure 1). The two sequences from Romania grouped in the same haplotype (H3), together with an *N. sylvestris* sequence from France (BOLD Id—GBMIX3166-16). We obtained moderate haplotype and nucleotide diversities, with values of $H_d = 0.750 \pm 0.112$ and $P_i = 0.00192 \pm 0.00142$, respectively. Unfortunately, the lack of other sequences from *N. sylvestris*, as well as other *Nemobius* species, does not allow us to draw any other conclusions regarding the systematics of the species.

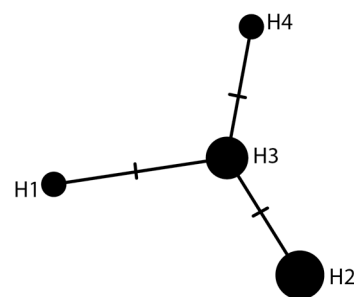


Figure 1. Median joining haplotype network of mitochondrial cytochrome oxidase subunit I (COI) haplotypes of *N. sylvestris* sequences available in the Bold database (see Table 2).

3.2. Bioacoustic Analysis

The wood cricket males stridulate during the day and at night, and typically a chorus of singers produces a continuous churring sound, with the calling song of each specimen being fragmented up into constantly repeated in short echemes [31]. The stridulation consists of a series of echemes or groups of 5–20 syllables, with a syllable repetition rate of 29–37/s. At an ambient temperature of 22 °C, each syllable lasts for about 8–16 ms (mean = 12.37 ± 1.63). Spectral analysis of the Romanian population shows that the carrier wave frequency consists of two loud dominant (at approximately 3–5.5 kHz and 11–13 kHz) and three quiet harmonics (at approximately 7–9 kHz, 15–17 kHz and 20–22 kHz), with the highest peak at about 4 kHz (Figure 2).

Analysis of syllable length across European populations revealed significant geographic variation (Figure 3; Table 3). Syllable length differed significantly among populations (Kruskal–Wallis: $\chi^2 = 28.6$, $df = 6$, $p < 0.05$; Figure 3). The Romanian population produced the longest syllables (mean 12.37 ± 1.63 ms), at approximately twice the duration observed in Western European populations. Specifically, crickets in Germany produced syllables with a mean length of 5.7 ± 0.92 – 6.3 ± 0.98 ms, and individuals in the populations in Netherlands produced syllables with a mean of 5 ± 0.86 – 6.65 ± 0.75 ms. The Italian (mean 5.8 ± 0.7 – 7.7 ± 1.08 ms), Spanish (mean 6.65 ± 0.67 – 7.2 ± 0.7 ms) and French (mean 6 ± 0.75 – 6.78 ± 0.87 ms) populations of *Nemobius sylvestris* showed intermediate values, while the Czech Republic exhibited a mean syllable length of 8.2 ± 0.89 ms.

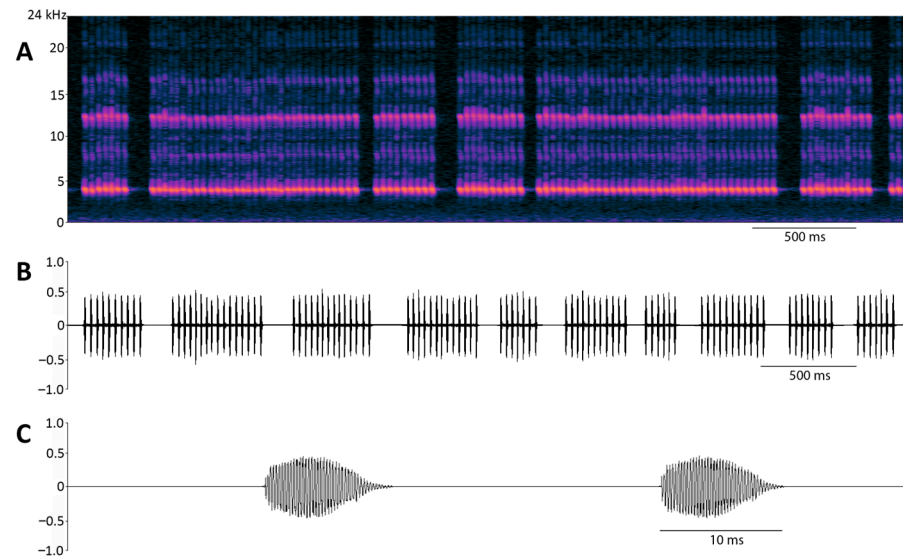


Figure 2. Graphic representation of *N. sylvestris* male calling song: (A) spectrogram; (B) oscillogram; (C) magnified oscillogram. Air temperature 22 °C. Bucharest, 24 October 2022.

