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Widespread Distribution of *Trypodendron laeve* in the Carpathian Mountains (Romania)

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Abstract: *Trypodendron laeve* Eggers, 1939 is a species of ambrosia beetle much less known than the other three *Trypodendron* species occurring in Europe. Its status (native or alien) in Central Europe has been a subject of debate over the past two decades. In Romania, the species was discovered in 2008 and the aim of the research presented in this paper was to investigate its distribution in the Carpathians, mainly at high altitudes (>800 m), in tree stands with Norway spruce (*Picea abies* [L.] H. Karst). Panel intercept traps baited with synthetic pheromone for *Trypodendron lineatum* (Olivier, 1795) were used in the spring of 2015, at 31 locations. Adults of *T. laeve* were caught in 20 of them. Additional observations were made within some studies using similar baits and *T. laeve* specimens were caught in eight locations. *T. laeve* was always trapped together with *T. lineatum*, and at some locations also together with *T. domesticum* (Linnaeus, 1758) and *T. signatum* (Fabricius, 1787). In all traps, fewer specimens of *T. laeve* were caught compared to *T. lineatum*. The species has a widespread distribution in the mountain regions, within forests composed of native tree species and generally located far away from commercial routes. There, it occurs together with other native species of the same taxonomic genus. It seems to be more abundant at high altitudes, but overall its populations are less abundant than those of *T. lineatum*.

Keywords: *Trypodendron laeve*; Carpathian Mountains; Romania; distribution

1. Introduction

The species of the genus *Trypodendron* Stephens, 1830 are ambrosia beetles, which make breeding galleries in wood, but feed on symbiotic fungi [1]. They infest the sapwood of weak, dying, and newly dead trees, logs, and stumps [2]. The beetles locate the suitable hosts using the ethanol released from wood undergoing anaerobic fermentation as a guiding cue [3]. However, they are able to distinguish broadleaved species from conifers. *Trypodendron* species living on conifers use the monoterpenes, mainly α-pinene, to recognize their hosts, the ethanol and α-pinene acting synergistically in attracting the beetles [4–6]. Then, the beetles aggregate by means of aggregation pheromones [7,8]. In fact, conifer living species use lineatin along with the two host volatiles as a very effective aggregation signal [9,10], while the adults of *T. domesticum* appear to be repelled by terpenes [5,11]. The tunnels excavated by insects and fungal staining can cause important economic loss through the degradation of the infested logs. In addition, some ambrosia beetle species attack apparently healthy trees [12] and consequently are important economic pests [13].

According to the latest reference work on bark beetles, the Holarctic region contains 13 species of *Trypodendron* [14], with four in Europe including *T. domesticum* (Linnaeus, 1758), *T. signatum* (Fabricius, 1787), *T. lineatum* (Olivier, 1795), and *T. laeve* Eggers, 1939 [15]. The first two species occur on broadleaved trees, with the second one seeming to prefer the oaks (*Quercus* spp.) [2]. In the Palaearctic region, *T. lineatum* was found on many species of *Picea* A. Dietr., 1824, *Pinus* L. 1753, *Abies* Mill. 1754, *Larix* Mill. 1754, and *Cedrus* Trew 1757 [16], while in North America, it is also occasionally found attacking species of *Alnus* Mill., *Betula* L. 1753, *Acer* L. 1753, and *Malus* Mill. 1754 [13,17]. The host tree species of *T. laeve* are less known. It was found in logs or dead trees of *Picea abies* (L.) H. Karst. 1881 [18–20] and *Pinus sylvestris* L. 1753 [20,21]. *Picea obovata* Ledeb. 1833 and *P. jezoensis* (Siebold & Zucc.) Carr. are also between its hosts [16].

While *T. lineatum* and the European species associated with broadleaved trees are quite well studied, *T. laeve* is less known, because its taxonomic status was only recently clarified. It was firstly described in Japan [22] and secondly (a few years later) in Norway, under the name *Trypodendron piceum* A. Strand, 1946 [18]. However, during the next four decades, the species was not reported in other parts of Europe. The standard taxonomic literature and identification keys used during that time, such as Balachowsky [23], Stark [24], Nunberg [25], and Pfeffer [26], did not include *T. laeve* or *T. piceum*. Consequently, *T. laeve* was "forgotten" or overlooked, particularly by applied entomologists. Later, Grüne [27] did not refer to *T. laeve* or *T. piceum* at all in the illustrated key of the European scolytids, and Schedl [28] regarded both names as synonyms for *T. lineatum*. Even Annila et al. [29] did not distinguish between *T. piceum* and *T. lineatum* [30], although it was known that the species was present in the Nordic countries.

Eventually, Holzschuh [19], comparing some specimens captured in 1982–1983 that looked different from those of *T. lineatum*, with specimens from the Schedl's collection of scolytids, identified those specimens as belonging to *T. laeve* and drew attention to the fact that they differ from *T. lineatum* both in the conformation of male genitalia and in the femur colour, suggesting the treatment of *T. laeve* as a distinct species. Pfeffer [31] mistakenly synonymised *T. piceum* Strand with *T. proximum* (Niijima, 1909), but it was corrected in Pfeffer [16,32], where he presents *T. laeve* as a separate species and the name *T. piceum* as a synonym for *T. laeve*, as Wood [33] suggested. Due to the original mistake in Pfeffer [31], *T. laeve* (=*T. piceum*) was mentioned by Martikainen [20] under the name *T. proximum*, but Mandelshtam and Popovichev [34] demonstrated that they are different species. This clarifies the taxonomic status of the *T. laeve* species in Europe, but not its origin.

In the 1980s, in Austria, various species of Siberian insects were reported, and Holzschuh [19] assumed that *T. laeve* is also an alien species that came to Europe with imported wood from Russia. The idea was reiterated in later works [35–37] and some authors mentioned this species as an invasive one in Europe [38,39]. However, the information gathered until the year 2000 about the distribution of the species in Fennoscandia led Martikainen [20] to reconsider the status of the species. Consequently, Kenis [38] regarded the species to be native to Scandinavia but alien to central Europe, where its distribution was less known. Apart from the above-mentioned reports from Austria, there were few reports from Germany [40] and the Czech Republic [41,42].

Currently, the species is also reported as being present in Estonia, Finland, Latvia, Norway, Poland, Slovakia, Sweden, Switzerland, the Central European Territory and North European Territory of Russia, China, and Japan [15], as well as from Romania [43] and the Russian Far East [44]. However, while the species distribution in the Fennoscandia is well documented [20,21,30,45–51], in Central and Eastern Europe, the knowledge is quite poor, although some studies have been done [43,52,53]. Consequently, the objective of the research presented in this paper was to determine the species distribution in Romania.

2. Materials and Methods

2.1. Field Research

To determine the species distribution of *T. laeve* in Romania, in the spring of 2015, with the help of field forestry personnel, 78 pheromone traps were placed in 31 locations situated along the Carpathian Mountains chain (Table 1).

Table 1. Location of study sites and tree stand characteristics where pheromone traps were set up.

No.	Location, County	Forest District, Production Unit, Compartment	Coordinates N/E	Elevation (m)/Aspect	Forest Composition (%)	Forest Age (Years)
Trap locations for detection of *Trypodendon laeve* in 2015						
1.	Vișeul de Sus, Maramureș	Vișeu, VI Mira, 9C	47.7227333 24.7672555	1520 NW	100 Pa	160
2.	Paltinu, Suceava	Vama, II Paltinu, 87A	47.6236667 25.4564722	1180–1250 E	100 Pa	80
3.	Cacica, Suceava	Solca, II Cacica, 9B	47.6322889 25.9207638	460 E	40 Aa 40 Pa 20 Fs	120
4.	Cârlibaba, Suceava	Cârlibaba, VI Cârlibaba, 129A	47.5716028 25.1482083	1100 NW	100 Pa	80
5.	Lunca Ilvei, Bistrița-Năsăud	Lunca Ilvei, I Lunca Ilvei, 150	47.3729028 25.0417055	1070 SW	60 Pa 30 Fs 10 Aa	120
6.	Almaș, Neamț	Gârcina, III Almaș, 28B	47.0176806 26.3233083	650 NE	40 Fs 20 Aa 20 Pa 20 Ap	55
7.	Stânceni, Mureș	Lunca Bradului, Trupul Gudea, 132B	46.864583 25.245783	1144 W	80 Pa 10 Aa 10 Fs	115
8.	Brateș_3, Neamț	Tarcău, VII Ața, 54A	46.7954444 26.0342027	983 NW	60 Pa 30 Aa 10 Fs	150
9.	Brateș_1, Neamț	Tarcău, V Bolovăniș, 179A	46.7748000 26.1507416	580 NE	60 Pa 30 Aa 10 Dt	80
10.	Brateș_2, Neamț	Tarcău, VI Brateș, 179A	46.7554583 26.0735944	818 NW	70 Pa 30 Aa	110
11.	Dărmănești, Bacău	Dărmănești, III Dărmănești, 79B	46.3614333 26.3454722	1020 E	50 Fs 30 Pa 20 Dt	100
12.	Sălătruc, Bacău	Lignum, UBII Lapos, 101, 102	46.351275 26.398533	820 E	50 Fs 30 Aa 20 Pa	100
13.	Poiana Uzul, Bacău	Dărmănești, III Bărzăuța, 54A	46.2393833 26.3076777	1160 N	90 Pa 10 Dr	100
14.	Soveja, Vrancea	Soveja, II Soveja, 64C	45.9973083 26.6106000	810 SE	80 Pa 20 Aa	75
15.	Covasna, Covasna	Comandău, IV Obârșia Bâscii, 99B,C	45.8171028 26.3339861	1340 N	100 Pa	30–130
16.	Brădăcești, Vrancea	Zăbala-Nereju, I Bârsești, 124	45.7158333 26.6588888	1060	60 Fs 40 Pa	110
17.	Predeal, Brașov	RPLP Kronstadt, III Postăvaru, 133A	45.5023472 25.6298277	1345 N	100 Pa	110
18.	Poiana Brașov, Brașov	RPLP Kronstadt, VI Tâmpa, 38A	45.5970556 25.5668472	1096 NW	40 Pa 30 Aa 30 Ld	110
19.	Moroeni, Dâmbovița	A.O.S. Carpathia, III Raciu, 56C	45.2785944 25.3500000	1200 NE	100 Pa	80
20.	Bădeni, Argeș	A.O.S. Carpathia, VII Bădeanca, 100A	45.3062000 25.2803972	1420 SW	90 Pa 10 Fs	90
21.	Căpățâneni, Argeș	Vidraru, VI Limpedea, 42B, 43B	45.3556778 24.6877555	1520 S/SW	100 Pa	125
22.	Cârțișoara, Sibiu	Arpaș, V Bâlea, 14H	45.6500000 24.6119444	1503 W	100 Pa	100
23.	Paltin, Sibiu	Sibiu, I Dealul Paltinului, 34G	45.5313889 24.1694444	1494 NE	100 Pa	120
24.	Bistra, Sibiu	Miercurea Sibiului, III Miercurea Sibiului, 164B	45.6716667 23.6638888	1490 N	100 Pa	100