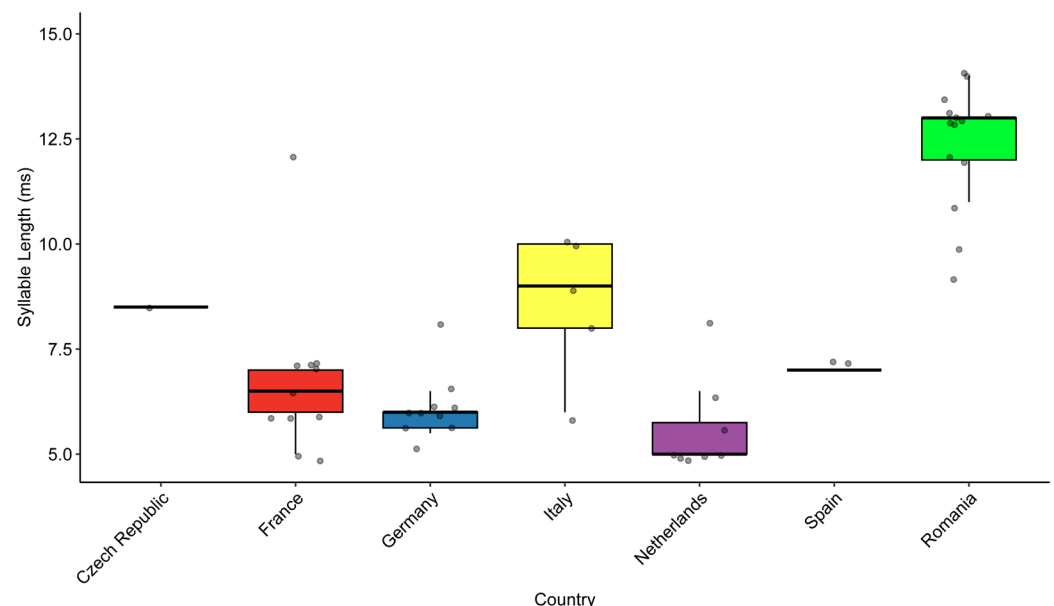
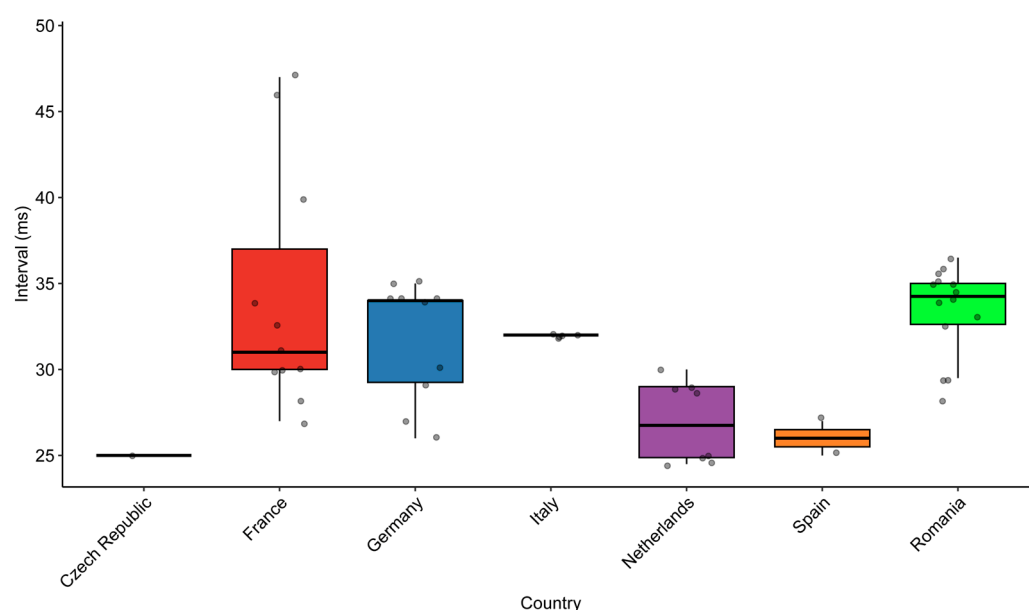