Table 1. *Cont.*

No.	Location, County	Forest District, Production Unit, Compartment	Coordinates N/E	Elevation (m)/Aspect	Forest Composition (%)	Forest Age (Years)
25.	Jieț, Hunedoara	Petroșani, V Jieț, 62A	45.4090278 23.5594361	1205 SE	60 Fs 40 Pa	150
26.	Cugir, Alba	Cugir, Valea Bosorog-Parva, 130A,B	45.6228889 23.4930194	1400 E/SE	100 Pa	95
27.	Poiana Mărului, Caraș-Severin	Oțelu Roșu, VI Obârșia Bistrei Mărului, 66A/66B/69B	45.3493333 22.6219722	1215 NW/NE/N	90 Pa 10 Aa/100 Pa/100 Pa	90/130/130
28.	Rusca Montană, Caraș-Severin	Rusca Montană, V Rusca Montană, 138A, 139	45.6071111 22.4974722	802 N/N	70 Pa 30 Fs 60 Pa40Fs	110 110
29.	Padiș_2, Bihor	Beliș, II Ponor, 69A	46.6338028 22.7409138	1135 N	100 Pa	115
30.	Doda Pilii, Cluj	Beliș, II Ponor, 143A	46.657875 22.7680111	1280 SE	100 Pa	160
31.	Padiș_1, Bihor	Beliș, II Ponor, 128A	46.650975 22.7502583	1440 NE	70 Pa 30 Fs	110
Trap location for other studies with synthetic pheromone of *Trypodendron lineatum*						
32.	Bobeica, Suceava	Cârlibaba, VII Buhăiescu, 49I	47.7132194 25.0763333	1200 S	100 Pa	115
33.	Putna_1, Suceava	Putna, II Putnișoara, 105A	47.827604 25.607495	650 SE	70 Pa 10 Aa 20 Fs	110
34.	Putna_2, Suceava	Putna, II Putnișoara, 121A	47.799167 25.603056	750 E	40 Pa 20 Aa 30 Fs 10 Ap	90
35.	Putna_3, Suceava	Putna, II Putnișoara, 131A	47.792221 25.611334	850 V	40 Pa 20 Aa 40 Fs	45
36.	Demacușa, Suceava	Tomnatic, I Demacușa, 50G	47.6701575 25.4389286	890–1000 NE	60 Pa 20 Aa 20 Fs	80
37.	Ciumârna, Suceava	Vama, III Dragoșa, 344A	47.6943417 25.5870722	800 SE	60 Pa 20 Aa 20 Fs	110
38.	Iacobeni, Suceava	Iacobeni, U.P. VI Botoş—Orata, 5A	47.4114722 25.3118611	835–1115 W	100 Pa	100
39.	Borca, Neamț	Borca, II Borca, 73A	47.1216389 25.7174388	1010 N	100 Pa	40

Aa—*Abies alba*, Ap—*Acer pseudoplatanus*, Fs—*Fagus sylvatica*, Ld—*Larix decidua*, Pa—*Picea abies*, Dr—Other conifers, Dt—Other broadleaved trees.

Trapping locations were chosen to be—generally—at altitudes above 800 m, within pure Norway spruce (*Picea abies*) stands or mixed stands of spruce and other native species, mainly silver fir (*Abies alba*), European beech (*Fagus sylvatica* L.), sycamore (*Acer pseudoplatanus* L.), and European larch (*Larix decidua*), most of them aged over 75–80 years. The selected sites were tree stands located at 50–100 m from the places where harvested logs were stored in 2014 before transport to a mill or nearby in one-year old clear-cut areas, but far from the main rail or car transport routes and woodworking factories.

At each location, three traps were placed in general, but at some points, there were only one to two traps (Table 2). The pheromone traps were of the Intercept® type. They were set up within the stand, at 10–15 m from the stand edge and at a distance of 50–100 m from each other.

The traps were primed with pheromone lures whose composition was optimized to attract beetles of *T. lineatum*, like in other studies [19,20,35,51,52], because there are no commercial products designed to attract *T. laeve*. The lures contained diluted lineatin in methylbutenol, ethanol, and alpha-pinene. This mixture diffused through a polyethylene film at a rate of 30 mg/day at 20 °C. The pheromone lures and traps were provided by the Research Institute in Chemistry "Raluca Ripan" Cluj-Napoca.

Table 2. Captures of *Trypodendon* species at the 31 sites monitored in 2015.

Site No.	Location	No. of Traps	Monitored Period	Start of Flight	No. Flight Days Lost	$T_{max.aver}$	Total Number of *Trypodendron* Beetles per Study Site				Ratio *T. laeve*/*T. lineatum*
							laeve	*lineatum*	*domesticum*	*signatum*	
1.	Vișeul de Sus	3	21.04–19.05.15	26.04.15	0		43	1832	1	0	1/42.6
2.	Paltinu	3	20.03–16.05.15	12.04.15	0		56	1270	314	0	1/22.5
3.	Cacica	3	03.04–18.05.15	24.03.15	3	15.9	0	63	9	12	
4.	Cârlibaba	3	07.04–25.05.15	26.03.15	1	13.9	217	4464	0	0	1/19.6
5.	Lunca Ilvei	3	26.03–14.05.15	26.03.15	0		3	60	118	0	1/17.0
6.	Almaș	3	04.04–07.05.15	25.03.15	2	14.9	0	474	18	3	
7.	Stânceni	3	15.04–29.05.15	26.03.15	4	14.4	46	4527	13	0	1/89.8
8.	Brateș_3	1	08.04–20.05.15	26.03.15	1	14.6	0	5	6	0	
9.	Brateș_1	1	01.04–12.05.15	24.03.15	4	15.1	0	12	3	2	
10.	Brateș_2	1	01.04–12.05.15	25.03.15	2	14.9	1	130	4	0	1/22.0
11.	Dărmănești	1	30.03–11.05.15	26.03.15	1	13.6	6	51	14	0	1/8.5
12.	Sălătruc	2	30.03–11.05.15	25.03.15	2	14.4	9	181	45	17	1/19.1
13.	Poiana Uzul	3	02.04–07.05.15	11.04.15	0		61	204	41	0	1/3.3
14.	Soveja	3	28.04–02.06.15	26.03.15	13	17.4	0	180	9	29	
15.	Covasna	3	28.04–26.05.15	11.04.15	9	14.2	11	3609	10	0	1/298.3
16.	Brădăcești	3	26.04–31.05.15	11.04.15	8	15.9	0	71	6	0	
17.	Predeal	3	23.05–04.07.15	11.04.15	19	15.5	0	4285	10	0	
18.	Poiana Brașov	3	30.03–11.05.15	26.03.15	1	13.3	178	1387	17	3	1/7.8
19.	Moroeni	3	02.04–21.05.15	11.04.15	0		0	69	5	0	
20.	Bădeni	3	02.04–23.05.15	11.04.15	0		67	722	348	56	1/3.2
21.	Căpățâneni	3	03.05–14.06.15	16.04.15	5	13.8	0	165	5	0	
22.	Cârțișoara	3	02.04–14.05.15	16.04.15	0		68	517	0	0	1/7.4
23.	Paltin	3	02.04–14.05.15	16.04.15	0		6	606	18	0	1/101.0
24.	Bistra	3	01.05–05.06.15	11.04.15	6	13.9	2	365	1	0	1/151.5
25.	Jieț	3	20.04–25.05.15	26.03.15	6	15.7	83	818	67	0	1/7.7
26.	Cugir	3	23.04–08.06.15	11.04.15	4	14.8	1	769	0	0	1/671.0
27.	Poiana Mărului	3	14.04–19.05.15	26.03.15	4	15.9	1	88	12	0	1/68.0
28.	Rusca Montană	3	14.04–19.05.15	25.03.15	7	17.1	0	64	2	0	
29.	Padiș_2	1	07.05–10.06.15	25.03.15	16	16.5	3	262	19	0	1/31.3
30.	Doda Pilii	1	07.05–10.06.15	26.03.15	13	16.1	1	364	12	0	1/364.0
31.	Padiș_1	1	07.05–10.06.15	26.03.15	10	15.6	0	427	22	0	
	Total	78					863	28,041	1149	122	

Since February 2015 and the first ten days of March 2015 were warmer than normal, the installation of the traps in the forest was scheduled for March, so that they would be operational before the maximum daily temperature reached 13 °C, but in the second half of March and the beginning of April, the weather had become very variable, including snowing periods, and in many cases, the installation of traps was postponed for April or even May.

The traps were kept in the field for four to eight weeks and the captures were generally checked weekly, and in some cases every two weeks. The collected insects were preserved in ethanol until their identification.

Additional data on the presence of the *T. laeve* species in other sites was obtained from the processing of biological material captured in eight other studies using the same type of pheromone, and in one case (at Borca, Neamţ county), a flying beetle collected on logs.

2.2. Determining the Date of the Commencement of the Flight and the Number of Missed Flight Days

Given the way in which field traps were installed, for a fair interpretation of the results, it was necessary to indirectly determine whether they were set up before or after the beginning of flight, and the respective number of missed flight-friendly days.

For this purpose, the maximum daily air temperature (T_{max}) values for January–May 2015 have been extracted from the E-OBS data base [54] version 15.0, for the sites where the traps had been installed. The average altitude of the area covered by the grid cell (0.25° × 0.25°) corresponding to each site has been obtained from the same data base.

Then, the maximum daily temperature was corrected for the difference between the elevation of the field location and the mean elevation of the grid cell, taking into account a mean thermal gradient for the maximum daily temperature during the spring of 0.86 °C/100 m for the Eastern Carpathians, 0.92 °C/100 m for the Southern Carpathians, and 0.79 °C/100 m for the Western Carpathians [55].

Daily corrected maximum temperatures ($T_{max.correct}$) were then compared to the thermal threshold (13 °C) at which, according to the data published by Martikainen [20], the flight of *T. laeve* species begins. The first day of the year when this threshold was reached was considered to be the start date of the flight.

If the trap installation was made after that date, the time interval between the start of the flight and the day of the trap installation was considered a delay time, expressed in days of delay. In the period of delay, the maximum daily temperature was often below the thermal threshold and the insects did not fly. Subtracting from the total number of days of delay those in which the maximum corrected temperature was below 13 °C resulted in the number of days actually missed and for those days, the average maximum temperature ($T_{max.aver}$) was computed.

The same procedure was used for *T. lineatum* for which a temperature threshold of 15 °C [20] was taken into account.

2.3. Studies of Collection Material

In order to find out if there are specimens of *T. laeve* in the country collections, the main collections of Scolytinae in the country have been checked, namely those from the Museum of Natural Sciences Suceava—"Ştefan Negru" collection, presented in the catalogue published by Vasiliu et al. [56]; from the Brukenthal Museum in Sibiu—a collection presented by Negru [57]; and from the Faculty of Biology of Babeş-Bolyai University in Cluj-Napoca—the non-catalogued Orest Mark's collection.

Species identification was done using the key published by Pfeffer [16].

2.4. Data Processing

Since our primary goal was to determine whether *T. laeve* is present in the locations where traps were installed, the results are given as the total number of captures per site. However, in order to facilitate the interpretation of data on *T. laeve* captures, taking into account the captures of *T. lineatum*, a species collected at all sites and which uses the same substrate with *T. laeve* [18–20], the potential correlation between the captures of these species was analysed. For this analysis, only the places with *T. laeve* captures were taken into account. Because the data were not normally distributed (Shapiro-Wilk test), even after their log or square root transformation, Spearman's rank order correlation was run.