Figure 3. Geographic variation in the syllable length of *N. sylvestris* calling songs in Europe ($p = 2.2 \times 10^{-6}$).

Syllable repetition interval also varied significantly across populations (Kruskal–Wallis: $\chi^2 = 18.4$, $df = 6$, $p = 0.0021$; Figure 4; Table 3), though with less pronounced differentiation than the syllable length. The Romanian population showed moderate-to-high repetition intervals (mean = 33.49 ± 1.79 ms), similar to the German populations (mean 26.2 ± 1.2 – 34.15 ± 1.17 ms), and significantly different from the populations in the Netherlands (mean 25.1 ± 0.97 – 29.85 ± 2.66 ms) and in Spain (mean 25.15 ± 0.75 – 27.3 ± 1.34 ms), which exhibited faster repetition rates. In contrast, individuals from France displayed the widest range of variation (mean 29.23 ± 1.37 – 46.65 ± 1.27 ms); while their group median was intermediate, the population included notable outliers, with the longest intervals observed in the study (>46 ms). Wood crickets from Italy (mean 32.5 ± 1.64 – 31.65 ± 0.99 ms) displayed intermediate syllable repetition interval values while the crickets in Czech Republic produced the shortest intervals (mean 25.85 ± 3.05 ms).

Table 3. Descriptive statistics for oscillographic parameters of *N. sylvestris* calling song (n = number of measured elements).

Locality	Syllable Length (ms)				Syllable Repetition Interval (ms)			
	Min	Max	Mean $\pm$ SD	n	Min	Max	Mean $\pm$ SD	n
Bucharest, Romania (9♂♂)	8	16	12.37 $\pm$ 1.63	280	25	39	33.49 $\pm$ 1.79	280
Průhonice, Czech Republic (1♂)	7	9	8.2 $\pm$ 0.89	20	23	33	25.85 $\pm$ 3.05	20
Creuse, France (1♂)	5	8	6.65 $\pm$ 0.75	20	45	49	46.65 $\pm$ 1.27	20
Corrèze, Noailles, France (2♂♂)	4	8	6.33 $\pm$ 0.91	40	27	32	29.9 $\pm$ 1.2	40
Corrèze, Chanteix, France (2♂♂)	4	7	6 $\pm$ 0.75	40	30	43	36.98 $\pm$ 1.5	40
Cordes-sur-Ciel, France (2♂♂)	5	8	6.78 $\pm$ 0.87	40	26	34	29.23 $\pm$ 1.37	40
Hautes-Pyrénées, Vielle-Aure, France (1♂)	4	6	5.35 $\pm$ 0.67	20	27	31	29.75 $\pm$ 0.97	20
Bayern, Erlangen, Germany (1♂)	4	9	5.75 $\pm$ 0.94	120	31	38	34.15 $\pm$ 1.17	120
Bayern, Bayreuth, Germany (1♂)	7	10	8.3 $\pm$ 0.86	20	28	30	29.2 $\pm$ 0.7	20
Kreisfreie Stadt Jena, Germany (1♂)	4	8	5.7 $\pm$ 0.92	20	24	29	26.8 $\pm$ 1.32	20
Ober-Mörlen, Germany (1♂)	5	7	5.85 $\pm$ 0.67	20	28	33	29.7 $\pm$ 1.34	20
Jena, Germany (1♂)	4	8	6.3 $\pm$ 0.98	20	24	28	26.2 $\pm$ 1.2	20
Monte Pigna, Italy (1♂)	5	7	5.8 $\pm$ 0.7	20	29	35	32.5 $\pm$ 1.64	20
Appennino Tosco Emiliano, Italy (1♂)	6	9	7.7 $\pm$ 1.08	20	29	34	31.65 $\pm$ 0.99	20
Veluwezoom, The Netherlands (2♂♂)	4	7	5.48 $\pm$ 0.64	40	27	31	28.78 $\pm$ 0.93	40
Bergherbos, The Netherlands (4♂♂)	4	9	5.99 $\pm$ 0.89	80	22	30	25.58 $\pm$ 1.25	80
Ede, The Netherlands (1♂)	6	8	6.65 $\pm$ 0.75	20	24	27	25.1 $\pm$ 0.97	20
Plasmolen, The Netherlands (1♂)	4	6	5 $\pm$ 0.86	20	25	34	29.85 $\pm$ 2.66	20
Xinzo, Spain (1♂)	6	8	7.2 $\pm$ 0.7	20	24	30	27.3 $\pm$ 1.34	20
Albariza, Spain (1♂)	5	8	6.65 $\pm$ 0.67	20	24	26	25.15 $\pm$ 0.75	20