To understand if *T. laeve* responds differently than *T. lineatum* to the pheromone we used, a fact that could have affected *T. laeve* captures, the proportion of males in the total catches of the two species was analysed. It has been assumed that the sex ratio in nature for both species is the same, 1:1, as is known for *T. lineatum* [58]. Only locations where at least 30 specimens of each species were captured were considered, and the proportions have been calculated for each place. Testing for the difference between the two proportions was done using the Z-test [59], because theoretically, both sexes have the same chance of being captured.

A significance level of 0.05 was taken into account both for correlation analysis and for the comparison of two proportions. Statistical calculations were made with XLSTAT 2012 version (Addinsoft: Paris, France).

2.5. Maps

The distribution map for each *Trypodendron* species was obtained in ArcMap 10.2.2 software (Esri: Redlands, CA, USA). The projected coordinate system used was GCS WGS 1984.

3. Results

At the 31 sites where traps were installed to detect *T. laeve* in the spring of 2015, a total of 30,175 specimens of *Trypodendron* were captured, of which 863 (2.9%) were *T. laeve*, 28,041 (92.9%) *T. lineatum*, 1149 (3.8%) *T. domesticum*, and 122 (0.4%) *T. signatum* (Table 2).

T. laeve was trapped at 20 places, while *T. lineatum* was captured at 31, *T. domesticum* at 29, and *T. signatum* at seven places (Figures 1–4).

There was a strong positive and statistically significant correlation (r_s = 0.6225, p = 0.0041) between *T. lineatum* and *T. laeve* captures.

The number of *T. laeve* captures varied greatly from one place to another, being between 0.33 and 72.3 beetles/trap, with most of them in Cârlibaba (25.1%) and Poiana Braşov (20.6%), where the traps were set up in the spring of 2015 sufficiently early in relation to the altitude of the place and the evolution of the weather; in both cases, only one day of flight was lost.

Where the traps were placed before the flight or at most four days favourable to flight were lost, and the maximum daily temperature of those days did not exceed, on average, 15 °C, the ratio between the number of *T. laeve* and *T. lineatum* (caught during the *T. laeve* flight period) was, with only two exceptions, greater than 1:25.0. Where more than four flight-favourable days have been missed, the number of *T. laeve* captures has considerably decreased both in absolute terms and in relation to *T. lineatum* captures. In the places where more than 10 flight-favourable days have been lost, at most one to three beetles have been caught (e.g., at Doda Pilii and Padiş_2).

T. laeve was not captured at Soveja, Padiş_1, and Predeal, where the traps were installed at the latest time.

Some beetles of *T. laeve* were also caught in other locations (Table 3 and Figure 1), in traps baited with synthetic pheromone for *T. lineatum*, but—in the most cases—captures were very low compared to those of *T. lineatum*. However, in Putna, at 850 m above sea level, the captures of *T. laeve* were higher than those in Carlibaba and Poiana Brasov.

The proportion of males was 45.2–81.7% and 32.6–50.7% in *T. lineatum* and *T. laeve* captures, respectively, at the locations monitored in 2015. At all locations, except one (Bădeni), the male proportion in *T. laeve* captures was statistically significantly lower than in *T. lineatum* captures (z = 2.26–5.56; $p < 0.05$). In one of the eight additional studies using traps with similar baits (Putna, in 2017), the male proportions were 78.0–84.4% in *T. laeve* captures and 76.8–78.4% in *T. lineatum* captures, and the differences between proportions were not statistically significant (z = 0.18–1.10; $p > 0.05$).

No specimens of *T. laeve* were found in the coleopteran collections analysed in the study.

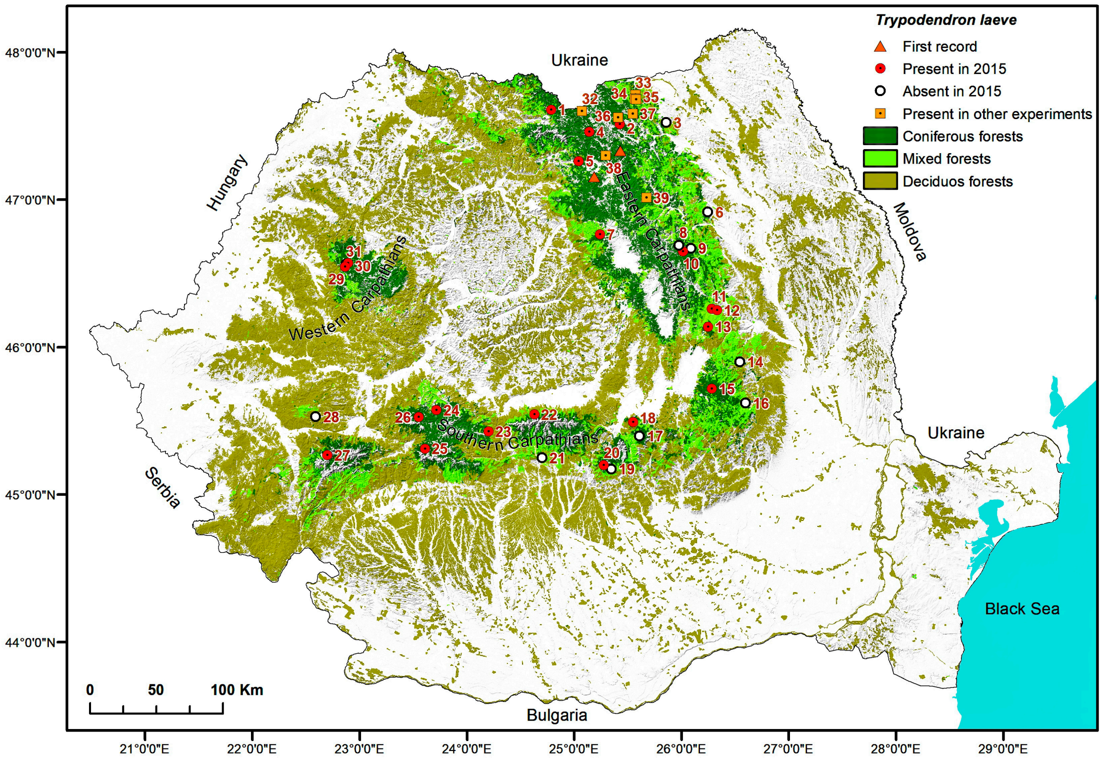

Figure 1. Sampling locations surveyed for the occurrence of *Trypodendron laeve* in 2015 and locations where it was found on other occasions mentioned in this study.

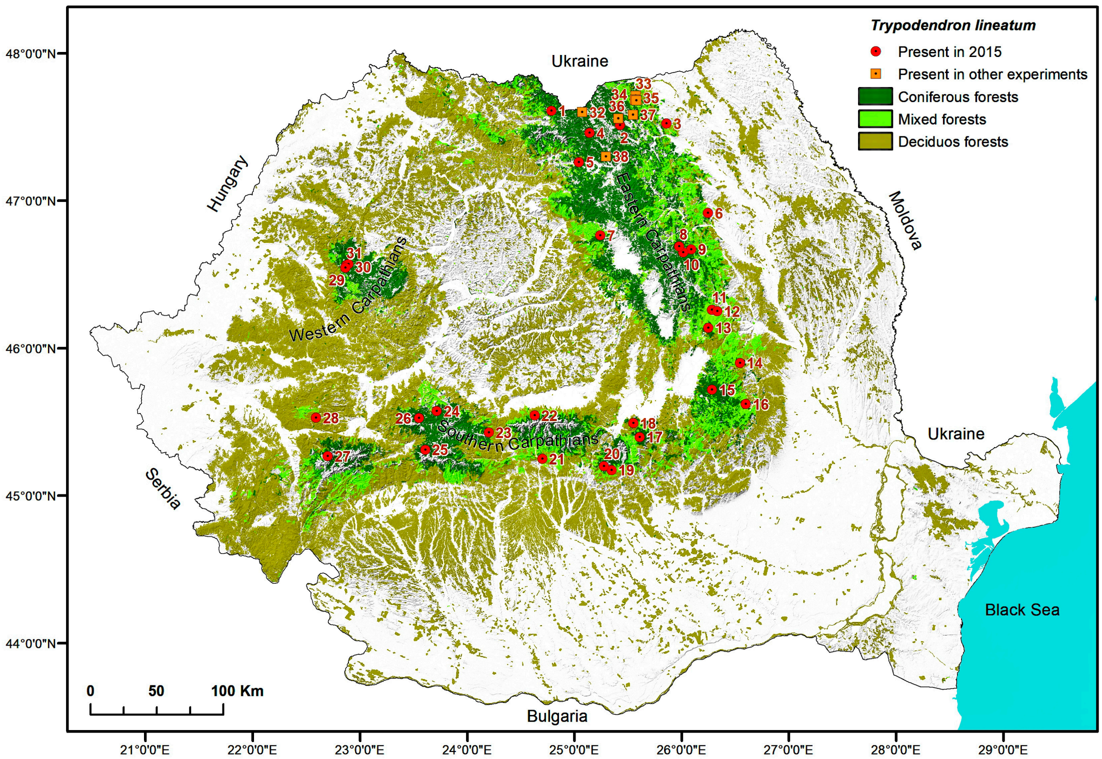

Figure 2. Sampling locations where *Trypodendron lineatum* was found in 2015 or on other occasions mentioned in this study.

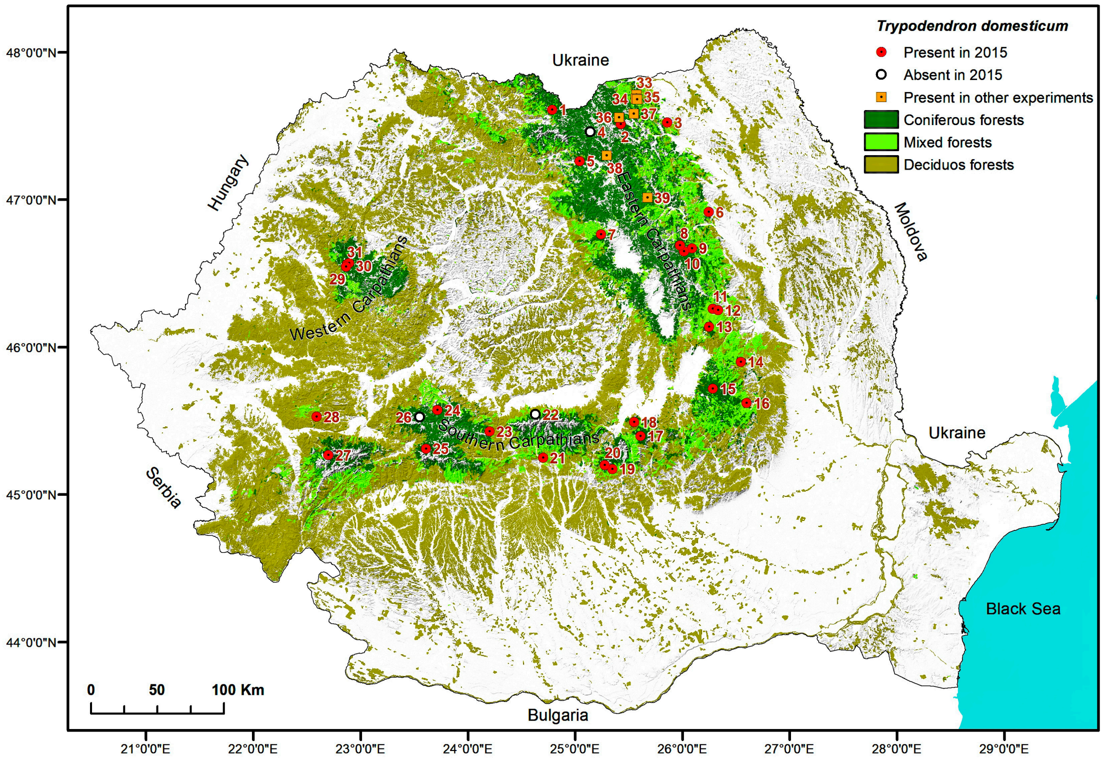

Figure 3. Sampling locations where *Trypodendron domesticum* was found in 2015 or on other occasions mentioned in this study.