**Figure 4.** Geographic variation in the syllable repetition interval of *N. sylvestris* in Europe ($p = 0.0015$).

A significant positive correlation was observed between median syllable length and median repetition interval across all populations ($r = 0.37$, $p = 0.0076$; Figure 5), indicating that populations producing longer syllables tended to have somewhat longer intervals between syllables. The Romanian population occupied a distinctive position in the bivariate acoustic space, characterized by both long syllables (10–13 ms) and moderate-to-long repetition intervals (30–37 ms). In contrast, crickets in Western European populations (Germany, Netherlands, France and Spain) formed a relatively tight cluster, with shorter syllables (5–8 ms) and variable repetition rates (25–35 ms). The Italian populations showed intermediate characteristics, partially bridging the gap between Western European and Romanian populations, while the crickets recorded in Czech Republic exhibited moderate syllable lengths with the shortest repetition intervals.

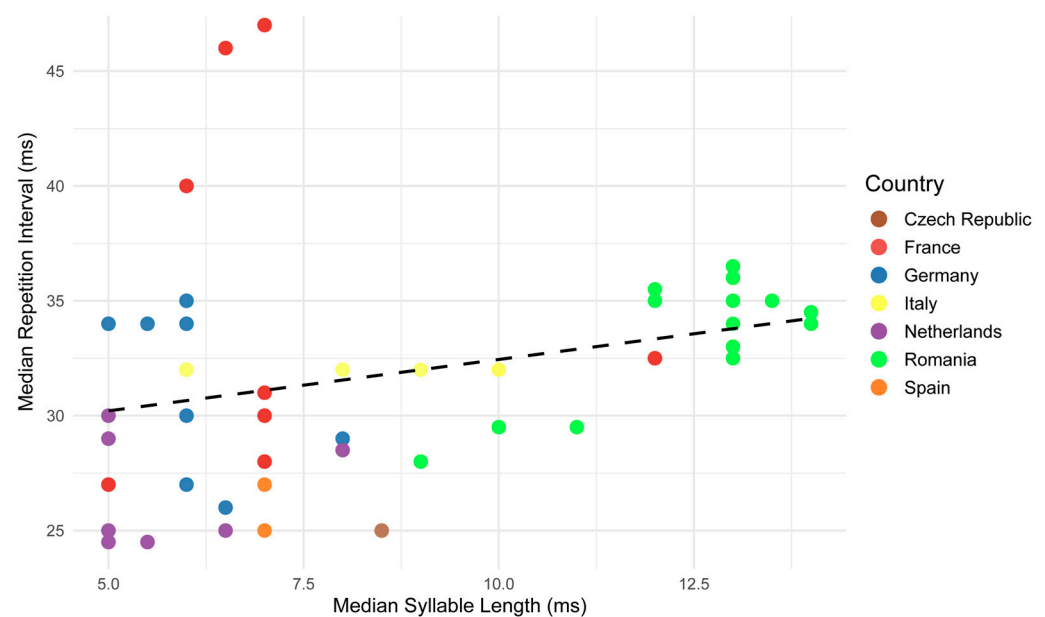


Figure 5. The correlation between median syllable length and median repetition rate in the calling song of *N. sylvestris* in the studied populations across Europe ($r = 0.37$, $p = 0.0076$). The dashed line represents a linear regression line illustrating the positive monotonic trend between syllable length and repetition rate.