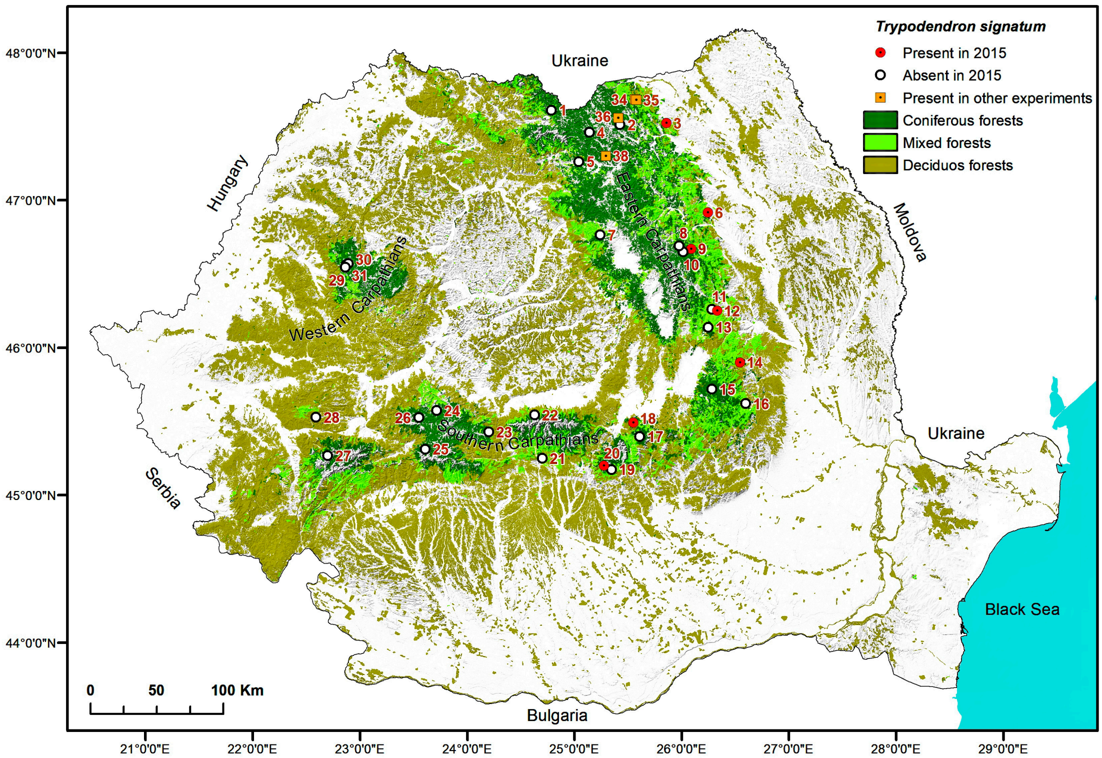

Figure 4. Sampling locations where *Trypodendron signatum* was found in 2015 or on other occasions mentioned in this study.

Table 3. Captures of *Trypodendron* species in other studies that have been conducted with synthetic pheromone of *T. lineatum*.

Site No.	Location	No. of Traps	Monitored Period	Total Number of *Trypodendron* Beetles per Study Site			
				laeve	*lineatum*	*domesticum*	*signatum*
32.	Bobeica	3	19.04–16.09.14	1	37,291	0	0
33.	Putna_1	3	13.03–24.08.17	5	353	19	0
34.	Putna_2	3	13.03–24.08.17	41	686	42	4
35.	Putna_3	3	13.03–24.08.17	237	4125	47	5
36.	Demacușa	20	12.04–15.06.16	1	19,366	524	1
37.	Ciumârna	20	06.05–09.06.15	1	14,095	57	0
38.	Iacobeni	20	13.04–16.06.16	3	31,906	47	3
39.	Borca	-	23.03.17	1	-	2	-
	Total	72		290	107,822	738	13

4. Discussion

Adults of *T. laeve* were captured along the entire Carpathian Mountains chain in Romania and in nine of the sampling locations the catches were substantial (more than 30 individuals). At some sites, the catches were very low, and in one third of all sampling sites, no specimens of *T. laeve* were trapped.

Considering that *T. laeve* fly very early and the intense flight takes only about three weeks [21,37,51], it is quite normal for the number of specimens to be smaller where the traps have not functioned throughout the flight period (as was the case for 23 of 31 places), or even to capture nothing where the traps have been set up after the end of the flight (e.g., in Predeal).

On the other hand, the abundance of *Trypodendron* individuals in a given location is dependent on the abundance of the ephemeral substrate in which these species develop, varying with it. Where the host resource is reduced, the populations decline, while they increase where the available host habitats increase [13,51,60]. Consequently, relatively small or missing *T. laeve* captures from some places where the installation of the traps was not delayed (Lunca Ilvei, Moroeni) or where only few favourable days for flight were lost (Sălătruc, Dărmăneşti, Paltin, Brateş_1–3, Almaş, Cacica) could be due to the poverty of the suitable breeding material (wind–thrown trees, stumps, coniferous trees killed by bark beetles). This is suggested by *T. lineatum*'s low captures from the same places, with the captures of the two species (*T. laeve* and *T. lineatum*) being closely correlated according to our results and to data from other studies [61], because both species colonize coniferous wood. The results from Putna confirm this hypothesis. While the traps at the elevations of 650 m and 750 m were set up in tree stands with only a few stumps (all older than one year), at an 850 m altitude, the traps were located at about 50 m apart from the logs (about 100 cubic meters) abandoned in the forest from the previous spring (2014) and many more beetles have been captured.