4. Discussion

Parks that represent remnants of original ecosystems may harbor relict populations of specialist species that have been extirpated from the surrounding landscape, populations that can serve as a source material for future restoration efforts and maintain genetic diversity that may be lost elsewhere [41]. Unlike rural forest fragments embedded in intensive agricultural landscapes, urban parks are often protected from the pesticide exposure and nutrient enrichment that threaten many invertebrate populations [42,43]. The formal designation as a park provides a degree of legal protection against conversion to other land uses, offering stability that may be lacking in rural areas subject to changing agricultural or forestry economics [44]. However, urban parks also face unique challenges including recreational pressure, edge effects from surrounding urban infrastructure, invasive species, altered microclimates, and management practices oriented toward aesthetics rather than biodiversity [45,46].

Nemobius sylvestris is widely distributed in the western and central parts of Europe; however, comprehensive studies on the species' stridulation are scarce [31,34]. Our study reveals substantial geographic variation in the acoustic signals of *N. sylvestris* across its European range, with the Romanian population exhibiting the most distinctive acoustic

characteristics. The highly significant differences in syllable length ($p < 0.05$) and significant variation in repetition interval ($p = 0.0021$) among populations demonstrate that acoustic parameters are not uniform across the species' distribution, suggesting population-specific divergence in calling song structure. The population studied in Romania stands out as a clear acoustic outlier, producing syllables approximately twice as long as those from Western European populations (Germany, Netherlands, Spain). The maintenance of the general positive correlation between syllable length and repetition interval ($r = 0.37$, $p = 0.0076$) across all populations, including Romania, indicates that biomechanical constraints on sound production are conserved. The divergence in syllable length is remarkably large and highly unlikely to be an artifact of thermal differences alone. While cooler temperatures can slightly extend the syllable duration, the differences between Western European populations and the crickets from Romania indicate an intrinsic morphological or neurological divergence rather than thermal plasticity. The magnitude of this syllable length discrepancy provides robust preliminary evidence of a distinct acoustic phenotype in Bucharest, justifying future bioacoustic investigations.

The position of the population from Romania in the bivariate acoustic space supports the concept of an acoustic "dialect" within *N. sylvestris* (Figure 3). Cricket populations at the margins of their geographic distribution often produce unusual songs: aberrant acoustic behavior has variable effects on reproductive success and population viability, ranging from maintained or enhanced mating success to potential reproductive isolation. The oceanic field crickets *Teleogryllus oceanicus* (Le Guillou, 1841) show significant among-site differences in multiple calling song traits across Australia and Oceania, island populations that produce greater variances in song, with especially high variability in Hawaii [47]. Similarly, marginal populations of *Gryllus bimaculatus* De Geer, 1773 in northern Europe exhibit calling-behavior differences linked to the colder climates at the distribution edge [48]. Chirp and pulse differences between *G. bimaculatus* populations persist in laboratory rearing, indicating genetic differentiation rather than plastic environmental responses [49]. In *Dianemobius nigrofasciatus* (Matsumura, 1904), the sound produced in urban versus rural populations differs in dominant frequency and chirp structure, showing environment-dependent divergence in calling song and female behavior [50].

The Median-Joining network exhibits a distinct star-like topology, usually associated with recent demographic expansions from a small effective population size or a single ancestral lineage (Figure 1). The network is dominated by a central haplotype (H3) from which other variants radiate, separated by one mutational step. This weak genetic divergence indicates that the sampled European populations share a relatively recent common ancestor, despite the geographic distances involved. Unusually for a flightless insect, this single haplotype is shared between individuals from France (Mauvron) and Romania (Bucharest). The lack of isolation by distance suggests that the Romanian population may be the result of a swift recent recolonization or of anthropogenic transport and both populations could have the same origin. However, the number of samples analyzed is too small to draw a definite conclusion. Also, only one mitochondrial marker was analyzed and although COI is a standard marker for delimiting geographically structured lineages and potential cryptic species [36,51,52], in shallow phylogenies the population structure should be combined with morphology or more mitochondrial and nuclear loci [53–56]. The network supports a scenario where the presence of *N. sylvestris* in these regions is a result of human activity, rather than representing deeply divergent cryptic lineages.

The discovery of *Nemobius sylvestris* in Bucharest contributes to understanding the city's urban ecology and highlights the conservation potential of its remaining green spaces. Bucharest has lost substantial natural habitat over recent decades, with green space coverage declining dramatically during periods of rapid urban expansion [8–10].

The population documented in our study represents an important addition to Bucharest's known urban biodiversity and underscores the value of the city's forest-derived parks as refugia for woodland specialists. Given the species' limited dispersal capacity and strong habitat requirements, this population is likely to represent one of the few remaining urban occurrences in the region. *Nemobius sylvestris* needs old and relatively large deciduous forests dominated by *Quercus* species to develop and breed. It was proved that the species, being ground-dwelling, needs a well-developed leaf litter layer and a large range of forest edge habitat where it can find both shelter and food [22]. In Romania, as in most of Europe, oak-based broad-leaved forests are a fragmented habitat, formed of series of "patches" of different sizes located at various distances from each other. In a study about the mobility of *N. sylvestris*, Brouwers and Newton [57] showed that the species has a low mobility of about 18 cm/min/adult individual and a low power of colonization of potential new suitable habitats that are more than 50 m further away from the source population.

Bordei park is part of the large chain of lakes and the forest belt in northern Bucharest, a remnant of a large centuries-old, deciduous forest, called Vlăsiei Woods, which remained prime until the beginning of the 20th century, when the city expanded northwards. The habitat where the wood cricket was found is a layer consisting of various herbaceous plants, the ivy *Hedera helix* and the leaf-litter of *Betula pendula*, *Fraxinus* sp. and *Platanus* sp. Recently, the privately owned forests surrounding Bucharest were subject to increasing anthropogenic degradation, under pressure by exponential residential development and abusive cutting and logging, altogether with the adverse management of forest regeneration. Following this, the parks from the northern string of lakes diminished and the wooden habitat was constantly fragmented, posing a major threat to species with very limited dispersal ability, such as *Nemobius sylvestris* [25]. Furthermore, the species may also be affected by the maintenance of herb layers, the collection of leaf-litter in autumn and the regular use of pesticides against mosquitos or ticks.

Our findings contribute to growing evidence that urban parks, particularly those derived from historical forest fragments, can serve as critical refugia for native biodiversity [4,58,59]. The conservation value of these urban green spaces extends well beyond their aesthetic and recreational functions, with essential roles in maintaining regional biodiversity, preserving genetic resources, and sustaining ecosystem processes [6]. The use of orthopterans as bioindicators of habitat quality in urban parks is well-supported by their ecological characteristics; these insects respond rapidly to vegetation structure, microhabitat continuity, and land-use intensity, making them sensitive indicators of environmental conditions. Therein lies the importance of protecting the last semi-wild areas in cities, as the construction of walkways and recreational sites in the parks has reduced or strongly degraded the habitats of many indigenous insect species.

Author Contributions: Conceptualization, I.-Ș.I., I.T. and E.I.I.; methodology, I.-Ș.I., I.T., C.-R.O., A.-F.P. and E.I.I.; formal analysis, I.-Ș.I., I.T. and E.I.I.; writing—original draft preparation, I.-Ș.I.; writing—review and editing, I.-Ș.I., I.T. and E.I.I.; funding acquisition, I.T. All authors have read and agreed to the published version of the manuscript.

Funding: I.T. was supported by Lucian Blaga University of Sibiu research grants LBUS-IRG-2023, no. 3567/24.07.2023.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors are grateful to Baudewijn Odé for his recommendation on using Xeno-canto database.

Conflicts of Interest: The authors declare no conflicts of interest.

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