It is known that *T. laeve* hibernates in tree bark and wood [20], while *T. lineatum* hibernates in soil [13], which allows the first species to fly when the soil is still covered with snow. This gives *T. laeve* a competitive advantage over *T. lineatum* in occupying the available substrate [62], but only when the soil is covered by snow or when the warming in the spring does not proceed very quickly, but gradually. If warming is very fast, there is no delay or only a very short one between the dates when the maximum daily temperature reaches 13 °C and 15 °C when the flight of *T. laeve* and *T. lineatum* respectively starts [20]. This happened in 13 of the places where traps were set up in 2015. The almost simultaneous beginning of the flight of the two species was observed in Austria at low altitudes by Krehan and Holzschuh [37] and in southern Sweden in the nemo-boreal zone, where there is little or no snow in winter, by Öhrn et al. [51]. Even in many parts of the very north or high altitude forests, the beginning of the flight is more or less the same for both species (Torstein Kvamme, personal communication). In this context, *T. laeve* is also disadvantaged by the fact that the adults of this species apparently do not produce a sister-brood [20,51], as is the case with *T. lineatum* [13,63].

The above-mentioned issues could explain why *T. lineatum* was captured in a much larger number (at least 3.2 times more) than *T. laeve*, even if only the flight time of *T. laeve* is taken into account and only a maximum of one to two days of *T. laeve* flight were missed. However, it is possible that the number of captures has also been influenced to a certain extent by a possible differential response of the two species to the synthetic pheromone used in the traps, as Öhrn et al. [51] suggested for their study. If the natural sex ratio in the case of the two species is the same, the different proportions of males in the total captures indicate quite a different response of the two species to the synthetic pheromone used in this study. Such differences have also been reported by Krehan and Holzschuh [37], Martikainen [20], and Lukášová and Holuša [61]. The fact that the two species do not respond in the same way to olfactory stimuli was also evidenced by Kvamme [21], who found that *T. lineatum* adults (especially the males) were attracted to alpha-pinene released at a rate of 1.2–1.4 mg/h, while those of *T. laeve* were not.

In the case of a very low population density in some places, the lack of *T. laeve* captures may also be the result of other factors, such as the low sampling effort (up to three traps and in some places only one) or the inappropriate placement of some traps, so that in five places, only one or two of three traps captured adults of this species. Observations made in other studies have highlighted the fact that traps that are not seen due to the foliage of young trees (with branches close to the ground) capture much less insects than those that are not surrounded by obstacles (Olenici, unpublished data). On the other hand, Öhrn et al. [51] noted that both *T. lineatum* and *T. laeve* were captured in larger numbers in traps placed in shade than in the sun-exposed traps. This may be the result of beetles responding better to olfactory stimuli under relatively still conditions [64], which they find inside the forest rather than in open settings [65].

Of the *Trypodendron* species which develop in the wood of broadleaved trees, it is worth noting that, although in many places the traps were located in coniferous stands, *T. domesticum* was found almost everywhere, suggesting that it is a common species in Romania, like *T. lineatum*. On the other hand, *T. signatum* was collected from a much smaller number of places.

It seems that *T. signatum* is a rare species compared to *T. domesticum*, previously being reported in Romania from only a few places (Haţeg, Huluzu in Latoriţa Mountains, Sibiu, Braşov, Şumuleu Ciuc, Mihăileni, Frumoasa—Harghita, Tazlău—Neamţ, and Remeţi—Maramureş) [56,66–68].

The lack of specimens of *T. laeve* in the collections of Scolytinae analysed by us may be due to several reasons. First of all, the scolytids have been much less collected and studied in Romania than in the countries with a rich entomological tradition. Secondly, entomological excursions and insect sampling are not usually done so early, when *T. laeve* is flying. Even nowadays, it seems unusual for many people, including practitioners, to search for insects during the winter months or when snow is still on the ground. Thirdly, until recently, when there were no pheromone lures, collecting bark and ambrosia beetles by an axe and chisel was a more difficult and uncertain task than today, especially for rare species. Eloquent is the fact that almost all the data obtained after 1980 on the presence of *T. laeve* in different places were obtained using traps baited with synthetic attractants [19,21,35,37,44,52,53]. Even in countries with a very rich entomological tradition, there is relatively little historical data. Specimens of *T. laeve* were found as early as the 19th century or the beginning of the 20th century only in Sweden and Finland [30], while in Germany, there is a single historical record which dates back to 1953 [40].

Summarizing the above, one can say that *T. laeve* is a widespread species in the Carpathian Mountains, where it coexists with at least two other indigenous species: *T. lineatum* and *T. domesticum*. It has a continuous range, accompanying the Norway spruce even where it has been extended into the altitudinal belt of beech forests, but appears to be more abundant in the spruce forests at high altitudes.

5. Conclusions

T. laeve has a widespread distribution in the Carpathian Mountains and it seems that the species is more abundant at high altitudes. Overall, its populations are less abundant than those of *T. lineatum*.

Author Contributions: N.O. conceived and designed the study; I.V. prepared the pheromone baits and tested them to establish the release rate; M.-L.D. and G.I. dealt with fieldwork, collected all the data about the places where the traps were installed, and verified the three entomological collections; N.O. identified the beetles and M.K. verified the correctness of the identifications; N.O. wrote the paper and M.-L.D. prepared the maps. All co-authors assisted the lead author in writing and revising the manuscript.

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